# Zwischenbericht 01/2014

## 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 cardio-vascular 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 aims to clarify the relation between cardiorespiratory fitness and platelet activation / platelet reactivity.
Sedentary women, women of average fitness and elite athletes (classification based on $VO_2$max 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:**
*Platelet function and cardiorespiratory fitness* were measured in healthy, young (23.4 ± 3 y), non-smoking women without any medication except oral contraceptives during follicular phase. Sedentary volunteers ($VO_2$max=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. Additionally, volunteers of average fitness (AF; VO2max=45.5 ± 4.7) and endurance athletes (EA; $VO_2$max=59.6 ± 5.9) 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 ($VO_2$max).

*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:**

Exercise training caused a significant increase in $VO_2$max (mean ± SD, ml/min/kg: ST1 39.75 ± 6.126; ST2 45.55 ± 5.191; p=0.0026) and Vmax (mean ± SD, km/h: ST1 10.52 ± 1.377; ST2 11.93 ± 1.346; p<0.0001).

Overall, there was a negative correlation between maximal running velocity at the end of the incremental exercise test (Vmax) and basal expression of platelet CD62P as well as agonist-induced expression of CD62P, CD40L and ROS formation.

Agonist-induced expression of CD62P and CD40L was increased (pre training) in sedentary volunteers compared to those of average fitness and endurance athletes; exercise training of sedentary volunteers over a period of 2 menstrual cycles led to an alignment of platelet function with volunteers of higher cardiorespiratory fitness ($EC_{50}$ TRAP mean CD62P expression: SC1: 5.026µM, SC2: 5.045; ST1: 6.026; ST2: 6.715; AF: 6.717; EA: 6.621; $EC_{50}$ TRAP mean CD40L-expression: SC1: 6.525µM, SC2: 6.27; ST1: 7.577; ST2: 8.388; AF: 8.425; EA: 8.345).

Generation of intracellular ROS were (not significantly) decreased after the exercise training, whereas the untrained control group showed significantly increased levels of ROS generation.

**CONCLUSION:**

A sedentary lifestyle favors a pro-inflammatory platelet phenotype. Endurance training of moderate intensity and duration normalizes platelet function, but high levels of cardio-respiratory fitness show no further benefit for platelet function. In sum these preliminary results are consistent with the proposed main hypothesis (the existence of an inverse relationship between cardiorespiratory fitness and platelet function) but suggest that a distinct level of cardiorespiratory fitness exists, above which no further benefit concerning platelet function can be expected. It is important to emphasize that these preliminary data are not yet complete and therefore have to be treated with caution.

**OUTLOOK:**

Each study group was calculated to consist of 15 apparently healthy women. As expected, there was a relatively high dropout in the training group – i.e., the (formerly) sedentary women. Currently, (data from) 1-3 volunteers are still missing in the respective groups. Additionally, plasma parameters have to be determined that might be relevant to platelet function, including plasma lipoproteins (triglycerides, VLDL, LDL, HDL) and platelet-related inflammatory markers/mediators (soluble P-selection, soluble CD40L).

The project will be completed within the next few months.

An abstract with the obtained data has been submitted for the ACSM's 61st Annual Meeting (May 27-31 2014; Orlando, Florida). Furthermore, data will be presented at the 2nd EUPLAN Conference on platelets (September 25-26th 2014; Le Bischenberg, France).

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