Influence of exhaustive exercise on the immune system in solid organ transplant recipients

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ABSTRACT

Prolonged exhaustive exercise has a great impact on the immune system of athletes and leads to a transient weakening of the immune system. A host of studies has documented changes of immune parameters in peripheral blood following exercise. Concerning the effect of exhaustive exercise in transplant recipients there is little knowledge at present. We analysed peripheral blood in healthy athletes and transplant recipients who participated in the „Euregio cycling tour 2009" before and immediately after they performed 81 km of cycling that included ascending more than 1800 m in altitude.

A full blood count and an automated differential count as well as microarray analysis were performed before, immediately after, and one day after exercise in 10 male patients carrying a kidney transplant and in 10 controls matched in age and gender.

Comparing the absolute increase in neutrophils in these two groups, we detected that the relative increase in neutrophils was significantly smaller in transplant recipients compared to their corresponding controls after exhaustive exercise. While both groups were comparable in performance, microarray analysis revealed a markedly different pattern of gene expression in transplant recipients compared to their controls. From the 130 genes that were significantly upregulated in controls immediately after exercise, only 12 genes were also upregulated in transplant recipients. 64 different genes were upregulated in transplant recipients only.

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Our findings may be related to the immunosuppressive medication that the transplant recipients took and therefore it should also be discussed that regular exercise might reduce the need for immunosuppressive medication in transplant recipients.

Key words: Exhaustive Exercise, Transplant recipients, Cycling, Immune system

INTRODUCTION

In the previous issue of "Exercise Immunology Review" we had the possibility to briefly introduce our project concerning transplant recipients and exhaustive exercise associated with the „Euregio cycling tour 2009" (11). We now are delighted to present a short summary of the first results from this study. It is known that moderate exercise has a positive effect on the immune system, while exhaustive exercise can lead to a transient immunodepression (13; 17). The response of athletes to exercise is a coordinated reaction of several organ systems (2). For instance white blood cells are activated and regulated following exhausting exercise. Many genes, such as inflammation-associated cytokines are known to be involved in this process (8; 15; 21; 22) . Increased levels of cortisol and adrenaline are also thought to participate in this kind of regulation (16). Furthermore, exhaustive exercise also leads to generation of reactive oxygen and nitrogen species (ROS, RNS) (14). In addition to their cell damaging potential, ROS play a physiological role in promoting lymphocyte apoptosis (9). Besides lymphocyte apoptosis through ROS, lymphocytopenia with reduced T cell proliferation and T cell production of IL-2 and IFN-gamma is also observed after exhaustive exercise, due to a decrease in the percentage of type 1 T cells and NK cells in the circulation at this time (7). All in all these impacts can lead to depression of immune function in athletes following exhaustive exercise. The impact of exhaustive exercise on the immune response in transplant recipients is unclear at present and of great interest for the transplant society, particularly since the immune system is strongly affected by the life-long required immunosuppressive medication after organ transplantation. To understand the relationship between the immune response and exercise it is crucial to investigate the influence on cell regulation before and after exhaustive exercise in healthy athletes and transplant recipients.

METHODS

The *"Euregio cycling tour"* is completed over 3 days and contains more than 300 km (day one: 110 km, day two: 90 km, day 3: 102 km). On day one cyclists have to cycle 110 km with a substantial ascent in altitude (Figure 1A). For the study blood samples were collected from the participants on the first two days. Blood samples (3 x 2.5ml whole blood) were drawn from seated subjects into EDTA and PAXgene™ Blood RNA Tubes (Qiagen, Hilden, Germany) at rest before (t0), immediately (up to 15 minutes) after cycling 81 km with an ascent of more than 1800 m in altitude from the starting point (t1), and one day after cycling (t2). The exact time of arrival of cyclists is unfortunately not available though all cyclists

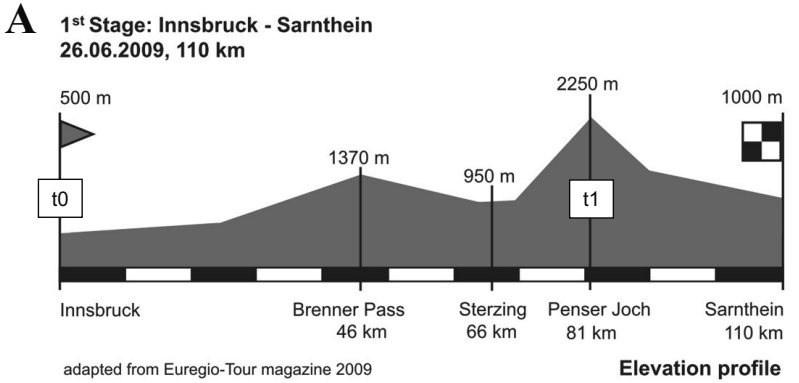

Figure 1A: The figure depicts the elevation profile of the "Euregio cycling Tour 2009", which participants had to surmount the first day. Altitude (in meters above sea level) and names of prominent locations are given.

did arrive within 45 minutes and the sequence of arrival was documented. For transplant recipients the positioning was recorded as places 2, 3, 7, 9, 11, 12, 16, 17, 19, 20 (average positioning: 11.6) and for controls the corresponding positioning was recorded as places 1, 4, 5, 6, 8, 10, 13, 14, 15, 18 (average positioning 9.4).

Candidate selection

Out of approximately 30 solid organ transplant recipients, 10 male patients, all of whom had received a kidney transplant in their past were selected for our study.

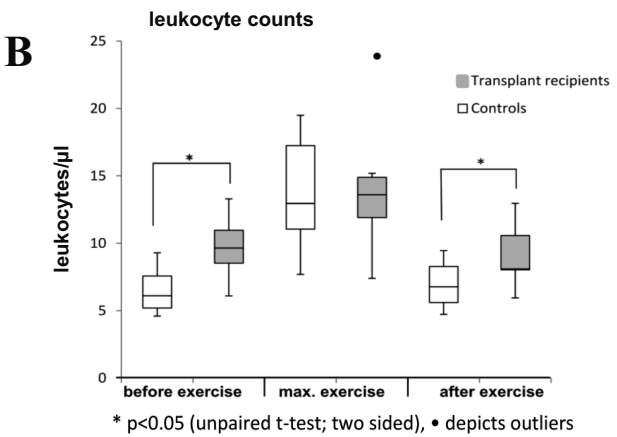

Figure 1B: The figure shows leukocyte counts of healthy control subjects (n=10) given in white, and transplant recipients (n=10) shown in grey, in a box and whiskers diagram. Both groups are shown before exercise (t0), at maximum exercise (t1) and one day after exercise (t2). Differences between both groups were assessed by a paired two sided t-test and are marked by asterisk when significantly different ($p<0.05$).

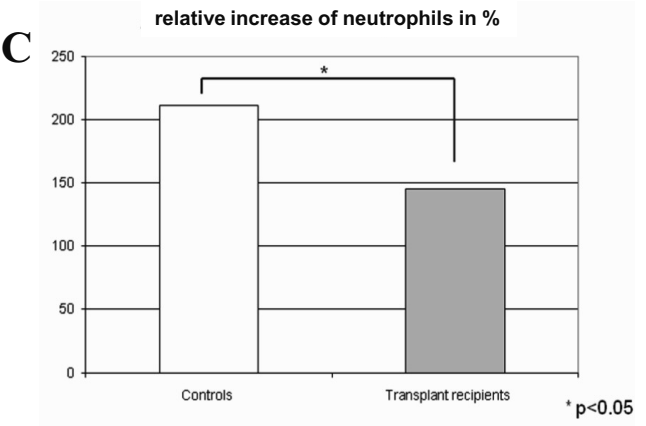

Figure 1C: The figure shows percental differences in the absolute increase of neutrophils of healthy control subjects (white) compared to transplant recipients (grey) comparing pre-exercise values to values after exhausting exercise (t0/t1). Differences between groups were assessed employing student´s t-test and marked by asterisk where significant ($p<0.05$).

Moreover, a healthy control group, consisting of 10 cyclists, matched in age and gender, was chosen. In the control group height was 178.4 ± 6.1cm, body mass 78.0 ± 9.8kg; body mass index (BMI) 24.5 ± 1.5 kg/m^2, age 49.2 ± 7.2 years and weekly training 2.7 ± 0.6 hours. In the transplant recipients height was 177.1 ± 9.1cm, body mass 71.3 ± 9.8kg, BMI 21.8 ± 2.2 kg/m^2, age 43.5 ± 2.2 years and weekly training 2.8 ± 0.4 hours. Only BMI showed a significant difference between groups ($p=0.006$) (an overview of data is shown in table 1). All kidney recipients had stable organ function and were in a healthy general status at the time of the tour as were the cyclists participating in the control group. Immunosuppressive medication used by the transplant recipients included FK 506 (Tacrolimus), Cyclosporine, Mycophenolate-Mofetil or Azathioprine.

Table 1:The table shows the anthropometric data of healthy control subjects and transplant recipients which were assessed in this study. Height, weight, body mass index (BMI) in kg/m^2 and age of cyclists as well as weekly training hours are given as means ± standard deviations, the respective minimum and maximum values are shown in square brackets. Inter group differences were assessed by student´s t-test and marked by asterisk when significantly different ($p<0.05$).

	Height (cm) [min-max]	**Weight (kg)**	**BMI (kg/m^2)**	**Age (years)**	**Weekly trainig (h)**
Controls (n=10)	**178.4 ± 6.1 [172-190]**	**78.0 ± 9.8 [71-85]**	**24.5 ± 1.5 [21.6-26.4]**	**49.2 ± 7.2 [39-63]**	**2.7 ± 0.6 [1-3]**
Transplant recipients (n=10)	**177.1 ± 9.1 [160-190]**	**71.3 ± 9.8 [50-82]**	**21.8± 2.2 [17.3-25.6]**	**43.5 ± 2.2 [25-66]**	**2.8 ± 0.4 [2-3]**
p-value	**0.72**	**0.07**	**0.006***	**0.24**	**0.69**

Each subject gave written informed consent prior to participation in the study. The experimental protocols were approved by the University Hospital's Human Ethics Committee according to the principles set forth in the Declaration of Helsinki of the World Medical Association (449/2008BO2).

Differential blood count

A full blood count and an automated differential count were performed at rest before (t0), immediately (up to 15 minutes) after (t1), and one day after exercise (t2). The total leukocytes and leukocyte subsets before, directly after and one day after exercise were compared in each individual. The absolute increase in neutrophils in the two groups was compared.

Transcriptome profiling

For expression profiling 400 ng of total RNA were linearly amplified and biotinylated using the Illumina® TotalPrep™ RNA Amplification Kits (Ambion) according to the manufacturer's instructions. Human HT-12v3 bead arrays (Illumina, San Diego, CA) were hybridized with 750ng cRNA for 18h at 58°C according to the Illumina® Whole-Genome Gene Expression with IntelliHyb Seal System Manual. Arrays were washed three times with buffer E1BC, High-Temp Wash Buffer and 100% ethanol, respectively, stained with streptavidine-Cy3 and again washed with buffer E1BC. Raw fluorescence intensities were recorded on a BeadArray Reader GX (Illumina). Average signal intensities, background correction, quantile normalization and quality control were performed with BeadStudio 3.1 software (Illumina).

All subsequent data analysis steps were performed on the software platform R 2.10.0 and Bioconductor 2.6.1 (6) with the packages "beadarray" (3; 5), "limma" (18; 19), "GOstats" (4). Initially, the expression data from all chips were normalized with VSN (10). The signal values were then averaged for the individual subgroups and differences in expression level were calculated. Differences between subgroups were extracted as contrasts and analyzed with the moderated F-test (empirical Bayes method) including a correction step for multiple testing with the 5%-FDR-based method of Benjamini and Hochberg. To attribute significant regulations to individual genes, a decision matrix was generated based on the function decide tests within the Limma option nestedF, where significant up- or down-regulations are represented by values of 1 or -1, respectively.

Due to the factorial design of the experiment, two parameters (patient group and time/treatment) have an impact on gene expression, while the influence of inter individual differences has to be taken into account. For both groups the factors time/treatment and donor was used to design a linear model capturing the influence of the different factors on gene expression levels. A non specific filter based on detection p-values was applied to remove non informative genes before the fitting of the linear models was performed. The coefficients describing the expression profiles of the remaining probe sets were calculated and the standard errors were moderated using an empirical bayesian approach. From the t statistic the resulting p-values were established and corrected for multiple testing with „Benjamini-Hochberg" (1).

Resulting gene lists were analyzed for over representation of gene ontology terms (www.geneontology.org) in the branches "biological process" and "molecu-

lar function" and KEGG pathways (www.kegg.jp) with conditional hypergeometric tests. Categories and pathways with a p-value ≤ 0.01 were considered significantly enriched.

RESULTS

All cyclists, consisting of the 10 transplant recipients and the 10 corresponding healthy control athletes finished the tour safely and successfully. Both groups were comparable in their performance and none of the athletes fell ill during the tour. All participants assessed here reached the destination of the tour within 45 minutes; within the first 10 participants a homogenous allocation of 40% transplant recipients and 60% controls was documented. We were able to show that the neutrophil counts at rest (t0), and also one day after exercise (t2) in transplant recipients were significantly higher than in their corresponding controls (Figure

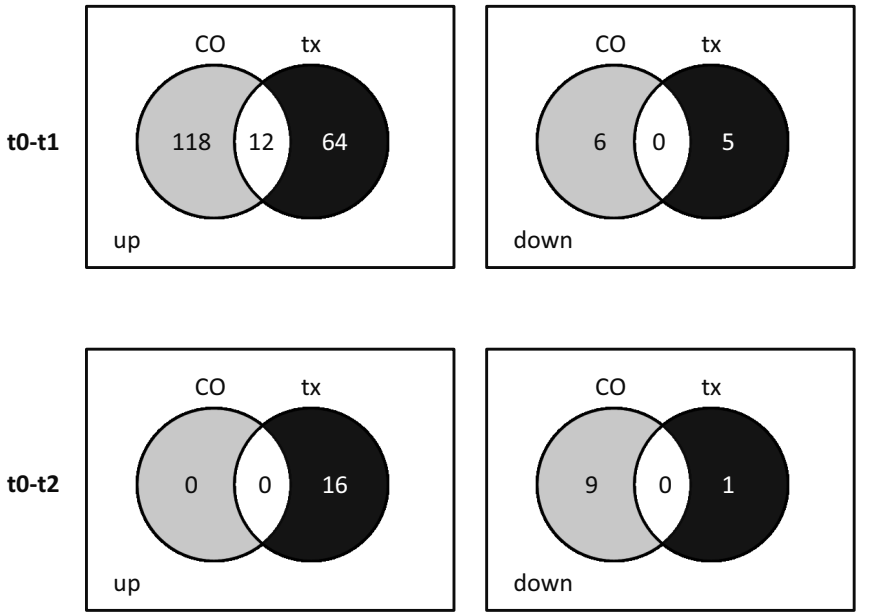

Figure 2: Circular charts of the amount of gene transcripts significantly regulated by exercise in transplant recipients (n=10) and corresponding healthy controls (n=10). The amounts of regulated genes detected are given as numbers in the circles, commonly up-/down-regulated genes are shown in overlapping circles (white). Gene regulations in transplant recipients are given in black in the circular diagrams, healthy control subjects are shown in light grey. The two circular charts at the top depict differences and analogies in regulated gene transcripts between rest (t0) and directly after exercise (t1); up-regulated gene transcripts are shown in the left diagram, down-regulated transcripts are depicted in the right diagram. The circular charts at the bottom depict differences and analogies in regulated gene transcripts between rest (t0) and one day after exercise (t2), up-regulated gene transcripts are shown in the left diagram, down-regulated transcripts are depicted in the right diagram.

1B). Directly after exhausting exercise (t1) the total amount of neutrophils was more or less identical in both groups (Figure 1B). The relative increase of neutrophils was however significantly smaller in transplant recipients compared with their corresponding controls after exhausting exercise due to higher basal levels (t0) (Figure 1C).

Microarray analysis revealed a significantly higher increase of differentially expressed genes in controls compared with transplant recipients, directly after exhausting exercise (t1). Whereas 76 significantly up-regulated and five down-regulated genes were detected in transplant recipients (t0-t1), we found an up-regulation of 130 and down-regulation of 6 significantly regulated genes in the corresponding controls (t0-t1). From all these differentially expressed genes only 12 up- and 6 down-expressed genes were regulated in common between these two groups. Comparing the gene expression in controls and transplant recipients before (t0) and one day after exhausting exercise (t2) there were no significant

Table 2 – Ontologic groups of gene transcripts differentially up-regulated in transplant recipients and corresponding healthy control subjects, as well as collectively up-regulated ontologic groups in transplant recipients and corresponding healthy controls.

up-regulated genes in transplant recipients	**p-value**
intracellular lipid transport	< 0.001
lactate metabolic process	0.002
muscle activity	0.004
reactive oxygen species	0.008
connective tissue replacement during inflammatory response	0.008
up-regulated genes in corresponding healthy controls	
immune response	< 0.001
inflammatory response	< 0.001
response to stress	< 0.001
signal transduction	< 0.001
acute-phase response	0.001
positive regulation of metabolic process	0.001
cell chemotaxis	0.001
regulation of lipid metabolic process	0.001
lipid localization	0.002
negative regulation of apoptosis	0.002
leukocyte migration	0.005
leukocyte activation during immune response	0.005
response to hyperoxia	0.007
up-regulated genes in both groups	
cyclooxygenase pathway	0.001
negative regulation of lipopolysaccharide-mediated signalling pathway	0.001
muscle cell migration	0.005
very-long-chain fatty acid metabolic process	0.008

differences detectable. (Data are given in Figure 2). Ontologic groups of gene transcripts differentially up-regulated in transplant recipients and their corresponding controls are given in table 2.

DISCUSSION

Following prolonged exhaustive exercise a number of peripheral immunological parameters have been demonstrated to change significantly (22). These changes comprise induction of cytokines and hormones, tolerance to pathogenic stimuli, changes in NK cell activity, changes in absolute cell counts and total leukocytes and leukocyte subsets (22). The transient immunodepression noticed directly after prolonged exhaustive exercise may be due to these changes. Most of the changes returned back to normal by the next day (12).

Exercise-induced changes of cell counts are known to influence gene expression levels of whole blood, but little is known about the behaviour of the immune system after exhaustive exercise in transplant recipients. Due to the need for lifelong immune suppression to avoid organ rejection it is of major interest for transplant recipients and the transplantation society to know how patients should deal with sports, especially those involving exhaustive exercise. There is evidence that exercise performance and maximal oxygen consumption are impaired after liver transplantation (20). In part, this may occur due to chronic deconditioning or myopathy due to immunosuppressive medication. In the present study we were able to show that the relative increase of neutrophils in transplant recipients was significantly smaller than in their corresponding controls after exhausting exercise. Nevertheless, the total number of circulating neutrophils after exhausting exercise was more or less identical in both groups. Accordingly and interestingly, the blood neutrophil counts at rest, and also one day after exercise in transplant recipients were significantly higher than the counts in their corresponding controls.

The data obtained by microarray analysis showed a significantly higher expression of differentially up-regulated genes in controls compared with transplant recipients. Concerning the distinct functional orientation of these differentially expressed genes, we were able to show for the first time that the control athletes demonstrated a higher immune response regulation than the transplant recipients. Transplant recipients showed a significant activation of genes related to cell metabolism, but not unexpectedly genes related to the immune response were missing. Reassuringly, most of the observed changes returned back to normal by the next day.

Being aware of the known the immunosuppressive effects of exhausting exercise in athletes, the question of the additional response to exercise in transplant recipients arises. On the one hand, it is possible that the immune system is impaired in a dual way by the effect of exhausting exercise and additionally by the immunosuppressive medication. This could lead to an increased risk of infection in transplant recipients after exercise. In this case regular exercise might also be an alternative solution to reduce the amount of immunosuppressive medication that is needed. On the other hand, it is possible that in transplant recipients, since inflammatory mechanisms are curbed by their medication, exercise has no pro-inflammatory effects and consequently no anti-inflammatory counter regulation.

In this case, exercise would be simply neutralized in its interaction with the immune system. In view of the fact that performance and workload were similar in both groups the results show clearly that the vast majority of gene regulation which is associated with exhaustive exercise in "healthy" persons is not related to performance metabolism but to regulation of inflammation. Only those few pathways which are shared between transplant recipients and controls may be primarily essential for exercise itself (table 2). We think it is possible that these few commonly regulated pathways may also contain genes which are essential for training adaptation, because training effects appear to take place in both groups in a similar way. Altogether, the exact mechanisms of how exhausting exercise affects the immune system in transplant recipients still remain unclear, but are of major interest and should be further investigated in the future.

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