[Original]

Physical Training Augments Plasma Catecholamines and Natural Killer Cell Activity

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Abstract: To elucidate the effects of a 10-week exercise training period on physical fitness and plasma catecholamine concentration at rest, and on natural killer cell activity in young healthy untrained females, subjects (20-22 years old, N=16) carried out physical training by a protocol which consisted of treadmill jogging at a work-intensity of 50% of their $\dot{V}O_{2\max}$, two hours a day three times a week for ten weeks. $\dot{V}O_{2\max}$ was increased significantly from 33.1 ± 3.4 ml/kg/min to 38.3 ± 6.6 ml/kg/min by the ten weeks of physical training ($P < 0.005$), and natural killer cell activity was also increased significantly from $31.9 \pm 14.3\%$ to $46.4 \pm 18.4\%$ ($P < 0.05$). The concentrations of epinephrine and norepinephrine before and after physical training were 18.3 ± 8.7 pg/ml and 20.4 ± 8.9 pg/ml, and 134.1 ± 52.2 pg/ml and 248.1 ± 106.8 pg/ml ($P < 0.005$), respectively. Changes in norepinephrine and epinephrine correlated with the change in $\dot{V}O_{2\max}$ ($r = 0.780$, $P < 0.005$; $r = 0.556$, $P < 0.05$). While the change in natural killer cell activity correlated with the change in epinephrine ($r = 0.623$, $P < 0.01$), the correlation of the change in natural killer cell activity with change in norepinephrine did not reach statistical significance ($r = 0.497$, $P = 0.0503$). From these results we concluded that physical training augments plasma catecholamine levels and natural killer cell activity at rest in young healthy females.

Key words: physical training, catecholamine, natural killer cell, fitness, oxygen uptake.

(Received 14 June 1999, accepted 8 October 1999)

Introduction

A substantial number of reports suggest that regular physical activity is associated with a reduced risk of coronary heart disease [1-7] and all-cause mortality including cancer [6-9]. The mechanism by which this protection occurs appears in part to be related to de-

creased blood pressure, lower body mass, higher high-density lipoprotein (HDL) cholesterol, lower total cholesterol/HDL ratio, and a lower prevalence of smoking associated with physical activity [10–12].

Regarding the effects of physical training on the catecholamine concentration, some earlier studies reported that catecholamine concentration was lower at rest in trained than in untrained subjects [13], and in response to given exercise loads, catecholamine concentrations are generally found to increase less in trained than in untrained subjects [14,15]. But recently some studies have reported that plasma catecholamine levels were not reduced at rest in trained compared with untrained subjects [14–16], and Dela *et al.* reported that epinephrine levels were higher in trained than in untrained subjects during sleep [17].

Natural killer cells are considered to play an important role in the first line of defense against viral infection and the spread of malignant disease [18, 19]. Natural killer cells are defined as major histocompatibility complex independent cytotoxic lymphocytes that do not express the cluster designation 3 cell-surface antigen. Their concentration and activity are influenced by physical stressors, including surgery [20], burns [21], acute myocardial infarction [22], hypovolemia [23], and hyperthermia [24]. Furthermore, natural killer cells are recruited to blood during exercise [25], but it is not clear whether physical training affects the natural killer cells activity or not.

The aim of the present study was to elucidate the effects of a 10-week exercise training period on physical fitness and plasma catecholamine concentration at rest, and on natural killer cell activity as an immune response parameter.

Subjects and Methods

The subjects for this study were 16 females aged from 20 to 22 years old. They were healthy and relatively sedentary students who were not in the habit of taking regular exercise. None of the subjects smoked or were taking medication, and none were in the midst of menstruation on the sampling day. Written informed consent was obtained from each subject before the study.

To measure the physical fitness, each subject performed a graded treadmill run to exhaustion according to the Bruce protocol [26]. We analyzed the expiratory gas with a gas analyzer (Aerometer : AE-280 Minato Medical Science Co. , Ltd. , Osaka, Japan) with a turbine transducer. The breath by breath data were averaged for 15 sec throughout the exercise test. When the heart rate did not reach more than 90% of the predicted maximal heart rate (220-age) or when the ratio of expired CO₂ and inspired O₂ did not exceed 1.1, we did not employ the peak value of the oxygen uptake as a maximal oxygen uptake ($\dot{V}O_{2\max}$). According to these criteria, no tests were excluded.

The physical training protocol involved treadmill jogging at 50% $\dot{V}O_{2\max}$ intensity for two hours a day three times a week over a period of 10 weeks. 50% $\dot{V}O_{2\max}$ was set by the

heart rate determined $\dot{V}O_{2\max}$ was previously measured.

After fasting overnight, 10ml of blood was obtained by venipuncture after 30min bed rest at a room temperature of 23°C. Five ml of blood was kept at 4°C with a 0.7ml of CPD buffer (sodium citrate 2.63g, citrate 0.327g, glucose 2.32g/100ml) to measure natural killer cell activity by ^{51}Cr releasing assay [27]. After centrifugation of the remaining blood at 1000g for 15 min, the plasma was frozen at -20°C until assayed. Plasma epinephrine and norepinephrine were measured by high performance liquid chromatography before and after the 10-week physical training period. To determine cholesterol levels, another 10ml of blood samples were collected in tubes containing ethylenediaminetetraacetate. Cholesterol, triglyceride content in the plasma and LDL and HDL cholesterol levels were measured enzymatically.

Statistics

All data are expressed as mean $\pm$ standard deviation. Paired t test for paired observations was employed whenever appropriate. Simple linear regression was performed when correlation was considered. Differences were considered statistically significant at P values < 0.05 .

Results

Physical characteristics of all subjects are shown in Table 1. The average body weight was 47.5 ± 3.6 kg, and their body weight after ten weeks was 47.7 ± 3.6 kg, so body weight did not change significantly in this study. Table 2 shows the total cholesterol, low-density lipoprotein (LDL) cholesterol, HDL cholesterol and triglyceride concentrations of the subjects. The physical training did not result in any obvious changes, however the $\dot{V}O_{2\max}$ rose significantly from 33.1 ± 3.4 ml/kg/min to 38.3 ± 6.6 ml/kg/min ($P < 0.005$, Fig. 1). Physical training augmented plasma norepinephrine at rest significantly (248.1 ± 106.8 pg/ml vs 134.1 ± 52.2 pg/ml; $P < 0.005$, Fig. 2.), but epinephrine did not show any clear statistical change (20.4 ± 8.9 pg/ml vs 18.3 ± 8.7 pg/ml). On the other hand, natural killer cells activity increased significantly from $31.9 \pm 14.3\%$ to $46.4 \pm 18.4\%$ ($P < 0.05$, Fig. 3). In three persons, $\dot{V}O_{2\max}$ deteriorated and their epinephrine levels also decreased after 10 weeks of physical training, and norepinephrine levels decreased in one of these three subjects.

The change in norepinephrine was calculated as the difference in concentration of norepinephrine before and after physical training, and change in epinephrine correlated with change in $\dot{V}O_{2\max}$ ($r = 0.780$, $P < 0.005$, Fig. 4.; and $r = 0.556$, $P < 0.05$, Fig. 5). The change in natural killer cell activity correlated with change in epinephrine ($r = 0.623$, $P < 0.01$, Fig. 6). In general, subjects whose $\dot{V}O_{2\max}$ increased greatly also showed large increases in catecholamine levels and natural killer cell activity, but the correlation of natural

killer cell activity with norepinephrine did not reach statistical significance ($r=0.497$, $P=0.0503$).

Table 1. Physical Characteristics of Subjects

Body height (m)	1.57 ± 0.036
Body weight (kg)	47.5 ± 3.6
BMI (kg/m^2)	19.3 ± 1.7
Systolic blood pressure (mmHg)	99.8 ± 8.8
Heart rate (bpm)	66.8 ± 10.9

BMI ; body mass index, bpm ; beats per minute.

Table 2. Plasma Lipids before and after Physical Training

	before PT	after PT
T-cho. (mg/dl)	172.6 ± 40.9	174.8 ± 35.3
LDL-cho. (mg/dl)	93.1 ± 28.5	93.0 ± 29.1
HDL-cho. (mg/dl)	56.2 ± 17.0	59.5 ± 18.6
TG. (mg/dl)	71.1 ± 30.7	76.1 ± 22.9

cho. ; cholesterol, PT ; physical training, T ; toral, TG ; triglyceride.

Fig. 1. $\dot{V}O_{2\max}$ before and after physical training.

$\dot{V}O_{2\max}$ levels were augmented significantly in all but three subjects, whose $\dot{V}O_{2\max}$ deteriorated after physical training. Error bar shows the standard deviation.

PT ; physical training, $\dot{V}O_{2\max}$; maximal oxygen uptake.

Fig. 2. Plasma norepinephrine concentrations before and after physical training. Plasma norepinephrine concentrations were augmented by physical training. Error bar shows standard deviation. PT ; physical training.

Fig. 3. Natural killer cell activity before and after physical training. Natural killer cell activity was augmented significantly. Error bar shows standard deviation.

Fig. 4. Correlation between change in $\dot{V}O_{2\max}$ and change in norepinephrine.
 Δ norepinephrine correlated with $\Delta \dot{V}O_{2\max}$ significantly ($r = 0.780$, $P < 0.005$).

Fig. 5. Correlation between change in $\dot{V}O_{2\max}$ and change in epinephrine.
 Δ epinephrine correlated with $\Delta \dot{V}O_{2\max}$ significantly ($r = 0.556$, $P < 0.05$).

Fig. 6. Correlation between change in epinephrine and change in natural killer cell activity.

Δ natural killer cell activity correlated with Δ epinephrine significantly ($r=0.623$, $P<0.01$), but the correlation with Δ norepinephrine did not reach statistical significance ($r=0.497$, $P=0.0503$).

Discussion

The present study shows that periodical physical training augments plasma norepinephrine and natural killer cell activity at rest (Fig. 1, 2, 3). It has been well documented in previous studies that for a given sub-maximal workload both plasma norepinephrine and epinephrine are reduced in endurance-trained rats compared with their sedentary counterparts [28, 29]. In humans, however, it is controversial whether plasma catecholamine levels will be reduced at rest in trained compared with untrained subjects [13–16]. In contrast to some earlier studies using fluorometric catecholamine assays [13, 14], but in agreement with studies using more precise radioenzymatic assays [14–16] or with microneurography, plasma catecholamine levels were not reduced at rest in trained compared with untrained subjects. Such differences in opinion may be the result of differences in the degree of physical fitness of the subjects. Dela *et al.* reported that epinephrine levels were higher in trained than in untrained subjects during sleep [17]. In our study, the physical training increased their physical fitness significantly, and this was accompanied by an increased plasma catecholamine level at rest. Furthermore changes in norepinephrine and epinephrine concentrations correlated with change in $\dot{V}O_{2\max}$. This means that plasma catecholamine levels are higher in trained states than in untrained states in the same subjects, at least in young females. In

general, periodical physical training brings an increase of $\dot{V}O_{2\max}$, and the degree of the rise of $\dot{V}O_{2\max}$ shows a positive correlation with load strength. Therefore, in the subjects whose $\dot{V}O_{2\max}$ did not increase after physical training, it is suspected that their physical training was insufficient. The change in $\dot{V}O_{2\max}$ shows a positive significant correlation with plasma catecholamine and natural killer cell activity (Fig. 4, 5 and 6). Using a spectral analysis of RR interval variability on an electrocardiogram, Furlan *et al.* reported that heavy dynamic exercise produces long lasting sympathetic activation in healthy subjects [30]. However, their results might be influenced by the time after the cessation of exercise. We took the blood samples in the morning after 30 min bed rest, so we concluded that physical training may augment plasma catecholamine at rest.

Natural killer cell activity has been reported to increase after a single exercise session [31, 32], and also has been reported recently after moderate exercise training by Nieman *et al.* [33]. Our study suggests that natural killer cell activity also increases after physical training. But in an *in vivo* study like this, the condition of the subjects before and after physical training is not always the same, for example, seasonal changes might affect the natural killer cell activity. We therefore can not exclude these limitations at this time.

Kappel *et al.* reported that intravenous administration of epinephrine resulting in plasma levels similar to those obtained during cycling recruits natural killer cells to blood, and that natural killer cell activity is mediated by epinephrine [34]. Therefore it is reasonable that the increased levels of plasma catecholamines at rest may also augment the natural killer cell activity by recruiting natural killer cells to blood. Our finding that change in catecholamine correlated with change in natural killer cell activity is supported by Kappel's findings which showed acute effects of catecholamine. Our findings of increasing natural killer cell activity after a 10-week exercise period are also in agreement with the report by Nieman *et al.* after 15-week exercise [33]. Regular physical activity is reported to be associated with a reduced risk of all-cause mortality including cancer [6–9] and natural killer cells are considered to play an important role in preventing the spread of malignant disease [18, 19]. In our study physical training increased natural killer cell activity, and this may be one reason for the reduced risk of all-cause mortality, including cancer, but this remains to be established.

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運動トレーニングは安静時血中カテコラミンとnatural killer細胞活性を増加させる

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要 旨: 運動トレーニングによるfitness, 安静時血中カテコラミン濃度およびnatural killer細胞活性への影響を検討した。対象は特にスポーツ活動のない健康若年女性(20-22歳)16名である。トレーニングプロトコールとして, 最大酸素摂取量50%相当の運動強度でトレッドミルによるジョギングを1日2時間, 週3回10週間行った。最大酸素摂取量は10週間の運動により 33.1 ± 3.4 ml/kg/minから 38.3 ± 6.6 ml/kg/minへ有意に増加し($P < 0.005$), natural killer細胞活性は $31.9 \pm 14.3\%$ から $46.4 \pm 18.4\%$ へ有意に増加した($P < 0.05$)。トレーニング前後の安静時epinephrineとnorepinephrineはそれぞれ 18.3 ± 8.7 pg/mlと 20.4 ± 8.9 pg/ml, 134.1 ± 52.2 pg/mlと 248.1 ± 106.8 pg/ml($P < 0.005$)だった。norepinephrine増加量とepinephrine増加量はそれぞれ最大酸素摂取量増加量と正相関があった($r = 0.780$, $P < 0.005$; $r = 0.556$, $P < 0.05$)。またnatural killer細胞活性増加量とepinephrine増加量は正相関があった($r = 0.623$, $P < 0.01$)。これらの事より我々は若年女性において運動トレーニングは安静時血中カテコラミンとnatural killer細胞活性を増加させると結論した。

J UOEH (産業医大誌) 21 (4): 277-287 (1999)