Correlation between Cardiorespiratory Fitness and Platelet Function in Healthy Women

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ABSTRACT

HEBER, S., A. ASSINGER, R. POKAN, and I. VOLF. Correlation between Cardiorespiratory Fitness and Platelet Function in Healthy Women. *Med. Sci. Sports Exerc.*, Vol. 48, No. 6, pp. 00–00, 2016. **Purpose**: Low cardiorespiratory fitness (CRF) represents a major risk factor for atherosclerosis, and platelets play a key role in the development of this chronic inflammatory disease. Therefore, the purpose of this study was to assess the relationship between CRF and platelet function. **Methods**: CRF and different aspects of platelet function were assessed in healthy, young, nonsmoking women. Results were compared between groups of low (LF), medium (MF) and high CRF (HF). Measurements were repeated in group LF after a supervised endurance training program lasting two menstrual cycles and obtained results were compared with groups MF and HF. CRF was quantified by maximal oxygen consumption ($\dot{V}O_{2max}$) determined by an incremental treadmill exercise test. $\dot{V}O_{2max}$ criteria for groups were (mL·min^{-1}·kg^{-1} bodyweight): LF < 45, MF 45–55, HF > 55. Platelet activation state and platelet reactivity were assessed by basal and agonist-induced surface expression of CD62P and CD40L as well as the intraplatelet amount of reactive oxygen species. **Results**: In group LF, basal platelet activation as well as agonist-induced platelet reactivity were increased compared with groups MF and HF. Between groups MF and HF parameters of platelet function were roughly equal despite a pronounced difference regarding CRF. Exercise training improved CRF in group LF and aligned platelet function to levels observed in groups MF and HF, although CRF still markedly differed. **Conclusions**: Low levels of CRF favor a proinflammatory platelet phenotype. A relatively low dose of exercise is sufficient to normalize platelet function, whereas superior levels of physical activity and CRF do not provide any further substantial benefit, but also no appreciable adverse effects. **Key Words**: ENDURANCE EXERCISE TRAINING, RUNNING, ATHEROSCLEROSIS, SEDENTARY LIFESTYLE, P-SELECTIN, CD40L

Physical inactivity with a concomitant lack of cardiorespiratory fitness (CRF) represents the most important independent risk factor for atherosclerosis (4,13,17,25,39). Atherosclerosis constitutes a chronic inflammatory disease, and platelets (in part by virtue of their proinflammatory capabilities) play a key role in its development (12,18,21,30,33). Depending on their activation state, platelets are able to express distinct (glyco)proteins (e.g., CD62P, CD40L) which facilitate their binding to leukocytes and the endothelium, resulting in leukocyte extravasation (2,3), impairment of endothelial function, and eventually leading to vascular inflammation.

A relationship between CRF and platelet function has not been precisely defined. Cross-sectional studies comparing sedentary with active individuals yielded contradictory findings, because they show either no difference between groups (10,26,31,37) or an increase in platelet activation in sedentary (8) or highly trained (23) individuals. In contrast, longitudinal studies were able to show that several weeks of exercise training performed with (formerly) sedentary subjects reduce platelet activation and reactivity (compared to the pretraining state) in diseased as well as in healthy populations (reviewed in Heber and Volf [15]). However, the lack of norm values for platelet function, applicable for scientific purposes, requires the inclusion of appropriate control groups for interpretation. Although commonly assumed, there is no evidence that pretraining values observed in sedentary volunteers represent a state of platelet hyperreactivity or that training results in "normalization" of platelet function.

Thus, the main purpose of our study was to define the overall relationship between CRF and platelet function. We aimed to clarify if individuals with low CRF exhibit increased platelet activation compared with active individuals with medium CRF, and further, if markedly higher levels of CRF entail any further benefit.

Therefore, we analyzed platelet function in three groups of volunteers, who differed regarding their levels of physical activity and CRF. We hypothesized to find highest levels of platelet activation in sedentary individuals with low fitness (group LF), less platelet activation in individuals performing

recreational sports on a regular base with medium fitness (group MF), and least platelet activation in competitive runners with high fitness (group HF).

Further, we aimed to relate platelet function in individuals with low CRF observed after several weeks of exercise training to platelet function observed in individuals with medium and high CRF. Thus, volunteers with low CRF underwent a supervised exercise training program for several weeks to determine to what extent exercise training diminishes platelet activation.

METHODS

Experimental Design

This study was approved by the ethics committee of the Medical University of Vienna and informed consent was obtained from each volunteer. Sixty-two women were included in this partly longitudinal, partly cross-sectional study. Inclusion criteria were: apparently healthy, eumenorrhea according to anamnesis, 18–30 yr of age. Exclusion criteria were smoking and regular intake of antiplatelet drugs. Volunteers both with and without oral contraception were included. According to their $\dot{V}O_{2max}$ reached in an incremental exercise test, volunteers were classified to be of low fitness (LF), medium fitness (MF) or high fitness (HF). $\dot{V}O_{2max}$ criteria for groups were <45, 45–55, and >55 mL·min^{-1}·kg^{-1} bodyweight for groups of low, medium, and high fitness, respectively.

In volunteers of groups MF and HF, CRF and platelet function were assessed at one time point. Volunteers of group LF were distributed to either a training (LFT) or a control (LFC) subgroup and were tested at two time points, at the beginning and at the end of a period spanning two menstrual cycles with exercise training (LFT) or no intervention (LFC).

Volunteers of group LF were requested not to change any aspect of their lifestyle—except for training habits if grouped to LFT. All platelet function tests were carried out in follicular phase to avoid possible bias due to influences of the menstrual cycle on platelet function. Two participants of LFT dropped out of regular training within the first 2 wk and were thereafter allocated to LFC.

Cardiopulmonary Exercise Testing

CRF was measured by means of an incremental exercise test on a treadmill with continuous breathing gas analysis and HR monitoring.

Before the exercise tests, blood pressure was determined, a 12-lead ECG at rest, heart ultrasound, and a lung function test were carried out to exclude contraindications for exercise testing (40). After explanation of the protocols and procedures as well as volunteer's familiarization with the treadmill, the breathing gas analyzer (Oxycon mobile; Jaeger, Wuerzburg, Germany) was calibrated for volume and O_2/CO_2 concentration according to the manufacturer's instructions.

Subsequently, the test was started at a running velocity of 6 km·h^{-1}, which was increased after each completed minute

until volitional fatigue or dyspnea; the slope was kept constant for all tests at 0.9%. To achieve maximal cardiorespiratory response without muscular exhaustion, each test duration was designated to be at least 8 min and 17 min at maximum (40). Hence, the increment per minute was chosen according to the subject's expected maximum running velocity appraised by anamnesis. Individuals reporting no or very low levels of exercise training were expected to exhibit low levels of CRF, thus increments of 0.6 km·h^{-1}·min^{-1} were chosen. For those reporting regular exercise training within the scope of nonprofessional recreational sports, increments of 0.8 km·h^{-1}·min^{-1} were chosen, and for volunteers who reported to be involved in competitive endurance sports, increments of 1.0 km·h^{-1} per min were applied. In case minimum duration was not reached or maximum duration was exceeded, tests were repeated with an adjusted increment at the earliest 72 h later to assure adequate recovery.

During exercise tests, respiratory gas exchange measures were recorded in breath-by-breath mode and averaged for 30 s. HR was measured continuously in 5 s intervals using Polar Vantage NV Telemetry (Polar Electro Oy, Finland). Maximum oxygen consumption ($\dot{V}O_{2max}$) was defined as the mean oxygen consumption during the last 30 s before volitional exhaustion. Ventilatory thresholds 1 and 2 based on a three-phase model (29) were determined visually as described in Simonton et al (28). In particular, VT1 was defined as the nadir of the respiratory equivalent for oxygen ($\dot{V}E/\dot{V}O_2$), VT2 as the nadir of the respiratory equivalent for carbon dioxide ($\dot{V}E/\dot{V}CO_2$). Maximal running velocity ($\dot{V}_{max}$), oxygen uptake at ventilatory threshold 2 ($\dot{V}O_2VT2$) and running velocity at ventilatory threshold 2 ($\dot{V}VT2$) were used as secondary measures for CRF.

Exercise Training

For each volunteer, the exercise period started immediately after completion of the first blood sampling/exercise test and ended after completion of the second set of tests after two menstrual cycles. Volunteers exercised three times per week with at least 1 d of recovery in between and were allowed to miss a maximum of two training sessions. Exercise training was performed under supervision, only in exceptional cases volunteers were allowed to perform an unattended training session.

Intensity was prescribed by means of walking/running velocity slightly below velocity at VT2 (16). Target duration per training session was 40 min of walking or running at target intensity. In case volunteers were not capable to achieve a total of 40 min per session, they were gradually acquainted to 40 min on an individual basis, where necessary by the use of intervals.

Echocardiography

Echocardiographic images were acquired in physically resting volunteers according to current guidelines (19) by a single experienced sonographer by the use of a commercially

available ultrasound system (Vivid 7; GE Medical Systems, Horten, Norway). LV volumes were calculated using the Simpson's biplane method from apical two-chamber and apical four-chamber images.

Blood Sampling and Preparation

Blood was collected via venipuncture of an antecubital vein using a 21G needle (0.8 × 19 mm; Greiner bio-one, Kremsmuenster, Austria) into 3.8% sodium citrate Vacuette tubes (Greiner bio-one), which were immediately gently inverted. To avoid bias, all blood samples were taken by the same physician between 7:30 AM and 9:30 AM from volunteers after overnight starvation and exercise abstinence for at least 24 h, and volunteers did not take any medication likely to interfere with platelet function within the last 12 d. Whole blood was centrifuged at 120g, 24°C for 20 min (Allegra™ X-12R Centrifuge; Beckman Coulter, Vienna, Austria) to generate platelet-rich plasma (PRP). Only the upper two thirds of the PRP fraction were used in order to avoid contamination with other cell types.

Platelet Function Tests

All determinations were performed in triplicates. Platelet function tests were carried out to quantify platelet activation levels without any stimulus (referred to as basal), and to estimate their tendency to become activated by a physiologically relevant stimulus (referred to as platelet reactivity). For measurement of basal platelet activation, PRP was incubated with phosphate-buffered saline (PBS). For assessment of platelet reactivity, platelet agonist thrombin receptor activating peptide (TRAP)-6 (dissolved in PBS) was incubated with platelets at different concentrations (final concentrations: 2, 4, 6, 7, 8, 10, and 12 μM) for 10 min. TRAP-6 represents a peptide (SFLLRN) which activates platelets by binding to protease activated receptor 1 receptors, thereby mimicking the action of thrombin, one of the most important platelet activators and key enzyme in plasmatic coagulation.

Determination of P-selectin expression. After incubation with PBS (± TRAP-6), PRP was fixed with formaldehyde at a final concentration of 1% for 15 min and subsequently incubated with a phycoerythrin-labeled antibody against P-selectin (PE antihuman CD62P, clone AK4; BioLegend, San Diego, CA) for 30 min.

Determination of CD40L expression. After incubation with PBS (± TRAP-6), PRP was incubated for 30 min with a fluorescein isothiocyanate labeled antibody against CD40L (FITC mouse anti-human CD154, clone TRAP-1; BDBiosciences, Schwechat, Austria) and subsequently fixed with 1% formaldehyde (f.c.).

Quantification of generation of reactive oxygen species. After incubation with PBS (± TRAP-6), PRP was incubated for 10 min with dihydrorhodamine 123 (Enzo Life Sciences, Lausen, Switzerland) at a final concentration of

AQ3 5 μM and subsequently fixed with 1% formaldehyde (f.c.).

Subsequently, cells were analyzed with a FACSCalibur (BDBiosciences) flow cytometer by the use of Cell Quest Pro software. In particular, 1×10^4 platelets were analyzed using scatter plots with log-transformed forward scatter (a measure for cell size) on the y-axis and fluorescence (e.g., of fluorophore-conjugated antibody bound to the platelet surface) on the x-axis. Within the diagram, a region (gate) was defined in the vicinity to a population of resting platelets (reflecting a region of higher fluorescence). Binding of fluorophore-conjugated antibodies to relevant activation-associated epitopes (or increased fluorescence of dihydrorhodamine 123, respectively) causes platelets to be plotted at higher fluorescence intensities and consequently within the predefined region. The percentage of platelets within the gate (in relation to the total number of analyzed platelets) represents a measure of platelet activation and % gated cells were used for further statistical analysis as explained below. Supplemental Digital Content 1 shows representative plots obtained with resting and activated platelets in the context of EC_{50} estimation (see Figure, Supplemental Digital Content 1, dose–response curves, http://links.lww.com/MSS/A654).

Statistical Analysis

Differences of metric variables among all groups were tested by the use of multivariate ANOVA and univariate ANOVA. For preselected pairwise comparisons Fisher least significant difference test was used followed by correction of P values according to Bonferroni–Holm. For all-pairwise comparisons, ANOVA was followed by Tukey test. 95% confidence intervals as well as (corrected) P values are given. Approximate normal distribution and approximately equal standard deviations were assessed by inspection of boxplots as well as Shapiro–Wilk testing. Where necessary, data were log-transformed. Dichotomous variables were tested by chi-square tests.

Effects of exercise training in group LF were estimated by multivariate ANCOVA and univariate ANCOVA with baseline levels as covariates. Differences between LFT and LFC are given in the respective unit with uncorrected 95% confidence intervals and corresponding P values corrected according to Bonferroni–Holm. ANCOVA models were tested graphically by residual plots.

As a measure for platelet sensitivity toward TRAP-6, best-fit values for EC_{50} in terms of CD62P expression and ROS formation and percent CD40L-positive platelets in response to 6 μM TRAP-6 were used for further statistical analysis by ANCOVA and ANOVA. Best-fit values for EC_{50} of TRAP-6 were estimated for each volunteer separately (at each time point, where applicable) by fitting a four-parameter dose–response curve to flow cytometry data (% gated cells) as a function of TRAP-6 concentration. The four parameters are: bottom, top, EC_{50}, and hill slope. The equation for the curve is $Y = \text{Bottom} + (\text{Top-Bottom})/(1 + 10^{\wedge}((EC_{50} - X) \times \text{HillSlope}))$. Accuracy of curve fitting and approximate normal distribution were verified by means

of inspection of the graph and its residual plot. Two exemplary dose–response curves are shown in Supplemental Digital Content 1 (see Figure, Supplemental Digital Content 1, dose–response curves, http://links.lww.com/MSS/A654).

Additionally, Spearman correlation coefficient was used to quantify associations between maximal oxygen consumption and parameters of platelet function. To adjust for the potential confounders, BMI, age, and the intake of oral contraceptives, also partial Spearman correlation coefficients were calculated.

To detect a potential nonlinear effect of $\dot{V}O_{2max}$ on parameters of platelet function, regression analysis was performed with parameters of platelet function as dependent variables, and $\dot{V}O_{2max}$ and the quadratic term of $\dot{V}O_{2max}$ as predictors.

For sample size calculation, a difference of 5% basal CD62P expression between groups was considered relevant. Based on a standard deviation of 5% in each group and a level of significance of 0.05, a sample size of 16 per group was needed to detect the relevant difference with a power of 0.8.

All reported P values are a result of two-sided tests. P values of 0.05 or less were considered significant. (*$P \leq$ 0.05; **$P \leq$ 0.01; ***$P \leq$ 0.001; ****$P \leq$ 0.0001).

Statistical analysis was performed with IBM SPSS Statistics 22 and Graph Pad Prism 5.

RESULTS

Group characteristics. According to their $\dot{V}O_{2max}$ levels, 62 volunteers were grouped into group LF ($n = 34$), group MF ($n = 16$), and group HF ($n = 12$). There was a pronounced difference in median self-assessed exercise duration per week (within 6 months before study enrolment) between these groups. As shown in Table 1, groups did not differ significantly with respect to age, BMI, or percentage of volunteers taking oral contraceptives.

Parameters of CRF. Maximal velocity ($\dot{V}_{max}$), velocity at ventilatory threshold 2 ($\dot{V}VT2$) and oxygen uptake at VT2 ($\dot{V}O_2VT2$) were lowest in group LF and highest in HF, confirming their disparity regarding CRF (Fig. 1A; see Table, Supplemental Digital Content 2, pairwise comparisons of groups low fitness and medium fitness, http://links.lww.com/MSS/A655). In line with this, endurance athletes also had the highest heart stroke volume at rest. Maximal HR (HR_{max}), HR at VT2 (HRVT2) and maximal RER (RER_{max}) did not differ between groups (descriptive statistics listed in Table 1).

Platelet function in volunteers of low, medium and high fitness. A MANOVA showed that parameters of platelet function differed significantly between the three groups (MANOVA; Pillai's trace V = 0.559, F(10, 110) = 4.93, $P < 0.0001$). Subsequently conducted separate univariate ANOVAs revealed that basal platelet activation (reflected by CD62P expression) and platelet reactivity (measured by agonist-induced increases in the expression of CD62P and CD40L as well as agonist-induced ROS formation) were increased in group LF compared to MF and HF. There were no significant differences regarding basal expression of CD40L between groups (data not shown).

Notably, parameters of platelet function were roughly equal between groups MF and HF, despite the fact that there was a pronounced difference regarding CRF between these groups (Figs. 1A and B; see Table, Supplemental Digital Content 2, pairwise comparisons of groups low fitness and medium fitness, http://links.lww.com/MSS/A655; see Figure, Supplemental Digital Content 1, dose–response curves, http://links.lww.com/MSS/A654).

Correlation between CRF and parameters of platelet function. Whereas correlations were found between $\dot{V}O_{2max}$ and basal expression of CD62P or agonist-induced expression of CD62P and CD40L as well as ROS formation, basal CD40L expression was not correlated with $\dot{V}O_{2max}$. Partial correlation coefficients with age, BMI, and the intake of oral contraceptives as control variables were found to be very similar to correlation coefficients without control variables.

Regression analysis consistently revealed significant effects of $\dot{V}O_{2max}$, but not for its quadratic term, with regard to basal CD62P expression (P for $\dot{V}O_{2max}$, P for $\dot{V}O_{2max}^2$; $P = 0.002$, $P = 0.617$), EC$_{50}$ of TRAP in terms of CD62P expression ($P < 0.0001$, $P = 0.878$) and ROS formation ($P < 0.0001$, $P = 0.578$) as well as for CD40L expression induced by 6 μM of TRAP ($P < 0.007$, $P = 0.909$). Scatter plots with respective correlation coefficients and linear regression lines are depicted in Figure 2.

Effects of exercise training on CRF and platelet function in group LF. 17 volunteers of group LF were distributed to LFT (training) and 17 to LFC (control). There was no significant difference regarding self-assessed weekly exercise duration during 6 months before study inclusion between these groups. During the training period, volunteers

TABLE 1. Group characteristics of volunteers with low CRF (LF), with medium CRF (MF) and with high CRF (HF).

	Unit		LF	MF	HF	P	Significance
Participants		n	34	16	12		
Weekly exercise[a]	h·wk^{-1}	Median (IQR)	0.25 (0.0–0.5)	2.25 (1.5–4.0)	11 (7.0–14.25)		
Age	yr	Mean ± SD	22.9 ± 2.8	22.4 ± 3.3	24.2 ± 2.8	0.29	n.s.
BMI	kg·m^{-2}	Mean ± SD	21.2 ± 2.2	20.9 ± 2.4	19.7 ± 1.4	0.12	n.s.
Contraceptives	n (%)		19 (55.9)	8 (50)	4 (33.3)	0.4	n.s.
SV/kg	mL·kg^{-1}	Mean ± SD	0.95 ± 0.15	1.13 ± 0.16	1.28 ± 0.20	<0.0001	****
HR$_{max}$	bpm	Mean ± SD	194 ± 8	198 ± 10	190 ± 9	0.08	n.s.
HRVT2	bpm	Mean ± SD	177 ± 12	180 ± 11	173 ± 9	0.25	n.s.
RER$_{max}$		Mean ± SD	1.11 ± 0.6	1.14 ± 0.8	1.14 ± 0.8	0.32	n.s.

[a]Self-assessed weekly exercise duration during 6 months prior to study inclusion.
SV/kg, left ventricular stroke volume (mL) at rest per kg body weight.
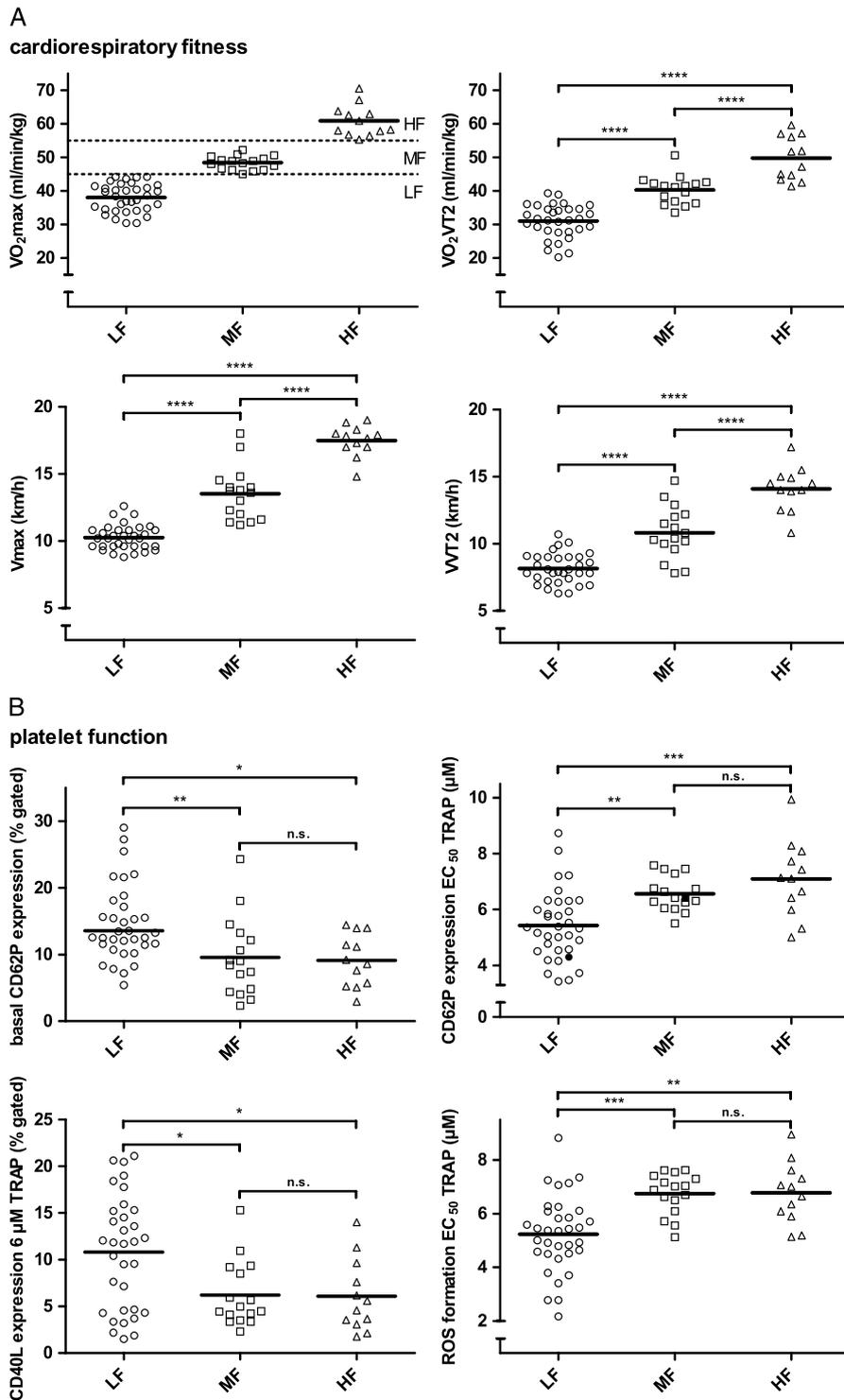*$P \leq$ 0.05; **$P \leq$ 0.01; ***$P \leq$ 0.001; ****$P \leq$ 0.0001.

FIGURE 1—CRF (A) and platelet function (B) in individuals with low (LF, *n* = 34), medium (MF, *n* = 16) and high CRF (HF, *n* = 12). Solid horizontal lines indicate means; dotted horizontal lines indicate predefined $\dot{V}O_{2max}$ limit values separating groups. Representative dose–response curves are plotted in Supplemental Digital Content 1 (dose–response curves, http://links.lww.com/MSS/A654; filled data points in B). *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq$ 0.001; ****$P \leq 0.0001$.

of group LFT completed 93.86% ± 2.15% (mean ± SD) of prescribed exercise training sessions. They performed 97.98 ± 14.85 (mean ± SD) minutes of exercise per week, which significantly affected CRF, resulting in increased $\dot{V}O_{2max}$, $\dot{V}_{max}$, $\dot{V}O_2VT2$, and $\dot{V}VT2$ levels (Fig. 3A). Stroke volume at rest as well as BMI were not affected by exercise training (descriptive statistics of LFT and LFC at both time points given in Table 2, effects of training estimated by ANCOVA listed in Table 3). Despite equal levels of CRF in LFT and LFC group, LFT group showed a tendency to lower levels
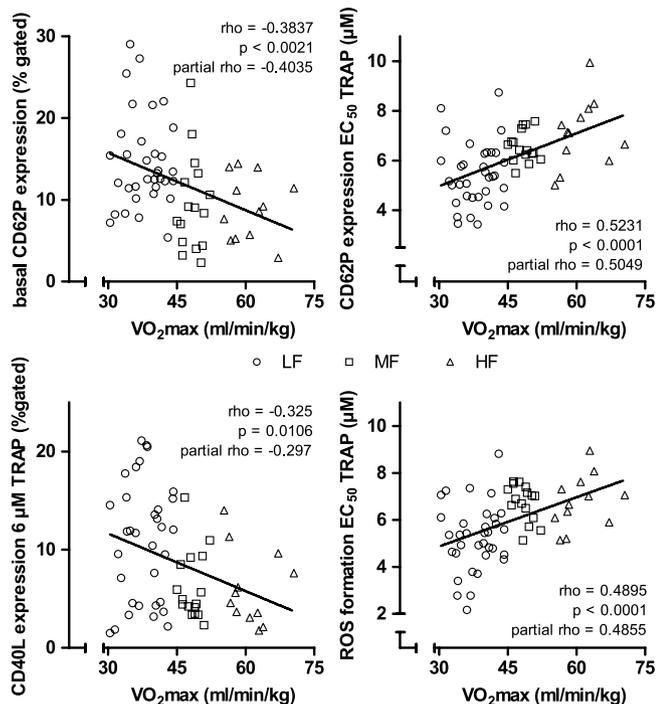
FIGURE 2—Scatter plots with linear regression lines of $\dot{V}O_{2max}$ and parameters of platelet function with Spearman correlation coefficients, respective *P* values and partial Spearman correlation coefficients with age, BMI, and the intake of oral contraceptives as control variables. LF: *n* = 34; MF: *n* = 16; HF: *n* = 12. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.

of basal platelet activation and platelet reactivity compared with volunteers of group LFC already before exercise training.

Exercise training of LFT group affected both CRF and platelet function. After a MANCOVA, which confirmed the significant effect of exercise training on parameters of platelet function in general (MANCOVA; Pillai's trace V = 0.735, F (5,21) = 11.68; $P < 0.0001$), separate univariate ANCOVAs for each measured parameter of basal platelet activation and platelet reactivity confirmed that exercise training significantly reduced basal surface expression of CD62P and platelet reactivity (TRAP-induced CD62P expression, CD40L expression and ROS formation), but had no significant impact on basal surface expression of CD40L (Fig. 3B, Tables 2 and 3).

CRF and platelet function in group LF after exercise training in relation to groups MF and HF. Although exercise training performed with group LF clearly improved CRF ($\dot{V}O_{2max}$, $\dot{V}_{max}$, $\dot{V}VT2$, $\dot{V}O_2VT2$), obtained values were still markedly lower than those observed in groups MF and HF (Fig. 3A; see Table, Supplemental Digital Content 3, pairwise comparisons of all groups, http://links.lww.com/MSS/A656).

Exercise training also led to decreased basal platelet activation (surface expression of CD62P) and platelet reactivity (increased EC_{50} of TRAP in terms of CD62P expression and intraplatelet ROS formation as well as CD40L expression to 6 μM TRAP).

Notably, this is in contrast to the situation observed with CRF, as parameters of platelet function after exercise training are comparable to those observed in groups MF and HF (depicted in Fig. 3B; see Table, Supplemental Digital Content 3, pairwise comparisons of all groups, http://links.lww.com/MSS/A656). Volunteers of the control group exhibited higher levels of basal platelet activation and platelet reactivity than groups MF and HF at all points in time.

DISCUSSION

Results of this study show for the first time that basal platelet function and platelet reactivity are related to CRF. Here, we show that i) young, healthy women with low CRF exhibit a hyperreactive platelet phenotype compared with fitter individuals; ii) modulation of platelet function achieved by a relatively low dose of exercise training applied for several weeks reflects a normalization of platelet function; and iii) platelet function in women with very high levels of CRF resembles that observed in women with medium levels of CRF.

In our first approach, we analyzed platelet function in three groups of volunteers differing in CRF. The disparity in CRF was confirmed by oxygen uptake and running velocity (maximal and at VT2). These observed differences were very unlikely caused by different motivation of volunteers during exercise tests, because maximal HR and maximal RER were similar in all groups. Additionally, clear differences in left ventricular heart stroke volume at rest indicate long-term adaptations to exercise training in groups MF and HF.

Platelet reactivity and basal platelet activation state were measured in all volunteers. Thereby, we focused on platelet activation events which are critically involved in atherogenesis (21): surface expression of CD62P facilitates the binding of platelets to leukocytes and the endothelium, thereby promoting leukocyte extravasation (2,3), which finally leads to vascular inflammation. CD40L promotes atherogenesis by modulating function of endothelial cells, leukocytes, and other platelets (1,14,22,24), whereas intraplatelet ROS represents an important second messenger in platelets, relevant for CD40L expression and the regulation of the fibrinogen-binding capability of glycoprotein receptor GPIIb/IIIa (32) that is essential for platelet aggregation.

Compared with groups MF and HF, platelets of group LF showed increased CD62P expression even in the absence of a platelet agonist, indicating increased basal platelet activation. Further, platelets of group LF were also characterized by an increased propensity to become activated by platelet agonist TRAP-6, evident by increased agonist-induced CD62P and CD40L expression as well as ROS formation, indicative for increased platelet reactivity.

However, in contrast to our hypothesis, there was no difference in platelet function between groups MF and HF, indicating that very high levels of CRF do not provide any
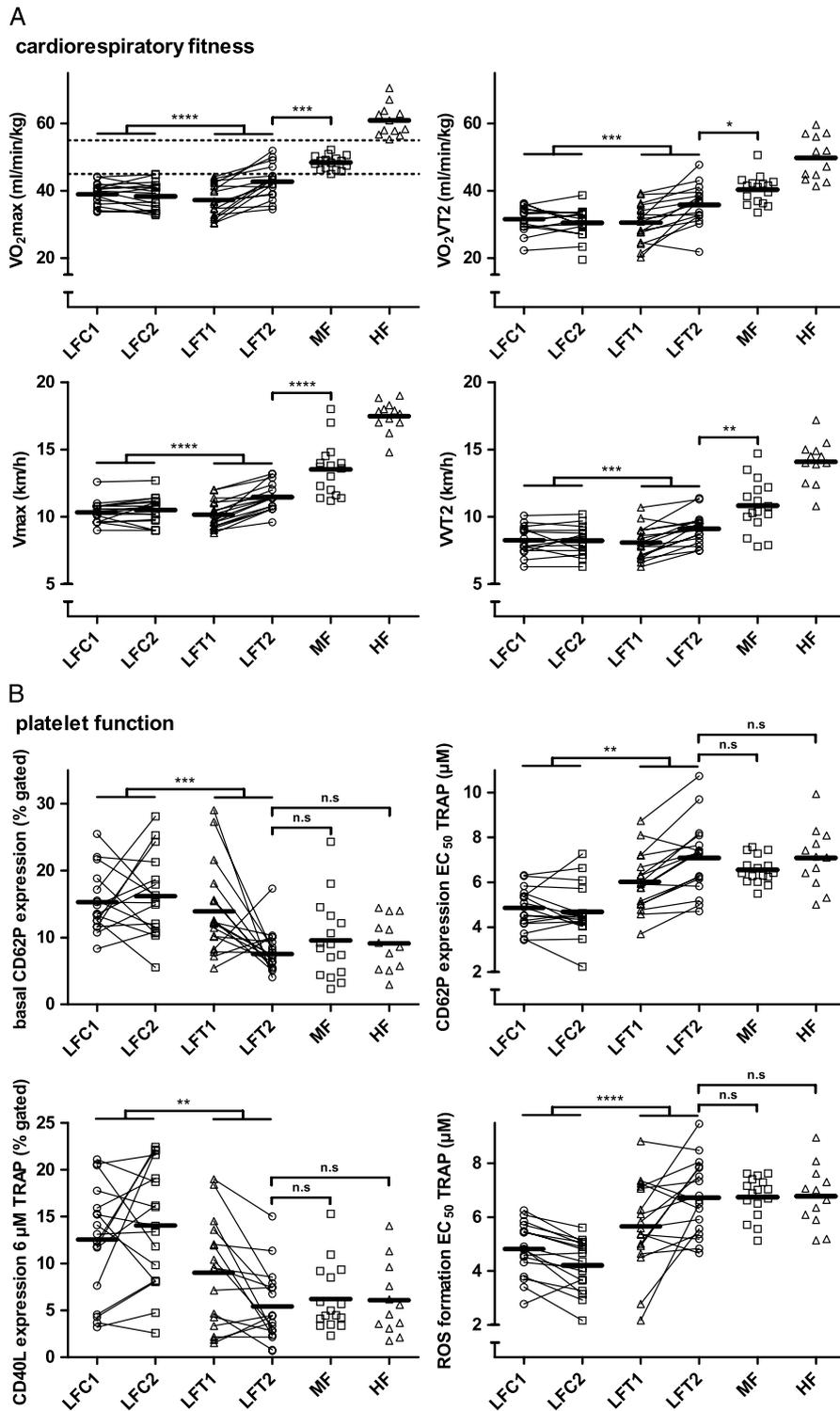
FIGURE 3—**Effects of exercise training in training group (LFT, *n* = 17) or no intervention in control group (LFC, *n* = 17) on parameters of CRF (A) and on parameters of platelet function (B) in relation to volunteers with medium CRF (MF, *n* = 16) and high CRF (HF, *n* = 12). *Horizontal solid lines* indicate means; *dotted horizontal lines* indicate predefined $\dot{V}O_{2max}$ limit values separating initial groups. *$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.**

substantial benefit with respect to platelet function compared with moderate levels of CRF.

To quantify the degree of monotone correlation between $\dot{V}O_{2max}$ and parameters of platelet function, Spearman correlation coefficients were calculated, showing significant,

moderate associations between $\dot{V}O_{2max}$ and parameters of platelet function. The fact that partial correlation coefficients were almost equal to coefficients without control variables indicates that our results were not biased by volunteer's BMI, age, or the intake of oral contraceptives. Further characterization of

		Unit		Before Training/No Intervention		After Training/No Intervention	
				Training Group	Control Group	Training Group	Control Group
CRF	n			17	17	17	17
	BMI	$kg \cdot m^{-2}$	Mean ± SD	21.45 ± 2.39	20.99 ± 2.11	21.39 ± 2.42	20.88 ± 2.11
	Weekly exercise[a]	$h \cdot wk^{-1}$	Median (IQR)	0.25 (0.00–0.50)	0.00 (0.00–0.30)	—	—
	$\dot{V}O_{2max}$	$mL \cdot min^{-1} \cdot kg^{-1}$	Mean ± SD	37.11 ± 4.79	38.91 ± 3.39	42.74 ± 4.92	38.35 ± 4.14
	$\dot{V}_{max}$	$km \cdot h^{-1}$	Mean ± SD	10.16 ± 1.03	10.36 ± 0.81	11.48 ± 1.00	10.52 ± 1.00
	$\dot{V}VT2$	$km \cdot h^{-1}$	Mean ± SD	8.07 ± 1.07	8.26 ± 1.07	9.12 ± 1.12	8.24 ± 1.04
	$\dot{V}O_2VT2$	$mL \cdot min^{-1} \cdot kg^{-1}$	Mean ± SD	30.59 ± 5.76	31.47 ± 3.99	35.84 ± 5.98	30.47 ± 4.45
	SV/kg	$mL \cdot kg^{-1}$	Mean ± SD	0.95 ± 0.12	0.98 ± 0.17	0.94 ± 0.18	0.97 ± 0.13
	HR_{max}	bpm	Mean ± SD	195 ± 7	193 ± 9	195 ± 6	193 ± 11
	HRVT2	bpm	Mean ± SD	179 ± 11	175 ± 12	181 ± 10	175 ± 12
	RER_{max}		Mean ± SD	1.11 ± 0.07	1.10 ± 0.06	1.13 ± 0.09	1.13 ± 0.08
Platelet function	Basal CD62P	% gated	Mean ± SD	13.92 ± 6.71	15.2 ± 4.54	7.98 ± 3.04	16.18 ± 6.18
	Basal CD40L	% gated	Median (IQR)	0.94 (0.38–2.50)	1.03 (0.82–2.52)	0.89 (0.55–1.34)	0.92 (0.70–1.77)
	CD62P (EC_{50} TRAP)	μM	Mean ± SD	6.02 ± 1.30	4.84 ± 0.91	7.09 ± 1.59	4.69 ± 1.22
	CD40L (6 μM TRAP)	% gated	Mean ± SD	9.00 ± 5.73	12.53 ± 6.03	5.42 ± 3.87	14.06 ± 6.43
	ROS (EC_{50} TRAP)	μM	Mean ± SD	5.66 ± 1.66	4.82 ± 0.99	6.37 ± 1.37	4.21 ± 0.95

[a]Self-assessed weekly exercise duration during 6 months before study inclusion.
[b]Left ventricular stroke volume (mL) at rest per kg body weight.

the relationship between $\dot{V}O_{2max}$ and parameters of platelet function by regression analysis provided no indication for a nonlinear effect of $\dot{V}O_{2max}$.

Our subsequent aim was to test if several weeks of exercise training in group LF lead to alignment of platelet function to levels observed in groups MF and HF.

Group LFT performed a supervised exercise training for slightly more than one and a half hours per week, which was sufficient to increase CRF. This can be appreciated by increased oxygen uptake and running velocity (both maximal as well as at VT2). Because stroke volume at rest was not affected by exercise training, increased CRF was probably due to peripheral, muscular adaptations. Maximal RER at the end of each exercise test and maximal HR were not increased after exercise training, confirming that detected ameliorations in CRF represented indeed training effects and were not biased by stronger motivation during the second exercise test.

Notably, exercise training was not sufficient to align CRF levels to group MF—nevertheless, platelet function after exercise training aligned with that seen in groups MF and HF. These results are commensurate with the findings obtained in the cross-sectional part of our study.

Our results are in principal accordance with previously published work as they confirm the ability of exercise training to decrease platelet activation in healthy volunteers (11,34–36,38). Moreover, we compared for the first time the pre-training state as well as the platelet-inhibiting effects mediated by exercise training with the situation observed in fitter individuals. This study design led to the central finding that a correlation between CRF and platelet function exists, and that platelet function differs between individuals with low and medium levels of CRF, but not markedly between those with medium and high levels of CRF. Thereby, our findings also allow the conclusion that low CRF is associated with a proinflammatory and prothrombotic platelet phenotype and that endurance training results in normalization of platelet function. The fact, that platelet function observed in fitter individuals represents the physiological situation would also be expected from an evolutionary perspective, as high levels of physical activity represent the normal state (6,7,9). Consequently, increased platelet activation and reactivity in group LF can be seen as a nonphysiologic state (i.e., pathological platelet hyperactivation and hyperreactivity).

Epidemiological studies indicate that very low levels of physical activity result in high prevalence of cardiovascular

				Uncorrected 95% CI			
		Unit	Estimated Effect	Lower Bound	Upper Bound	P	Significance
CRF	BMI	$kg \cdot m^{-2}$	0.05	−0.16	0.26	0.63	n.s.
	$\dot{V}O_{2max}$	$mL \cdot min^{-1} \cdot kg^{-1}$	5.76	3.36	8.16	<0.0001	****
	$\dot{V}_{max}$	$km \cdot h^{-1}$	1.12	0.66	1.58	<0.0001	****
	$\dot{V}VT2$	$km \cdot h^{-1}$	0.99	0.47	1.51	0.0005	***
	$\dot{V}O_2VT2$	$mL \cdot min^{-1} \cdot kg^{-1}$	5.51	2.46	8.56	0.0009	***
	SV/kg	$mL \cdot kg^{-1}$	0.05	−0.06	0.16	0.34	n.s.
	HR_{max}	bpm	2.95	−0.12	6.04	0.059	n.s.
	HRVT2	bpm	5.42	0.85	9.99	0.02	*
	RER_{max}		0.05	−0.06	0.16	0.34	n.s.
Platelet function	Basal CD62P expression	% gated	−8.29	−11.79	−4.79	0.00015	***
	Basal CD40L expression[a]	% gated	0.75	0.46	1.24	0.256	n.s.
	CD62P (EC_{50} TRAP)	μM	1.16	0.49	1.84	0.0029	**
	CD40L (6 μM TRAP)	% gated	−6.63	−10.03	−3.23	0.0012	**
	ROS (EC_{50} TRAP)	μM	2.11	1.38	2.84	<0.0001	****

P values regarding platelet function were corrected according to Bonferroni–Holm method. Descriptive statistics of each subgroup listed in Table 2.
[a]Log-transformed data were used for ANCOVA.
*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$; ****$P \leq 0.0001$.

T2

mortality. A limited extent of physical activity drastically reduces cardiovascular mortality, while there is hardly any further benefit from higher levels of physical activity (5,20,27). In light of the established role of platelets in atherogenesis, our findings may suggest that platelets play a role in this relationship, reported by epidemiological studies.

Our findings and conclusions should also be seen in context of the strengths and limitations of this study. The direct assessment of various aspects of platelet function represents a definite asset of this study. Nevertheless, this demanding and time-consuming methodology also limited sample size and power of our study. Therefore, we might have been unable to detect minor differences in platelet function (e.g., between individuals with medium and high CRF).

On the other hand, it should be stated that group LFT tended to have lower levels of platelet reactivity than LFC

already before exercise training. Importantly, this effect occurred despite practically equal levels of baseline CRF in these groups and was therefore not predictable.

In conclusion, our study shows that young, healthy females with low levels of CRF exhibit a hyperreactive and thus proinflammatory platelet phenotype. In this study population, a relatively low dose of exercise is sufficient to normalize platelet function, whereas superior levels of CRF do not provide any further substantial benefit, but also no appreciable adverse effects.

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