Plasma Sulpho-conjugated Catecholamine Responses to Moderate Steady-state Exercise

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Abstract

OGAKI, T., SAITO, A., HOTTA, N., UEDA, N., KANAYA, S. and FUJINO, T., Plasma Sulpho-conjugated Catecholamine Responses to Moderate Steady-state Exercise. Adv. Exerc. Sports Physiol., Vol.7, No.3 pp.87-92, 2001. The purpose of this study was to investigate the time course of the plasma sulpho-conjugated catecholamines (CA) during moderate steady-state exercise. Six male university runners exercised at 70% of maximal oxygen uptake for 60 min on a cycle ergometer. The plasma free and sulfated CA were measured before exercise, every 10 min during exercise and at 30 min and 60 min after exercise. The concentrations of plasma free noradrenaline (NA), adrenaline (AD) and dopamine (DA) compared with the pre-exercise levels were elevated significantly at 10 min ($p<0.01$), 50 min ($p<0.01$) and 20 min ($p<0.05$), respectively. The plasma NA sulfate (NA-S) and AD sulfate (AD-S) concentrations increased gradually with exercise duration and were significantly elevated over pre-exercise values at 20 min ($p<0.01$) and 30 min ($p<0.05$), respectively. The plasma DA sulfate (DA-S) concentration showed a transitory decrease after 10 min and 20 min of exercise ($p<0.05$) and then increased gradually with exercise and reached a statistically significant level by the end of exercise ($p<0.001$). All three sulfated CA concentrations remained elevated for 60 min after exercise ($p<0.01$-$p<0.001$). The ratio of free AD and NA to total (free + conjugated) AD and NA concentrations reached steady-state levels after 20 min of exercise. We conclude that the concentrations of all three CA sulfates increase during exercise when plasma free CA concentrations reached sufficient levels. In addition, our results suggest that the plasma free CA, especially free AD and NA released during exercise may undergo sulphoconjugation during exercise as well as after exercise. Moreover, we noted that the plasma DA-S concentration decreased temporarily during an early stage of exercise, although the mechanism remains unclear.

Keywords: sulpho-conjugation, noradrenaline, adrenaline, dopamine

Introduction

Circulating catecholamines (CA) in plasma exist in free forms and as sulphoconjugates. In humans, approximately 95% to 99% of plasma dopamine (DA) and approximately 70% of plasma adrenaline (AD) and noradrenaline (NA) exist as sulphoconjugates[6, 11, 14, 22]. The plasma free CA concentration increases linearly with the exercise duration and exponentially with the exercise intensity[9, 12] and returns nearly to pre-exercise values within minutes due to the free CA's short plasma half-life of 1-3 min[20, 23]. Most of the free CA were readily conjugated by phenolsulphotransferase (PST) when either released or infused into the blood[10, 14, 21, 24].

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The responses of free CA to exercise have been well studied, but the responsiveness of noradrenaline sulfate (NA-S), adrenaline sulfate (AD-S) and dopamine sulfate (DA-S) during exercise remains unclear. Some investigators have studied the changes of plasma sulfated CA concentrations during exercise or immediately after exercise [5, 11, 17, 25, 26], but the study results have been equivocal. Furthermore, Strobel and Weicker[28] have stated that the determination of the free CA and sulfated CA should be carried out separately, because the processes for the analytical recovery of free CA and sulfated CA differ. In previous studies, the concentration of the sulfated CA was determined by measuring the difference between the total CA and the free CA concentration in each blood sample. Therefore, Strobel and Weicker[28] have argued that the results of previous studies on plasma sulfated CA with reference to clinical disorder or physical stress were erroneous.

Strobel et al.[29], who measured plasma free CA and sulfated CA concentrations separately, reported that plasma NA-S and AD-S concentrations increased at 20 min or 40 min of exercise with 62% and 77% of maximal oxygen uptake ($\dot{V}O_{2max}$), respectively. In that study, however, they did not measure the plasma DA-S, which makes up an absolute majority of plasma CA. Moreover, NA-S and AD-S concentrations were measured only at 20-min intervals during exercise.

The purpose of the present study was to re-examine the time course of all three plasma sulfated CA concentrations during moderate steady-state exercise using a pertinent analysis method. To achieve this, we measured CA sulfate concentrations at more frequent intervals than those used in the study of Strobel et al[29].

Materials and Methods

Subjects

Six male university runners participated in this study after giving their informed written consent. The study was approved by the Ethical Committee of the Institute of Health Science, Kyushu University. The mean age, height, body mass and percentage body fat of the subjects were 20

±0.5 (SEM) years, 166.5±1.0 cm, 57.8±1.0 kg and 11.0 ±0.5%, respectively. The mean $\dot{V}O_{2max}$ was 3.69±0.10 l/min, or 63.9±1.9 ml/kg/min. None of the subjects was taking any medication for at least 6 weeks prior to the study or had a history of metabolic or circulatory disease.

Experimental protocol

All the subjects participated in an exhaustion test and a steady-state exercise trial on a cycle ergometer. After familiarization with the ergometer and experimental procedures, the subjects undertook the exhaustion test to determine the relationship between workload and oxygen uptake ($\dot{V}O_2$) and to measure their $\dot{V}O_2$max. The exhaustion test consisted of three stages of steady-state exercise and incremental exercise to exhaustion on a cycle ergometer (Bodyguard-990. Jonas Øglaend A.S., Norway). After a warm-up period, the test was started at an exercise intensity of 75-100 W with a constant pedaling rate (50 rpm) for 4 min and then was increased by 50 W every 4 min for 8 min. Thereafter, the exercise intensity was increased by 12.5 to 25 W every minute until the subject reached a state of exhaustion.

One week later, the subjects reported to the laboratory at 9 a.m. after overnight fasting of at least 10 h. They were transported to the testing site by car or motor-bike to minimize the influence of physical activity on the morning of the trial. They were told not to take part in any exercise training for 1 day before the trial and not to make any changes in their lifestyle or dietary habits.

Each subject emptied his bladder before his body weight was obtained, and then a rectal probe was inserted to a depth of 15 cm. An indwelling catheter was inserted into an antecubital vein and kept patent with a dilute heparin-saline solution (10 U/ml). Thereafter, the subjects sat quietly in an armchair for 45 min. The catheter was kept in place until the trial was finished.

The exercise trial began at 10 a.m. The subjects cycled a constant workload at the individual's 70%$\dot{V}O_{2max}$ for 60 min. The subjects were given 100 ml of water (30 ℃) every 15 min during exercise to avoid dehydration. After exercise, the subjects rested quietly in an armchair for 60 min. The laboratory was air-conditioned and held at a constant temperature of 24.2±0.2 ℃ and a constant relative humidity of 62±3%.

Measurements and analytical procedure

Both the electrocardiograph and heart rate (HR) were monitored continuously by a telemetric monitoring system (DS-882, Fukuda Denshi Co., Japan) throughout the test period. The rectal temperature (Rec-T) was recorded every minute throughout the test period using a portable data-recording machine (VMM-67, Vine Co., Japan). Expired gas samples (60 s) were analyzed continuously using a mass-spectra gas analyzer (WSMR-1400, Westron Co., Japan) and an automatic respiratory analyzer (RM-300i, Minato Ikagaku Co., Japan) during the 30-min rest period before exercise began and throughout the exercise period. These analyzers were calibrated before each measurement. Expired gas samples were also collected at 25-35 and 55-65 min during recovery periods.

A blood sample was collected before exercise after 30 min of rest following the insertion of the catheter, after every 10 min of exercise, and at 30 and 60 min after the cessation of exercise. The blood samples were collected with subjects in the sitting position in an armchair or on a cycle ergometer. Each venous blood sample was immediately dispensed into two ice-chilled tubes. One tube contained 5 mg of glutathione and 9 mg of ethylene glycol bis (ß-aminoethyl ether)-N,N,N',N'-tetraacetic acid for sulfated CA. The other tube was a heparinized 7-ml tube containing 8.4 mg of ethylenediaminetetraacetic acid-2K (EDTA-2K) for free CA. After mixing the blood by gentle inversion and storing it on ice, that plasma was separated from the blood cells by centrifugation (3,000 g) at 4℃ for 10 min. The plasma was then frozen and stored at −40℃ until it was assayed. The plasma free CA and sulfated CA concentrations were determined separately by high-performance liquid chromatography (HPLC). All assays were carried out using an automated HPLC analyzer (HLC-725CA, Tosoh Co., Japan) with electrochemical detection following a previously published method by Strobel and Weicher[29] and a slightly modified method by Strobel et al.[27]. The intra-assay and inter-assay coefficients of variation varied between 2% and 9% and between 3% and 7%, respectively. The detection limit was 0.02, 0.03 and 0.04 nmol/l for AD, NA and DA, respectively.

The remaining blood sample was immediately placed into a nonheparinized tube and left to clot for 1 h and centrifuged for 10 min at 3,000 g. The plasma was stored at −40℃. Part of this serum was analyzed for total protein (STP), and percentage changes in plasma volume were measured as previously described[19].

Data analysis

Any statistical differences were tested using analysis of variance (ANOVA) and post hoc Fisher's PLSD test for multiple comparison. Significance was set at the 0.05 level of confidence. All results are presented as means±SEM unless otherwise stated.

Results

The $\dot{V}O_2$ value of each subject was constant throughout exercise for 60 min, and the mean value was 2.65± 0.05 l/min, which was 71.7±1.7%$\dot{V}O_{2max}$. The HR and Rec-T increased gradually with the increasing duration of exercise, and the mean HR and Rec-T value at the end of exercising were 175±4 beats/min (at rest; 56±3 beats/min) and 39.2 ± 0.1℃ (at rest; 36.6 ± 0.4 ℃), respectively. The body

weight loss was 1.2±0.3 kg (pre-exercise, 57.3±1.3 kg vs. post-exercise, 56.1±1.3 kg).

The STP concentration of each subject at any sampling point was used to eliminate the effect of plasma water loss from the determined values of plasma free CA and sulfated CA concentrations. The concentrations during exercise were relatively constant at the various sampling points, and the mean estimated hemoconcentration for a 10- to 60-min exercise session was 11.8±0.31%.

The plasma free AD, AD-S and total AD (the sum of free AD and AD-S) concentrations in the pre-exercise period, during exercise and during recovery from exercise are shown in Fig. 1. The concentrations of free AD, AD-S and total AD before exercise were 0.32±0.06, 0.63±0.13 and 0.96±0.17 nmol/l, respectively. During exercise, plasma free AD, AD-S and total AD concentrations increased with exercise duration and were significantly elevated over pre-exercise values at 30 min for AD-S ($p<0.05$), 40 min for total AD ($p<0.05$) and 50 min for free AD ($p<0.01$), respectively. The free AD and AD-S concentrations reached 12-fold (4.14±1.64 nmol/l) and 2.3-fold (1.47±0.23 nmol/l) at 60 min of exercise compared with the pre-exercise levels, respectively. In the recovery periods, the free AD concentrations rapidly decreased and reached pre-exercise levels by 30 min. However, the AD-S concentrations remained elevated for 60 min ($p<0.001$) after the cessation of exercise.

The concentrations of plasma free NA, NA-S and total NA in the pre-exercise period were 2.82±0.58, 4.79± 0.41 and 7.61±0.91 nmol/l, respectively. As shown in Fig. 2, a progressive increase in free CA, NA-S and total NA concentrations during exercise was observed, and statistical significance was achieved at 10 min for free NA ($p<0.05$) and total NA ($p<0.01$) and at 20 min for NA-S ($p<0.01$) compared with the pre-exercise levels, respectively. At the end of exercise, the free NA and NA-S concentrations were 11-fold (24.82±3.37 nmol/l) and 2.4-fold (11.58± 0.94 nmol/l) compared with concentrations before exercise, respectively. Compared with the pre-exercise levels, the NA-S concentrations were still elevated at 60 min after exercise ($p<0.001$), but this was not true of the free NA concentrations.

Plasma free DA, DA-S and total DA concentrations before exercise were 0.10±0.02, 16.77±1.87 and 16.87± 1.88 nmol/l, respectively. As is clear in Fig. 3, the free DA concentrations also exhibited a gradual rise during exercise. Statistical significant compared with the pre-exercise levels was achieved after 20 min of exercise ($p<0.05$-$p<0.001$). The concentration of free DA at the end of exercise was 13-fold (1.05±0.16 nmol/l) compared with the pre-exercise levels. On the other hand, plasma DA-S concentrations during exercise decreased significantly below resting values at 10 min and 20 min ($p<0.05$) and then increased significantly at the end of exercise ($p<0.001$). The

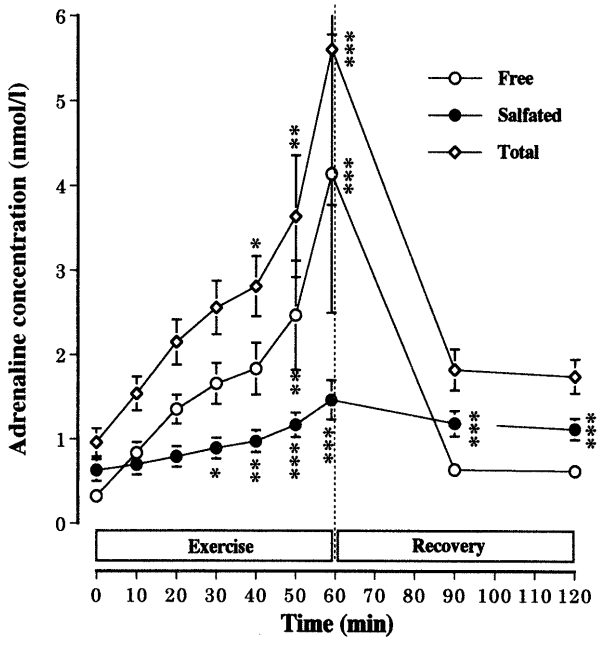

Fig. 1 Plasma free, sulfated and total (free+sulfated) adrenaline concentrations in the pre-exercise (0 min), during exercise (10-60 min) and post-exercise (90 and 120 min) (mean and SEM, n=6). *p<0.05, **p<0.01, ***p<0.001 Significant difference from the pre-exercise level.

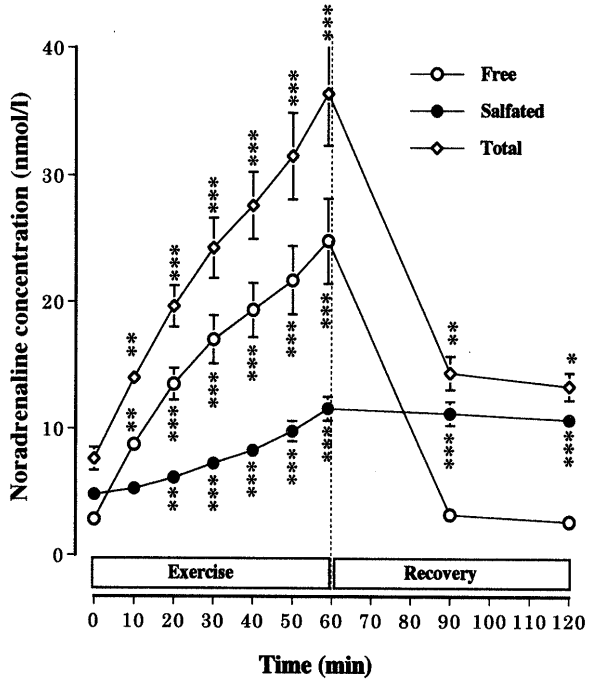

Fig. 2 Plasma free, sulfated and total (free+sulfated) noradrenaline concentrations in the pre-exercise (0 min), during exercise (10-60 min) and post-exercise (90 and 120 min) (mean and SEM, n=6).
*p<0.05, **p<0.01, ***p<0.001 Significant difference from the pre-exercise level.

DA-S concentrations were elevated after exercise and reached peak values at 30 min after exercise. The dynamics of the total DA concentration during exercise and during recovery from exercise was almost identical to that of the DA-S concentration.

We have calculated each free CA as a percent of the corresponding total CA. Fig. 4 depicts the profiles for change from pre-exercise levels during exercise and recovery. The percent free AD and NA at rest were 33.8±4.4 and 35.5±4.0%, respectively. Both measures increased substantially from pre-exercise levels by 20 min of exercise and then the levels remained stable for the remainder of the exercise (63-68% for AD and 68-70% for NA). ANOVA post hoc analysis (Fisher's PLSD and Bonferroni/Dunn) indicated that there were no significant differences in both measures at any points during 20-60 min of exercise (the significant level was not shown on the Fig. 4). At 30 min after cessation of exercise, the percent free AD returned to pre-exercise levels, although the free NA decreased to below pre-exercise levels. The percent free DA during exercise increased gradually with exercise duration, and the mean value at the end of exercise was 5.8± 0.9% of total DA (at rest 0.5±0.2%). The percent free DA returned to near pre-exercise level after 60 min of recovery.

Discussion

There were three main findings from this study. First, the plasma NA-S and AD-S concentrations increased gradually with exercise duration and were significantly elevated over pre-exercise values at 20 min for NA-S concentration and at 30 min for AD-S concentration. Second, the ratio of free AD and NA to total AD and NA concentrations reached a steady state during exercise. Third, plasma DA-S concentration was decreased temporarily after 10 and 20 min of exercise, whereas the DA-S concentration had increased a statistically significant amount by the end of exercise.

The responsiveness of CA sulfate during exercise remains unclear. Exercise protocols used to study plasma sulfated CA have ranged from light to high intensity and have had varying duration. Results have been equivocal in that increased[17,26], decreased[5,11], unchanged[17] or decreased and then increased[25] plasma NA-S or AD-S levels have been noted. The discrepancy might be explained in two ways. The first and more likely explanation for the discrepancies is the differences of exercise intensity and/or duration used in the various studies. In the studies that have resulted in significantly increased plasma NA-S and/or AD-S concentration during exercise or immediately after exercise[25, 26, 27, 28], plasma free NA and AD concentrations have been elevated 6-fold to 10-fold. In the present study, the concentrations of plasma free NA and AD, compared with pre-exercise levels, were elevated 11-fold for free NA and 12-fold for free AD at the end of exercise. On the other hand, in previous studies in which there was no elevation in plasma NA-S

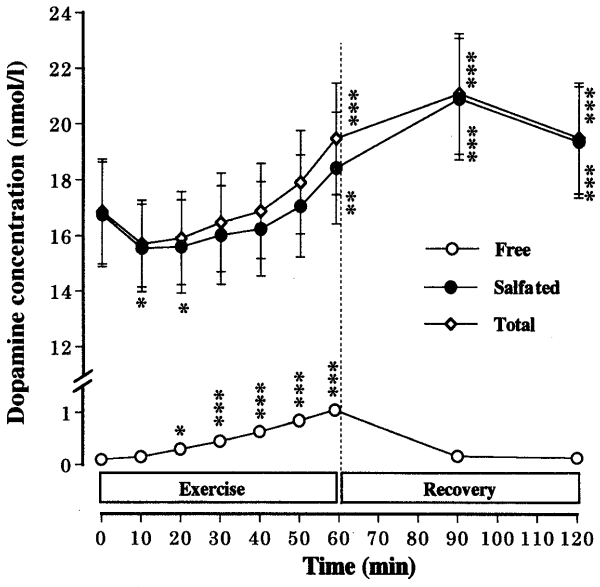

Fig. 3 Plasma free, sulfated and total (free + sulfated) dopamine concentrations in the pre-exercise (0 min), during exercise (10-60 min) and post-exercise (90 and 120 min) (mean and SEM, n=6). *p<0.05, **p<0.01, ***p<0.001 Significant difference from the pre-exercise level.

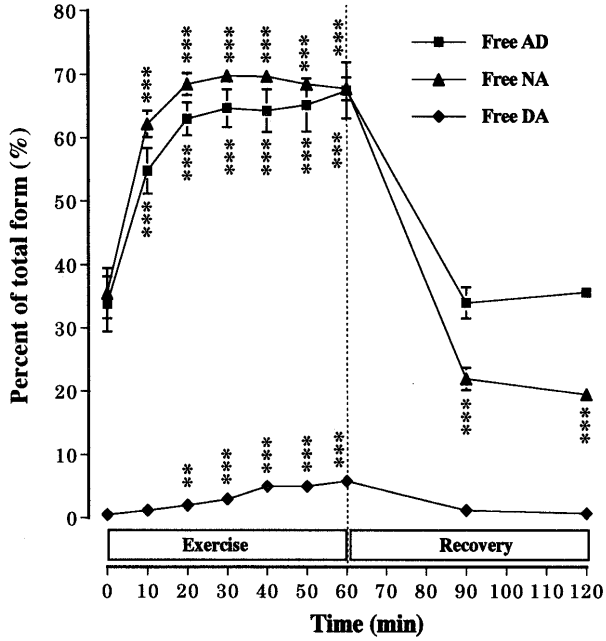

Fig. 4 A percent free adrenaline (AD), noradrenaline (NA) and dopamine (DA) to corresponding total (free + sulfate) in the pre-exercise (0 min), during exercise (10-60 min) and post-exercise (90 and 120 min) (mean and SEM, n=6).
**p<0.01, ***p<0.001 Significant difference from the pre-exercise level.

and/or AD-S concentration, exercise duration was only 8 min[21], 12 min[11] or 16 min[5], and the exercise intensity was 30%$\dot{V}O_{2max}$[26], 45% and 60% of $\dot{V}O_{2max}$[17] and 56%$\dot{V}O_{2max}$[4]. In those studies, the increase in the free NA and AD concentrations during exercise was only 2- to 5-fold. Thus, lack of elevation of NA-S and AD-S concentrations during exercise might have resulted from the insufficient exercise duration and/or low exercise intensity of those studies.

Also, in previous studies where the DA-S concentration did not increase, exercise duration was only from 8 min[21,25] to 22 min[28] or exercise intensity was only at 56%$\dot{V}O_{2max}$[4]. Odink et al.[17] found that the DA-S concentration increased during cycling at 75%$\dot{V}O_{2max}$, but the concentration did not change at 45% and 60% of $\dot{V}O_{2max}$ for 30 min. In the present study, plasma DA-S reached a statistically significant level, compared with the pre-exercise level, at 60 min of exercise at 70%$\dot{V}O_{2max}$, when plasma free DA concentration reached a 13-fold level compared with the pre-exercise level. Therefore, our result suggests that although plasma DA-S concentration was increased with exercise, sufficient exercise duration or significant elevation of free DA concentration was needed for the elevation.

The second explanation for the difference in study results might be related to the analysis method used for CA sulfate determination, as pointed out by Strobel and Weicker[29]. They have provided evidence that the previous method of determining sulfated CA as the difference between the total and the free CA was defective. Storobel et al.[27] used a pertinent analysis method, which was to measure plasma free and sulfated CA concentrations separately, and they reported that NA-S increased after 20 min of exercise at either 62% or 77% of $\dot{V}O_{2max}$ and AD-S increased after 40 min during steady-state exercise at 62%$\dot{V}O_{2max}$ or 20 min at 77%$\dot{V}O_{2max}$. Our results support and extend their finding, because we found that the plasma NA-S and AD-S concentrations were significantly elevated over pre-exercise values after 20 min for NA-S and 30 min for AD-S concentration.

In the present study, the plasma NA-S, AD-S and DA-S concentrations were significantly elevated over pre-exercise values at 10 min, 30 min and 60 min, respectively. The affinity for PST is the highest in free DA and the lowest in free AD[13,24]. Thus, the difference of response time of plasma sulfated CA to exercise cannot be explained by the affinity for PST activity. The difference could have resulted from the absolute concentration of free CA, because the plasma free NA concentration during exercise was 6- to 11-fold higher than that of free AD, and the free AD concentration was 3- to 5-fold higher than that of free DA (see Fig. 1).

Interestingly, the ratio of free AD and NA to total AD and NA concentrations reached a steady state during exercise, despite the fact that free AD and NA markedly increased with the increasing duration of exercise. Most of the free CA were readily conjugated by PST when either released or infused into the blood[10,14,21,24]. It has been shown that sulfated CA concentration, especially NA-S and/or AD-S concentrations are elevated not only for a long time after exercise[18,22,27] but also immediately after [6,25,26,28] or at 30 min after exercise[4]. We had hypothesized previously that the plasma free CA released during exercise undergoes sulfoconjugation after exercise[18]. In the present study, we confirmed also that plasma NA-S and AD-S concentrations increased significantly during exercise, which supported the findings of Strobel et al.[27]. Therefore, it is tempting to suggest that the free AD and NA released during exercise may undergo sulfoconjugation not only after exercise but also during exercise.

It is also interesting to note that the plasma DA-S concentration was decreased transiently after 10 and 20 min of exercise in our study. Sothmann et al.[25] also found that, although not significant, the DA-S concentration declined temporarily during the first 7 min of exercise at 78%$\dot{V}O_{2max}$ for 28 min. The concentration of plasma DA-S consists almost entirely (98-99.9%) of total plasma DA[6,11] and accounts for more than 70% to 80% of all three plasma CA[14,22]. Although free DA has the greatest affinity for PST among the three CA, arysulfatase enzyme can generate free CA[16]. Some authors have proposed that the DA-S can be converted directly into free DA, NA or AD in vivo[3,16] and to free NA in vitro[2,10], but other studies have not confirmed this[7,15]. Based on the results of the present study, we are also tempted to suggest that the DA-S may be cleaved at an earlier stage of exercise, augmenting free CA concentrations[4,21], and that deconjugation of DA-S at the onset of exercise could occur and account for a significant amount of plasma free NA and AD[4]. The roles of PST and arysulfatase enzyme during exercise remain unclear, however. PST activities have been detected in human platelets, liver, kidney, jejunum, lung, omentum and brain[1,30]. It has been shown that the blood volume in the abdominal organs such as the kidney and liver decreased during submaximal upright exercise, whereas lung blood volume increased[8]. Moreover, substrate inhibition of PST activity by high concentrations of free CA, by metabolites released during exercise or perhaps by a more rapid (and preferential) renal clearance of the conjugates are alternative explanations[5]. At present, we cannot explain why plasma DA-S concentrations decreased temporarily during an early stage of exercise, but we believe the factors mentioned above may have influenced DA-S dynamics during exercise.

In conclusion, sustained exercise of sufficient intensity and duration elicits increased all three plasma sulfated CA during exercise. These results suggest that the plasma free NA and AD released during exercise may undergo sulphoconjugation not only after exercise but also during exercise. We also find that plasma DA-S concentration decreases

transiently at an early stage of exercise, although the mechanism remains unclear.

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