

EFFECT OF TEN DAYS HIGH-INTENSITY TRAINING ON DNA DAMAGE

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The purpose of this study was to examine the effects of ten days high-intensity training on human PBMC DNA damage. 20 subjects were randomly assigned into two groups (n=10). The intervention group was performing daily cycle training for ten days, while the control group did not exercise at all. Blood samples were analysed the day before training starts in the morning after the last session and after four days of recovery. Daily training was quantified using the TRIMP the RPE scale and lactate concentration. Also the differences in the overall well-being was measured using the MDBF Two-way ANOVA showed no significant differences between the groups in DNA damage. Results have shown that the stress, initiated by the training was not represented in the PBMC.

KEY WORDS: Overtraining, oxidative stress, exercise

INTRODUCTION: Intensive exercise and inadequate recovery can cause an overtraining syndrome (OTS). OTS is described as a state of constant negative performance caused by stress in the athlete's environment which cannot be manipulated positively through short recovery (Meeusen et al., 2012). Moreover OTS is characterized by mood disturbance, and a prolonged maladaptation not only in performance but also "of several biological, neurochemical and hormonal regulation mechanisms" (Meeusen et al., 2012). Stress can also result in a dysregulation of catecholamines. This dysregulation can cause increased DNA damage (Morath et al., 2014) which was shown in post-traumatic stress patients. Also free radicals such as the superoxide-ion, which occur in the metabolism in the mitochondria, can cause DNA damage hence an exercise-induced increase of DNA damage is suggested (Finaud, Lac, & Filaire, 2006). Combining both, indicates that DNA damage could be a possible marker for OTS. Still, there is a great demand on diagnostic tools to identify an athlete experiencing OTS (Mackinnon, 2000). So the idea is to identify if there are any effects of high-intensity training on DNA damage. Hartmann et al, (1994) measured firstly increased DNA damage after exercise. Also other authors published results showing significant increase of DNA damage following training. For example Mars et al. (1998) showed significant increase of DNA damage after a treadmill test. Additionally Tanimura et al. (2008) and Tanimura et al. (2010) showed significant increased DNA damage after cycle ergometer training in a single bout test and after three consecutive days of training. In literature, however, there is a lack of studies using controlled trials to estimate exercise-induced changes in DNA integrity. In former studies it was often looked at testing protocols or single sessions of exercise. A whole block, as used in different states of a periodized training process, has not been studied yet. Therefore, the purpose of this study was to estimate the effects of ten days high-intensity training on DNA damage in human peripheral blood mononuclear cells.

METHODS: 20 healthy male subjects were included in the study (age: 30.6 (\pm 7,6) years; VO₂max: 54.4 (\pm 8.6) ml*kg⁻¹*min⁻¹; BMI: 22.6 (\pm 5.9)) and randomly divided into two groups (intervention group (IG) & control group (CG), n=20). Sample size was calculated with G*Power in advance (d = 1,42 (Morath et al., 2014). All subjects were regularly active and participate in training at least three times per week. Participants gave written informed consent and the study was performed with the approval of the ethic committee of Medical Association Baden-Württemberg.

Protocol of incremental test exercise

In order to establish an individual training protocol VO₂max was determined according to Laursen et al. (2002), using an electronically braked cycle ergometer (Lode Excalibur Sport,

Gröningen (NL)). After 5 min warm-up at self-selected speed the test started at 100W workload. Thereafter, workload was increased $15W \cdot 30 s^{-1}$ until volitional fatigue. Expired air was analysed breath-by-breath. A successful test was defined by the following criteria: 1) oxygen consumption increased almost linearly with rising workload and approached a plateau at the end. 2) 90% of the age predicted HR_{max} was reached and 3) the respiratory exchange ratio was > 1.10 . The endpoints from the incremental test were VO_{2max} and the P_{max} value which was defined as the minimum power output at maximum oxygen consumption. One day after the incremental test the participants performed the T_{max} Test according to Laursen et al. (2002) as well. After 5min warm-up at self-selected pace the participants performed a maximum sprint at P_{max} until a minimum rpm of 60 could not be sustained anymore. Endpoint of this test was the time they performed at P_{max} .

Based on the incremental tests a training protocol was designed (table 1). This training was repeated once a day and ten days in a row.

Table 1
Training Protocol: Pmax= maximum power output at minimum VO_{2max} ; Tmax= time to exhaustion at Pmax; HRmax= age predicted maximum heart rate

Sets	Intensity	Duration	Rest
8	Pmax	60%Tmax	65%HRmax

In every session the blood lactate difference (pre-post) was measured in capillary blood from the fingers. The capillary was put into 5ml glucose immediately after taking blood samples and analysed with BioSen S_line (EKF-Diagnostics) the same day. Also the heart rate (HR) was recorded (Polar RS800CX) during training sessions to calculate the training impulse (TRIMP, Banister, 1991). Furthermore, HR was classified in five categories (5: 100-90% HR_{max} ; 4: 90-80% HR_{max} ; 3: 89-70% HR_{max} etc.). Moreover, session RPE according to Foster et al. (2001) was evaluated directly after the training. CG did not train at all but used daily activities as usual.

Measuring DNA damage

Participants were asked to refrain from any type of exercise for at least 3 days. Blood samples from all participants were drawn in the morning one day before training starts, one day after the last exercise and after four days of recovery as well. To estimate the DNA damage an automated FADU-assay was performed. The procedure described by Moreno-Villanueva et al. (2009) was followed with minor modifications.

Psychological Testing

Besides the quantification of the training load a questionnaire was used (Steyer et al., 1997). The participants answered the question: "how do you feel at the moment?", followed by 24 adjectives like "fine" or "tired". Possible answers were given at a five points scale, where 5 means "absolutely" and 1 means "not at all". Negative items were analysed based on their inverted score. The result can vary from 24-120 points.

Statistics

Before the intervention the groups were analysed with a T-Test for unpaired groups. Effects of the intervention were estimated with a two-way ANOVA with repeated measurements (IBM, SPSS 22). The factors were time (3-steps) and Group (2-steps). Significant effects were corrected with Bonferroni post-hoc. Statistical significance was set with a p-value of $p < 0.05$.

RESULTS: ANOVA showed no significant main effects 'time' ($p=0.294$; $\eta^2=0.335$) or 'group' ($p=0.802$; $\eta^2=0.010$). Also no interaction was found ($p=0.766$; $\eta^2=0.085$). Training parameters revealed a high intensity of the daily training. In addition, the questionnaire showed a high impact of the intervention on the participants (figure 2).

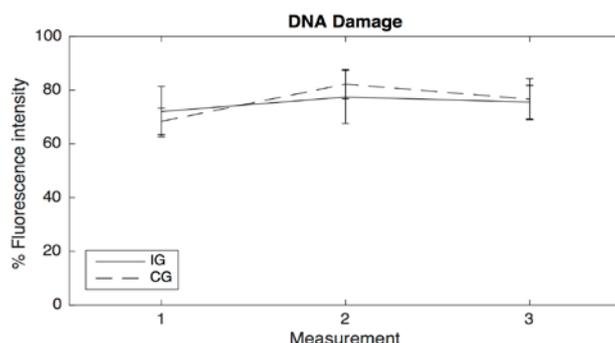


Figure 1: % fluorescence intensity. Intensity approaches double-stranded DNA in the sample. IG = intervention group; CG = control group. Values as mean \pm standard deviation.

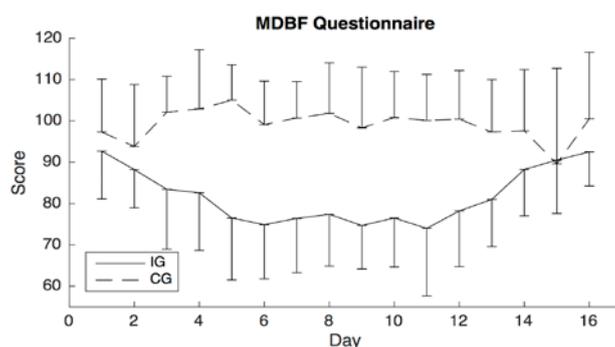


Figure 2: MDBF questionnaire values are shown as mean plus standard deviation (error bars). IG= intervention group; CG= control group.

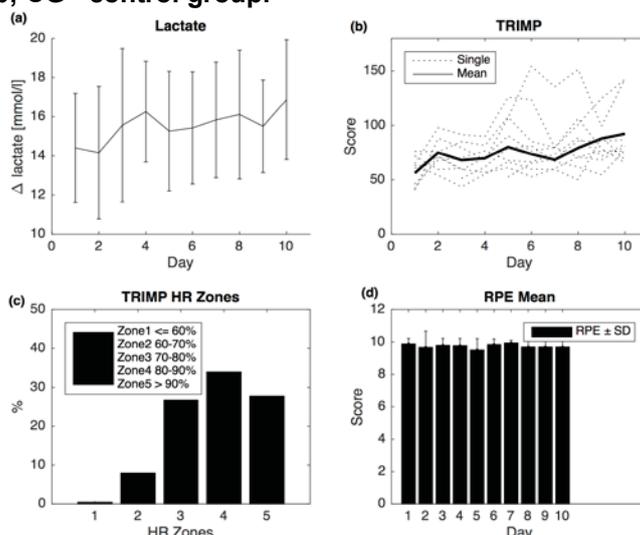


Figure 3: Control parameters of the daily training. (a) represents the mean lactate difference (pre-post) \pm SD (error bars). (b) shows the mean TRIMP and dashed the single trajectories. In (c) the HR zones are figured. Values are represented as mean over the whole training period and over all participants of the IG. (d) shows the mean RPE scale over the intervention \pm SD.

DISCUSSION: The purpose of this study was to examine the effect of ten days high-intensity training on DNA damage in young, healthy, physical active men. Based on the results, no effect could be found. The training had a high impact on subjective well-being. Moreover, the rest of the positive control parameters showed high intensity of every single training session. Those results contradict the results in literature (Wagner, Reichhold, & Neubauer, 2011). However, in this study DNA damage was examined for the first time in a randomized controlled trial. Also it was the first time that a whole block of high-intensity training was studied. So maybe interacting processes such as antioxidant capacities could have had an impact on DNA damage. Vezzoli et al. (2014) reported a significant reduction of oxidative stress after four weeks of training. Wadley et al. (2015) and Bogdanis et al. (2013) showed

significant increase in total antioxidant capacity after high intensity training. The fact that the training did not result in higher DNA damage could be a chance for diagnostics of overtraining. As a future approach athletes suffering from overtraining should be studied. If, in those future studies, an increased DNA damage can be seen in those athletes suffering from OTS, DNA damage could be a possible marker with discriminatory quality to distinguish between normal fatigue (like we produced in this study) and an acute OTS.

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