Effects of chronic exercise and deconditioning on platelet function in women

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Regular exercise and physical conditioning may reduce the risk of major vascular thrombotic events and protect individuals against cardiovascular diseases (24). Because platelets play a key role in thrombus formation, the protective effect of exercise against cardiovascular diseases may be partially due to alterations of platelet function. Results from our previous study of men indeed suggest that platelet adhesiveness and aggregability are suppressed by moderate intensities of acute exercise and exercise training (31, 32). However, similar studies in which women subjects were used have not been carried out, perhaps because of inconsistent results regarding the platelet function during different phases of the menstrual cycle (MC) (21, 23). We recently found that platelet function of women subjects in the midfollicular phase, but not in the midluteal phase, was affected by acute exercise (33). It is plausible to hypothesize that exercise training may also affect platelet function in women during the midfollicular phase.

In 1987, Busse et al. (4) reported that endothelium-derived relaxing factor (EDRF) could inhibit platelet activation. Recently, we found that exercise training could enhance agonist-stimulated EDRF release in rabbit or rat aortas (6–8). It is possible that exercise training in humans may also modulate platelet activities via altered EDRF release. Because EDRF has been identified as nitric oxide (NO) and its action is mediated by guanosine 3′,5′-cyclic monophosphate (cGMP) (3), we, therefore, assessed plasma NO metabolite (nitrite and nitrate) levels and platelet cGMP content to clarify the possible mechanisms for exercise-training effects on platelet function.

Materials and Methods

Subjects, exercise training, and deconditioning. This protocol had been previously reviewed and approved by an institutional committee for the protection of human subjects. Sixteen young, healthy female subjects who had regular MCs were studied after they had given their informed consent and understood the experimental procedures. These subjects had recorded three consecutive MCs before entering this program. They were randomly divided into control and training groups, eight for each group. None of these subjects engaged in any regular physical activity at least for 1 yr before this study. All subjects were nonsmokers and had never taken oral contraceptives. They abstained from any medication at least for 2 wk before this study and continued to abstain from any medication throughout the experimental period. There were no significant differences in their anthropometric data: age, 22.8 ± 1.3 vs. 21.3 ± 0.8 yr; height, 162.0 ± 1.5 vs. 158.8 ± 1.3 cm; weight, and 53.3 ± 0.9 vs. 53.4 ± 2.0 kg for the control and training groups, respectively.

Before the actual study, subjects were familiarized with exercise on a bicycle ergometer (Corival 400) to eliminate novel effects of a new experience. The training group then came to our laboratory to receive an exercise training program, which they started from the midfollicular phases for two MCs, whereas the controls were not trained during the experimental period. The training subjects were trained on a bicycle ergometer with an intensity of 50% of maximal oxygen consumption (VO2max), which was estimated by heart rates, for 30 min/day, 5 days/wk, for two MCs, and this was then followed by three MCs of deconditioning.

Exercise test and blood collection. At the beginning of the study, a progressive exercise test was performed by each subject at the midfollicular phase. During the experimental period, exercise tests were repeated every cycle in the training group until the end of deconditioning. In contrast, the control group performed exercise tests at the midfollicular phase twice, one at the beginning and the other at two MCs later. To avoid the short-term exercise effects, the training subjects performed exercise tests at least 48 h after training.

On the testing days, subjects arrived our laboratory at 1:00 PM to avoid possible diurnal influence (30). After a 30-min rest, 35 ml of blood samples in total were collected from a forearm vein for measurements of resting platelet function and plasma NO metabolites as described below. Immediately after the exercise test, another blood sample was drawn for
the measurements of postexercise parameters. Sodium di-
citrate (0.106 M, 1/8 of blood volume) was added as an anticoag-
ulant in the samples for platelet-function evaluation, and heparin (20 IU/ml) was used in the samples for measurement of plasma NO metabolites.

A progressive exercise test, described in detail in a previous
study (31), was conducted at 3:30 PM. The exercise protocol
consisted of 2-min unloaded pedaling, followed by a continu-
ous increment of workload, 10–20 W every 3 min, until
exhaustion. During the exercise test, the electrocardiogram
was continuously monitored and converted to the heart rate.
Resting blood pressure was measured by a sphygmomanom-
eter. Subjects' minute ventilation, O2 consumption, and CO2
production for every minute were obtained as described
previously (5).

Platelet adhesiveness. Platelet-rich plasma (PRP) was used
for the determination of platelet adhesiveness and aggregabil-
ity. It was prepared by centrifugation of 10-ml citrated blood
samples at 120 g for 10 min at room temperature. Platelet-
poor plasma was obtained as the supernatant after recentrifu-
gation at 1,600 g for 10 min.

Platelet adhesiveness was determined by using a tapered
parallel-plate chamber as described previously (31). PRP was
gently infused into the chamber and was allowed to settle on
the fibrinogen-coated surface for 12 min. The flow chamber
was then flushed with Tyrode-N-2-hydroxyethylpiperazine-N-
2-ethanesulfonic acid (HEPES) buffer (0.128 M NaCl, 2.7 mM
KCl, 0.5 mM MgCl2, 0.36 mM NaH2PO4, 12 mM NaHCO3, and
10 mM HEPES, pH 7.4) for 5 min at a flow rate of 0.027 ml/s,
which provided a shear stress range from 55 to 0 dyn/cm2. Ten
field locations along the central line were observed at inter-
vals of 5 mm from the downstream end with approximately
zero shear stress, and the number of remaining platelets per
0.16 mm2 was counted at each location. A simple linear
regression line for adherent platelets along with various
shear-stress fields was obtained. The slope of this regression
line was used as an index of platelet adhesiveness; i.e., the
less negative the slope, the greater the platelet adhesiveness.

Platelet aggregability. ADP-induced platelet aggregation
was evaluated by the percentage of reduction in single
platelet count as described in our previous study (32). Briefly,
the kinetics of platelet aggregation in PRP was assessed with
a platelet aggregometer (Hema Tracer 2, Niiko Bioscience)
after addition of various concentrations of ADP (Sigma Chemi-
cal). After the optical density had reached a steady value for
at least 1 min, the test tube was then removed from the
 aggregometer and left undisturbed for 90 min to allow
sedimentation of platelet aggregates. Forty microliters of
plasma from the upper suspension were used to measure a
single platelet count. Results were expressed as the percent
ratio of aggregated platelets to total platelets.

Measurement of platelet intracellular Ca2+ concentration
([Ca2+]i). Platelets were labeled with the Ca2+-sensitive
fluorescent dye fura 2-acetoxymethyl ester (AM) (Molecular
Probes) as described in our previous study (16). Briefly, PRP
was mixed with calcium-free HEPES buffer, which contained
0.05 IU/ml apyrase (Sigma Chemical) and 0.05 IU/ml hirudin
(Sigma Chemical), and was added to a polypropylene tube
with 0.4 g/ml albumin at the bottom to act as a cushion. After
centrifugation, platelet pellets were incubated with calcium-
free HEPES buffer containing fura 2-AM (4 mM), apyrase
(0.05 IU/ml), and hirudin (0.05 IU/ml) at 37°C for 40 min.
These fura-2-AM-loaded platelets were used to measure their
basal and ADP-induced [Ca2+]i by using a dual-wavelength
fluorescence spectrophotometer (Hitachi, excitation wave-
length 340/380 nm, emission wavelength 510 nm) (29).

Measurement of platelet cGMP contents. Platelet cGMP
contents were determined by enzyme-linked immunosorbant
assay (ELISA) by using a commercially available kit (Cayman)
(15). In brief, citrated PRP containing 10–4 M of 3-iso-
buty1-1-methylxanthine (a phosphodiesterase inhibitor;
Sigma Chemical) was mixed with ice-cold HEPES buffer and
centrifuged at 6,500 g at 4°C for 5 min. The pellet was
vortexed with ice-cold trichloroacetic acid (TCA; 6%) for 2
min. After centrifugation (10,000 g, 4°C, 15 min), TCA was
removed by washing four times with five volumes of water
saturated ether. The extracted samples were stored at −80°C
until measurement.

Determination of plasma NO metabolites (nitrite and ni-
trate). NO metabolites in plasma were determined by the
Griess reagent-based colorimetric method (13) by using com-
mercially available assay kits (Cayman). First, plasma nit-
rate was reduced to become nitrite. Then, nitrite was con-
verted into a deep purple azo compound with an absorbance
at 550 nm. Measured values represented the total amount of
plasma NO metabolites, i.e., nitrite plus nitrate.

Measurement of plasma progesterone. Plasma progesterone
levels were measured by using a progesterone ELISA kit
(Cayman) to confirm the menstrual phases.

Statistics. The statistical software package of StatView 4.0
(Macintosh) was used for analyses of our data. The compari-
son of variables between trained and control groups at the
beginning and 2 MCs later were analyzed by analysis of
variance (ANOVA), followed by Fisher's multiple-range test.
To compare the differences of various parameters in the
trained group along with the experimental period, data were
analyzed by the randomized-block ANOVA and Tukey's mul-
tiple-range test. Differences were considered significant
at P < 0.05. The results are expressed as means ± SE.

RESULTS

It was confirmed that all the experiments were
performed in the midfollicular phase, because resting
plasma progesterone levels in all subjects were low
(<2.6 ng/ml) on the experimental days. The length of
MCs did not differ between control and trained groups
either before or after training; i.e., 31 ± 1 and 30 ± 1
days/cycle for control and trained groups before train-
ing and 30 ± 1 and 30 ± 2 days/cycle for control and
trained after training, respectively.

Heart rates and blood pressures. Figure 1 shows that
before training, resting heart rates, systolic and dia-
stolic blood pressures, and maximal heart rates were
not significantly different between control and trained
groups. However, the trained group had significantly
lower resting heart rates and blood pressures than did
the control group after training (Fig. 1; P < 0.05).
Moreover, the trained group had lower resting heart rates
and blood pressure compared with pretraining values.
After one MC of deconditioning, the training
effects disappeared (Fig. 1).

Exercise performance. The trained subjects improved
their exercise performance, including elevating time to
exhaustion, maximal workload, maximal minute ven-
tilation, VO2max, and maximal CO2 production, after ex-
cise training (Fig. 2; P < 0.05). However, the training
effects were reversed back to the pretraining state after
deconditioning (Fig. 2). They also had significantly
greater exercise performance than did their controls
after training (Fig. 2; P < 0.05). In contrast, the control

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group did not alter exercise performance after two MCs (Fig. 2).

Platelet adhesiveness and aggregability. Our results showed that resting and postexercise platelet adhesive-
ness decreased after one or two MCs of training in the trained group (Fig. 3; P < 0.05). On the contrary, the control group did not alter platelet adhesiveness after two MCs (Fig. 3). Moreover, the trained group had significantly lower platelet adhesiveness than did the control after two MCs of training (Fig. 3; P < 0.05). The training effects on platelet adhesiveness, however, were reversed back to the pretraining state after deconditioning (Fig. 3). Before training, acute severe exercise increased platelet adhesiveness. This acute effect, however, no longer existed after training.

Figure 4 demonstrates that ADP-induced platelet aggregation was decreased by training. In contrast, the control group did not alter ADP-induced platelet aggregation (Fig. 4). Moreover, the trained group had lower resting and postexercise platelet aggregability than did the control after two MCs of training (Fig. 4; P < 0.05). This training effect was reversed back to the pretraining state after deconditioning. Although a short-term strenuous exercise increased platelet aggregation induced by 0.25 µM ADP at the beginning of this study, this acute exercise effect did not exist after training.

Platelet [Ca^{2+}]. Basal and ADP (0.5 or 2 µM)-evoked platelet [Ca^{2+}] values were decreased by training (Fig. 5; P < 0.05). These training effects were reversed back to the pretraining state after deconditioning. Conversely, they were not altered in the control group after two MCs of experimental period (Fig. 5). It was also noticed that the trained group had lower basal and
ADP-evoked platelet [Ca\textsuperscript{2+}]i than did the control after training (Fig. 5; \(P < 0.05\)).

Although a single bout of strenuous exercise enhanced basal and ADP-evoked platelet [Ca\textsuperscript{2+}]i at the beginning of this study (Fig. 5; \(P < 0.05\)), this phenomenon did not happen after training. In contrast, it consistently existed in the controls throughout the experiment (Fig. 5; \(P < 0.05\)).

Platelet cGMP. Figure 6 shows that resting and postexercise platelet cGMP contents were increased after training in the trained group (\(P < 0.05\)). These training effects were reversed back to the pretraining state after deconditioning. Conversely, platelet cGMP contents were not altered in the control group (Fig. 6). The trained group also had higher resting and postexercise platelet cGMP contents than the control after training (Fig. 6; \(P < 0.05\)).

Although platelet cGMP content was not changed by a single bout of strenuous exercise at the beginning, it was significantly increased by short-term strenuous exercise after training and was reversed back to the pretraining state by deconditioning (Fig. 6; \(P < 0.05\)). In controls, platelet cGMP content was not significantly changed by severe exercise throughout the experimental period (Fig. 6).

NO metabolites (nitrite and nitrate) in plasma. Figure 7 shows that before training, resting or postexercise plasma nitrite and nitrate levels did not differ between
control and trained groups. However, they were increased in the trained group after training (Fig. 7; P < 0.05). This training effect was reversed back to the pretraining state after deconditioning. Conversely, plasma nitrite and nitrate levels were not altered in the control group (Fig. 7).

DISCUSSION

Our results demonstrated that, in the follicular phase, two MCs of chronic moderate exercise training 1) increased female subjects’ aerobic capacities; 2) decreased platelet adhesiveness, ADP-induced platelet aggregation, and [Ca\(^{2+}\)]\(_i\) elevation; 3) increased platelet cGMP contents and plasma nitrite and nitrate levels; and 4) eliminated the acute severe exercise effects on platelet function. We also found that deconditioning reversed these training effects back to the pretraining state.

It is well known that the risk of primary cardiac arrest is transiently increased during vigorous exercise, whereas habitual physical exercise decreases the risk of primary cardiac arrest (9, 28). We have previously used male subjects to show that, whereas acute strenuous exercise enhances platelet adhesiveness and aggregation, acute moderate exercise and exercise training suppress these platelet-function parameters (31, 32). The present study is the first report to clearly demonstrate that platelets in women at the midfollicular phase, like those in men, can be suppressed by chronic exercise and be reversed back to the pretraining state after deconditioning. Based on this study and previous reports (31–33), exercise effects on cardiovascular diseases, being either protective or antagonistic, can be partially explained by the differential effects due to various intensities and durations of exercise. In addition, we also observed that acute exercise affected platelet function in women only at the midfollicular phase and not at the midluteal phase (33). This may be one possible reason to explain the epidemiological observation that premenopausal women have lower incidences of cardiovascular diseases than do men (17).

NO has potent antiplatelet effects. It stimulates platelet guanylate cyclase, elevates platelet cGMP levels, reduces agonist-induced rise of platelet [Ca\(^{2+}\)]\(_i\), and suppresses agonist-induced platelet aggregation (4). By similar mechanisms, NO also suppresses platelet adhesion (10, 15). Animal studies indicated that exercise training could enhance agonist-stimulated NO release (6–8). Whether chronic exercise can enhance NO release in humans has not been reported before. Our results showed that exercise training in women at the midfollicular phase could enhance plasma nitrite and nitrate and platelet cGMP levels and could suppress basal and ADP-induced platelet [Ca\(^{2+}\)]\(_i\) elevation. Therefore, exercise training apparently can increase endogenous NO release and elevate cGMP contents in platelets, which in turn may suppress platelet reactivity.

There are several possible explanations for the training-induced NO release. First, exercise training in animals increases norepinephrine (NE)-induced NO release (6, 8). Therefore, this NE-stimulated NO release may in turn diminish the NE-evoked platelet activation (25). This is consistent with our finding that training is able to prevent platelet activation by a single bout of strenuous exercise, which should be accompanied with NE elevation (19, 22). Second, increased blood flow during repetitive exercise may increase NO release. Augmentation of femoral arterial flow induced by peripheral vasodilation or by arteriovenous shunt can elicit vasodilation in vivo (26). Besides, in vitro studies demonstrate that blood vessel segments can release NO in response to either increased steady blood flow rate (27) or increased pulsatile frequency (14). However, whether vessels subjected to periodic elevation of blood flow, simulating the local blood flow change in exercise training, release more NO has not been investigated. Finally, low-density lipoprotein inactivates or inhibits EDRF release (1, 12) and chronic exercise decreases plasma low-density lipoprotein level (11, 34). Therefore, a third possibility is that exercise training changes the lipid pattern in favor of EDRF release, which inactivates platelets. Moreover, our results showed that deconditioning reversed the training effects on platelet function back to the pretraining state. This may be explained by the attenuation of blood flow (18) and the reversed alteration of lipoprotein patterns (20), which lower EDRF release after deconditioning.

In conclusion, platelet adhesiveness on fibrinogen-coated surface and ADP-induced platelet aggregation and [Ca\(^{2+}\)]\(_i\) elevation in women during the midfollicular phase can be desensitized by chronic exercise. Moreover, acute exercise-enhanced platelet activity decreases after training. These changes are likely to be mediated by NO. However, these training effects are reversible after deconditioning. Our findings give new insight into the possible protective effects of exercise training against the risk of cardiovascular disease in women.

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