Endbericht 06/2015

The woman's heart:
Influence of cardiorespiratory fitness on parameters of platelet function relevant to the development of cardiovascular disease

Background and Study Design:

This project investigates the influence of cardiorespiratory fitness on platelet function in different groups of female volunteers, classified by their physical fitness.
The rationale for this project is the fact that physical inactivity (resulting in inferior fitness levels) represents a strong and independent predictor of mortality resulting from cardiovascular disease (CVD), independent of body composition and other risk factors. This is especially relevant as CVD represents the leading cause of mortality and morbidity in Western countries.
Blood platelets play a central role in the development of CVD. Platelets and their activation state are not only involved in the final stages of atherosclerosis but rather play a key role in the development of this disease, as oxidative stress and inflammatory conditions result in platelet activation and – in turn - stimulated platelets are causally involved in the onset of inflammatory reactions, cell proliferation and immune responses.
While acute exercise is reported to result in platelet activation, one study has described that regular exercise training is able to modulate some aspects of platelet function towards a less pro-thrombotic state.
However, it remains unclear if the observed modulation of platelet function by endurance training in (formerly) sedentary subjects represents a "normalization" of platelet function or just a small (nevertheless, beneficial) step towards a physiologically "ideal" state of platelet activation and –reactivity.
Therefore, this project aimed to clarify the relation between cardiorespiratory fitness and platelet activation / platelet reactivity.
Sedentary women, women of average fitness and elite athletes (classification based on VO_2max obtained from exercise tests) have been included in this study.
Initial platelet function tests and exercise tests were performed before and after a period corresponding to two menstrual cycles of supervised exercise training in the group of (formerly) sedentary women. Platelet function and fitness status was measured once in the group of average fitness and in elite athletes. Additionally, a control group was included, consisting of sedentary women who maintained their sedentary life style.

METHODS:

Experimental design: 62 apparently healthy, non-smoking women, with and without oral contraception, were included in this partly longitudinal, partly cross-sectional study (age 23.00 ± 2.97 years; body mass index 20.85 ± 2.19; mean ± SD).
They were assigned to one of three groups: sedentary (S), average fitness (AF) and endurance athletes (EA). Groups were defined by means of the individual's maximal oxygen consumption. At first, volunteers were selected according to their self-reported exercise habit (i.e. sedentary lifestyle for group S and regular endurance running including competitions for group EA). Subsequently, an exercise test was performed to determine each individual's VO_2max. VO_2max criteria for groups were (in ml/min/kg bodyweight): sedentary < 45, 45-55 average fitness, and > 55 endurance athletes.

Platelet function and cardiorespiratory fitness were measured during follicular phase. Sedentary volunteers (VO_2max=39.6 ± 4.8 ml/min/kg) were studied before (ST1) and after (ST2) a period of

endurance training (running distinctly below ventilatory threshold 2 up to 40 min, 3 x / week) performed over two consecutive menstrual cycles. In parallel to this training group, a control group was included, consisting of sedentary women who maintained their sedentary life style. Additionally, volunteers of average fitness (AF; VO_2max=48.39 ± 2.06) and endurance athletes (EA; VO_2max=60.86 ± 4.67) were studied at one time point.

Cardiorespiratory fitness was quantified by an incremental treadmill exercise test until volitional exhaustion. Heart rate was recorded continuously, oxygen uptake and carbon dioxide emission were measured continuously utilizing an open air spirometry system for all tests in breath-by-breath mode in order to determine ventilatory threshold 1 and 2 as well as maximal oxygen consumption (VO_2max).

Platelet activation state and platelet reactivity were assessed by flow cytometry. Blood was drawn between 7 am and 9 am in order to avoid influences of the circadian rhythm and was centrifuged at 120g for 20 min to generate platelet rich plasma (PRP). Subsequently, PRP was incubated with different concentrations of TRAP-6 (0-12µM), fixed with formaldehyde and either stained with FITC anti-human CD40L, dihydrorhodamine 123 for detection of intracellular reactive oxygen species or PE anti-human CD62P in order to construct dose-response curves for TRAP in terms of intracellular ROS generation, CD40L- and CD62P expression.

RESULTS:

Parameters of cardiorespiratory fitness (CRF)

Maximal velocity (Vmax), velocity at ventilatory threshold 2 (VVT2), oxygen uptake at VT2 (VO2VT2) were lowest in sedentary individuals and highest in endurance athletes, confirming their disparity regarding CRF. In line with this, endurance athletes also had the highest heart stroke volume at rest. Maximal heart rate (HRmax), heart rate at VT2 (HRVT2), maximal respiratory exchange ratio (RERmax) and the proportion of individuals fulfilling the maximal effort criteria did not differ between groups.

Parameters of platelet function in relation to CRF

To test the hypothesis that basal platelet activation as well as platelet reactivity differ between groups of varying CRF, CD62P-expression and CD40L expression were measured without the addition of a platelet agonist as well as after incubation with different concentrations of TRAP-6. Moreover, the formation of reactive oxygen species (ROS) was quantified in response to stimulation with TRAP-6.

A MANOVA showed that the 3 groups differed regarding the abovementioned parameters of platelet function (MANOVA; Pillai's trace V = 0.0559, $F(10, 110) = 4.93$, $p < 0.0001$). Subsequent separate univariate ANOVAs revealed that basal platelet activation measured by CD62P expression (shown in Figure 1) as well as platelet reactivity measured by agonist induced CD62P-expression, CD40L-expression and agonist induced ROS formation (depicted in Figure 2) were increased in sedentary volunteers compared with the other two groups, whereas basal CD40L expression did not differ between groups. Notably, whereas there was a pronounced difference regarding CRF between groups with average fitness (AF) and endurance athletes (EA), parameters of platelet function were roughly equal (see also Figure 3).

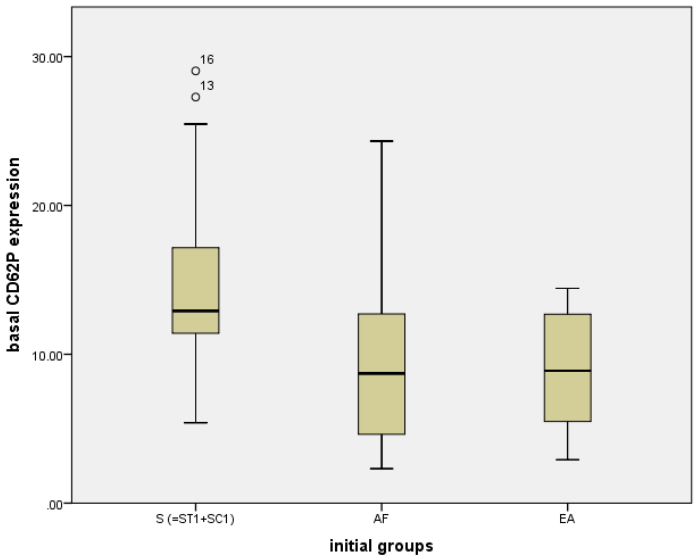

Figure 1: basal expression of CD62P on the platelet surface in the indicated groups

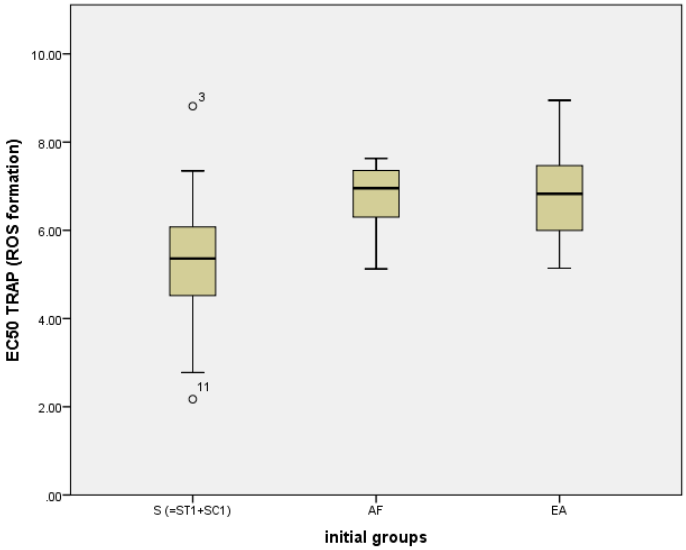

Figure 2: agonist-induced induction of intraplatelet reactive oxygen species in the indicated groups

Effects of exercise training on CRF and platelet function in sedentary volunteers

Exercise training caused a significant increase in VO_2max (mean ± SD, ml/min/kg:
ST1 37.11 ± 4.79 ⇨ ST2 42.74 ± 4.92; SC1 38.91 ± 3.39 ⇨ 38.35 ± 4.14; ANCOVA, *estimated effect of training +5.75 ml/min/kg,* 95% CI 3.36 – 8.16; p<0.0001 - see also Figure 3) and Vmax (mean ± SD, km/h: ST1 10.16 ± 1.03 ⇨ ST2 11.48 ± 1.346; SC1 10.36 ± 0.81 ⇨ 10.52 ± 1.00; ANCOVA, *estimated effect of training +1.12 km/h,* 95% CI 0.66 – 1.58; p<0.0001).
Overall, VO_2max was correlated with basal expression of platelet CD62P (Spearman r = 0.38) as well as agonist-induced expression of CD62P (EC_{50} TRAP, Spearman r = 0.52), CD40L (expression at 6µM TRAP, Spearman r = -0.32) and ROS formation (EC_{50} TRAP, Spearman r = 0.49) in terms of increased platelet activation / reactivity at lower VO_2max-levels.

Agonist-induced expression of CD62P and CD40L was increased in sedentary volunteers compared to those of average fitness (CD62P: p = 0.005; CD40L: p = 0.01) and endurance athletes (CD62P: p = 0.0002; CD40L: p = 0.02); ROS formation was increased in sedentary volunteers as well (S vs. AF: p = 0.0004; S vs. EA: p = 0.001).

Exercise training of sedentary volunteers over a period of 2 menstrual cycles led to a decrease in basal CD62 expression (% CD62P expressing cells; ANCOVA, estimated effect of training -8.29%; $p < 0.001$), agonist induced CD62 expression (EC_{50} TRAP; ANCOVA, estimated effect of training +1.16µM; $p = 0.003$), agonist induced CD40L expression (% CD40L expressing cells at 6µM TRAP; ANCOVA, estimated effect of training -6.63%; $p = 0.001$) and agonist induced ROS formation (EC_{50} TRAP; ANCOVA, estimated effect of training +2.11µM; $p < 0.0001$).

Overall, after 2 menstrual cycles of exercise training, parameters of platelet function in heretofore sedentary volunteers were not significantly different from those measured in groups AF or EA.

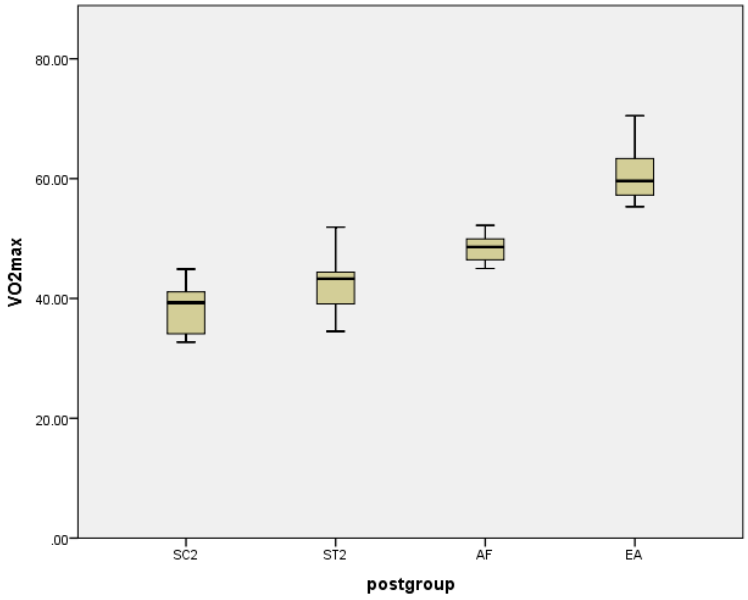

Figure 3: VO_2max of the indicated groups

CONCLUSION:

A sedentary lifestyle coincides with a pro-thrombotic and pro-inflammatory platelet phenotype. As such, platelet function significantly differs between sedentary and physically active volunteers. Noteworthy, platelets of volunteers with average and high cardiorespiratory fitness show comparable reactivity and basal activation state - therefore, our results suggest that a distinct level of cardiorespiratory fitness exists, above which no further benefit concerning platelet function can be expected. Consequently, a linear relation between CRF and platelet activation/reactivity can be excluded from the obtained results.

Endurance training of moderate intensity and duration is able to normalize platelet function. Interestingly, an alignment of platelet function between the (formerly) sedentary and the physically active groups was observed although the amount of exercise training was not sufficient to result in an alignment of CRF.

Therefore, it can be concluded that physical activity rather than physical fitness is the main determinant of platelet function. Given the central importance of platelets and their activation state in the development of atherosclerosis, these findings are in line with results of large epidemiological trials and appear highly relevant for preventive healthcare.

a.Univ.Prof. Dr. Ivo Volf
Institut für Physiologie
Medizinische Universität Wien