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# Preparation of an Electrochemical Luminescence Sensor for Monoamine Neurotransmitter (polydopamine) Detection after Endurance Training

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Electrochemiluminescence (ECL) analysis is characterized by high sensitivity and wide linear range while also providing convenient detection and simple instrumentation. Luminol, as the most commonly used luminescent reagent in chemiluminescence (CL) analysis, has been widely used in electrochemical luminescence analysis. Excitement of the dopamine (DA) system can directly promote people's curiosity and exploration, along with increasing exercise and other responses. If the DA system is inhibited, it will lead to a series of problems such as reduced movement and even a reduced desire for other life activities. In this work, a reaction of DA is catalysed by lactate dehydrogenase and coenzyme I to produce pyruvate, which reacts with pyruvate oxidase to form $H_2O_2$. ECL using luminol is sensitive to the above process. Based on this, a biosensor was developed to detect DA.

**Keywords:** Monoamine neurotransmitter; Electrochemical luminescence sensor; Endurance training; Modification; Nanomaterial

## 1. INTRODUCTION

Monoamine neurotransmitters play a very important regulatory role in the process of exercise, which is closely related to the body's energy metabolism, muscle coordination and exercise fatigue [1–3]. A lack of dopamine (DA) can lead to emotional depression and decreased motor performance. In contrast, moderate doses of DA can delay fatigue and improve performance [4–6]. Therefore, exercise also has an impact on the metabolism of DA in the body.

Freed et al. [7] conducted group comparison experiments with rats at different moving speeds. With increasing moving speed, DA in the rat caudate nucleus significantly increased. It is suggested that DA is positively correlated with exercise intensity. By observing the effects of swimming on the central nervous system of rats, Xu et al. found that single exercise stimulation could significantly increase DA

content in the nucleus accumbens of rats. Long-term exercise training could also enhance DA metabolism in the striatum, prefrontal cortex and nucleus accumbens of rats. Through experiments, Li et al. pointed out clearly that the significant increase in DA caused by exercise mainly occurred after the end of the exercise, while it was not as obvious during the exercise [8–11]. The reason may be that both DA synthesis and decomposition are promoted during the exercise, and the synthesis rate of DA after the exercise is significantly higher than the decomposition rate. Wang et al. observed the swimming exercise experiment in rats and found that the DA level in the brain for rats in the endurance training group was significantly higher than that for rats in the non-training group after a one-time exhaustion exercise. These changes suggested that the one-time exhaustion exercise could promote the activity of relevant enzymes in the anabolism of DA, thereby increasing the synthesis of DA to meet the needs of the metabolism of the body during exercise [12–14]. Extended tests of swimming endurance training made the rats develop stress adaptation and improve the stability of DA metabolism.

However, some scholars believe that the synthesis, release and decomposition of DA are simultaneously enhanced during exercise. Hattori et al. [15] studied the metabolic influence of track movement on rat striatum DA and found that movement promoted the metabolism of DA and was positively correlated with the speed of movement. In addition, they found that the activity of tyrosine hydroxylase (TH) in the striatum of rats in the speed training group increased by 35% compared with that before training; furthermore, striatal monoamine oxidase (MAO) activity increased by 60%. This indicates that both acute exercise and long-term training can significantly increase the activity of DA catabolism enzyme MAO and promote the catabolism of DA. This not only plays a positive role in maintaining and improving brain function but also delays the occurrence of motor fatigue [16–19]. Therefore, an increase in DA synthesis and catabolism during exercise is related to exercise intensity. The amount of DA in the blood may also reflect the exercise intensity and training level of an athlete [20–22].

The basic principle of electrochemical luminescence is to produce some special substances by applying certain electrochemical excitation signals on the electrode, which can react with the luminescent substances and provide enough energy to make the luminescent substances produce chemiluminescence [23–26]. Alternatively, the use of electrodes to provide energy directly causes the luminescent material to undergo a redox reaction, resulting in an unstable intermediate state material, which decomposes or returns to the ground state and causes luminescence [27–31]. In a typical chemiluminescent assay, the light emitted is usually of low intensity and does not last long enough to make an accurate detection and analysis. To address this issue, most commercially available luminol is supplied with an enhancer (e.g., modified phenol, naphthol, aromatic amine or benzothiazole), so the reaction can proceed for prolonged durations without any significant reduction in light output. Biosensors are a kind of new technology crossing into the areas of life science and information science, which consist of many disciplines, such as biology, chemistry, physics, medicine and electronic technology. As a new method for the direct or indirect determination of biomolecular, physiological or biochemical process parameters, biosensors are characterized by low sample consumption, high selectivity, fast analytical speed, simple and easy operation and low instrument price and can be used for online or even in vivo analysis [32–35]. At present, the research of enzyme biosensors has been extensive, but most of them are still in the stage of laboratory research and development. Therefore, the

development of enzyme biosensors with market potential has been the direction of scientists' efforts. We propose to use the ECL method to improve the sensitivity and specificity of DA actuators by combining an electrochemical luminescence method with an enzyme reaction. The purpose of this study is to combine an enzyme-catalysed reaction with an electrochemical luminescence of luminol to prepare an electrochemical luminescence biosensor with high sensitivity and selectivity and apply it to the detection of actual samples. The electrochemical luminescence of luminol on a platinum electrode was studied. A double enzyme electrochemical luminescence biosensor was prepared based on the above principles and applied to the detection of real samples. It demonstrated a low detection limit, high sensitivity and good reproducibility.

## 2. EXPERIMENTAL

Luminol, lactate dehydrogenase, coenzyme I, pyruvate oxidase and dopamine were purchased from Sigma. $K_3Fe(CN)_3$, disodium hydrogen phosphate, dihydrogen phosphate and chitosan were purchased from Sinopharm Co., Ltd. All chemicals were analytical grade.

Electrode modification: Carbon nanotubes (CNTs) have unique structures, large specific surfaces, and excellent physical and chemical properties. Many studies have reported that carbon nanotubes can be used to modify surfaces of electrodes. Lactate dehydrogenase (18 μL), 18 μL coenzyme (I), 15 μL pyruvate oxidase, 4 μL $KFe(CN)_3$ and 12 μL carbon nanotubes were mixed to form an electrode modifier solution. Then, 10 μL of the solution was dropped on the electrode surface and dried naturally.

Conductive polymer film-modified electrodes have been widely used in electrochemical biosensor preparation in recent years due to their large number of active sites. Among them, polypyrrole (PPy) is easy to use and forms films on electrode surfaces through electrical polymerization; moreover, the film thickness is controllable, thus, many studies have been conducted. A double enzyme was deposited on the surface of the working electrode by means of potentiostatic electropolymerization, and the bottom solution was a phosphoric acid buffer solution. Lithium perchlorate was added as the supporting electrolyte, and 1 μL pyrrole, LDH, NDA and PYOD were added. After electrodeposition for a period of time, the electrode was cleaned.

A PMT-II weak light measuring instrument and a BAS-100A electrochemical analyser were used for the ECL testing. A three-electrode system consists of a working electrode (platinum disc electrode), an auxiliary electrode (platinum wire) and a reference electrode (Ag/AgCl).

## 3. RESULTS AND DISCUSSION

Pulse waveform: The influence of excitation signals of various waveforms (sinusoidal wave, triangular wave, sawtooth wave, double-step wave, normal rectangular wave) on the luminescence behaviour of the luminol system was tested, and different waveforms were found. With a rectangular pulse, the peak shape of the generated ECL signal is stable, and the peak height changes little.

Pulse amplitude: There is weak light at 0.1 V, and the light intensity increases with increasing amplitude. When the pulse amplitude reaches approximately 0.9, the light intensity reaches the maximum and is relatively stable.

Period: Continuous light is generated when the period is a millisecond. As the reaction progresses, the intensity of the light decreases. When the period is a second, discontinuous light is generated, and the light intensity changes with pulse voltage. When the working electrode is at a high potential, light is generated. When the working electrode is at a low potential, there is no light signal, and the light signal period is consistent with the pulse period, which strongly indicates that the light generation is caused by electrolysis. The control period of this experiment is 3 s. A typical ECL signal recording is shown in Figure 1A.

Temperature is an important factor affecting the reaction rate. The changes in the ECL resulting from changes in the experimental temperature are shown in Figure 1B. At 30 °C, the ECL light intensity is the strongest, which is due to an increase in the reaction rate of luminol oxidation on the electrode to generate luminol radicals and an enhancement in ECL. On the other hand, when the temperature is high, the thermal movement of the free radicals has increased intense and there is an increased chance of annihilation between the free radicals; this reduces the free radicals in the system and weakens the ECL light intensity [36–39].

When the pH of the experiment changes, the light intensity of ECL first increases and then decreases (Figure 1C). When the pH = 8, the light intensity of ECL reaches its maximum, and the detection sensitivity is high under this condition. However, highly alkaline surroundings will cause decomposition of some species, which will further induce an unstable system and weaken the ECL signals [40]. Therefore, a PBS of pH 8 was selected for follow-up experiments.

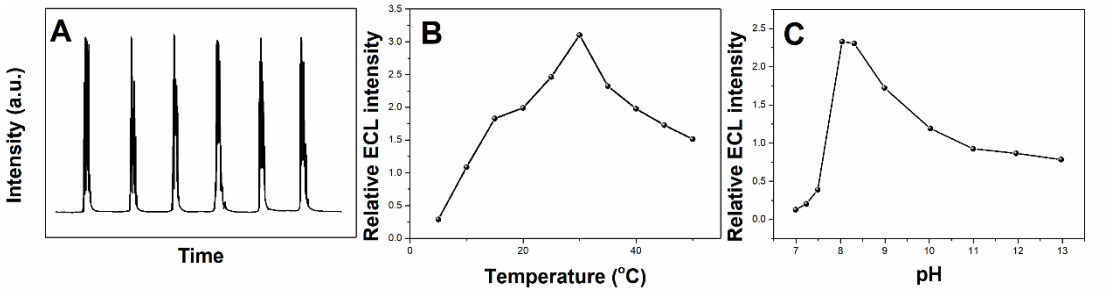


**Figure 1.** (A) ECL performance of Luminol. (B) The influence of changing the experimental temperature on the change of ECL. (C) The influence of changing the pH condition of PBS on the change of ECL.

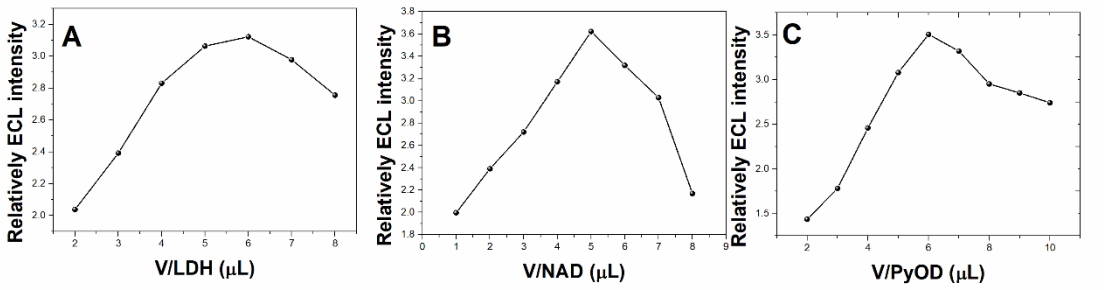


**Figure 2.** Effect of the amount of (A) acid dehydrogenase, (B) coenzyme (I) and (C) pyruvate oxidase on the change of ECL.

The enzyme concentration has a great influence on the sensor. When the amount of enzyme is small, the response of the enzyme electrode increases rapidly with the increase of enzyme. However, when the concentration of enzyme increases to a certain extent, the response signal reaches its maximum. The content of lactate dehydrogenase, coenzyme I and pyruvate oxidase loaded in the enzyme electrode was changed, and the relative luminescence intensity of luminol was changed as shown in Figure 2. When the concentration of lactate dehydrogenase is 6.7 mg/mL, coenzyme I is 8 mg/mL, and pyruvate oxidase is 3.2 μg/mL, the response of the biosensor to DA reaches its maximum. The excess of lactate dehydrogenase, coenzyme I and pyruvate oxidase hinders any further reaction for the limited surface area of the electrode and spatial steric effect occurs [41]. In addition, because the large surface area of the CNTs is responsible for the high output current, electrons can diffuse in the CNTs [42,43]. Therefore, the above concentration is selected in the subsequent experiments.

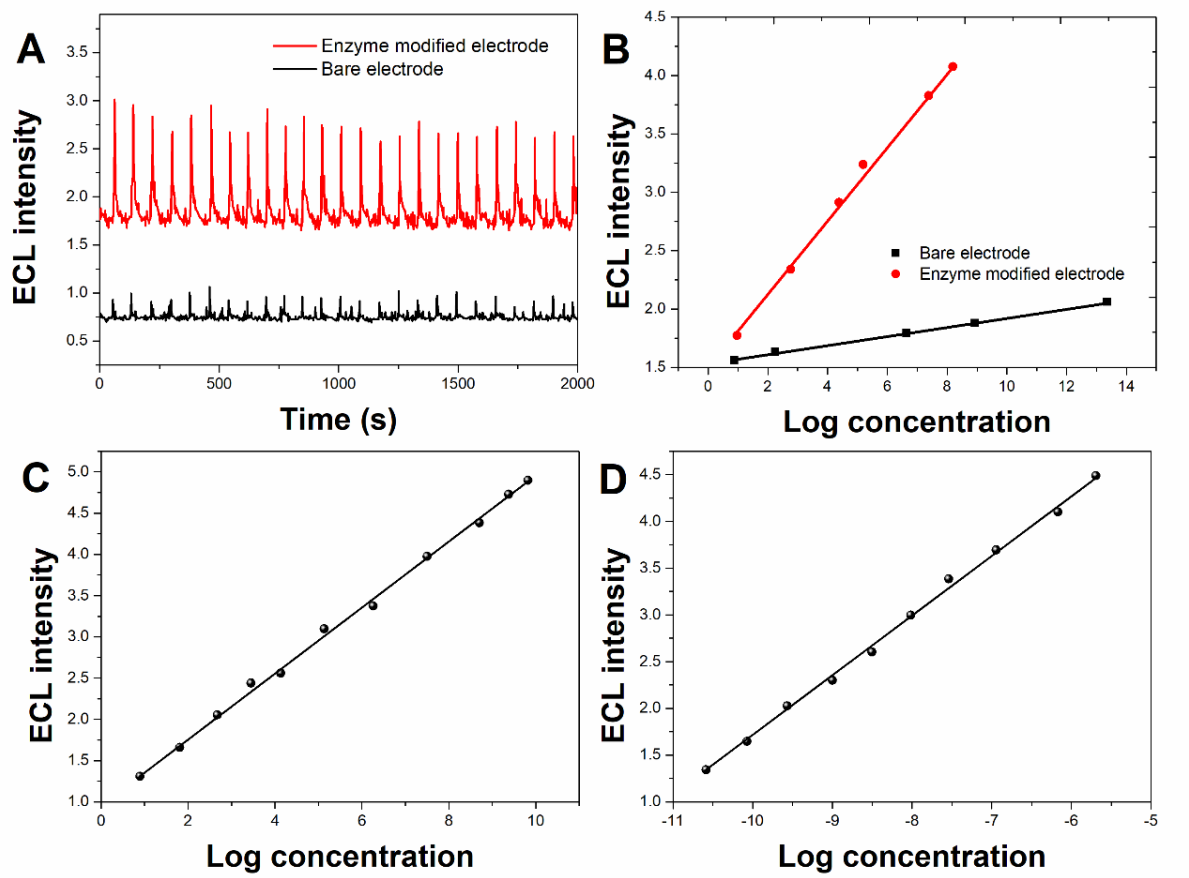


**Figure 3.** (A) Electrochemical luminescence curves of bare electrode and enzyme modified electrode in weakly alkaline system. (B) Comparison of DA response of electrode and bare electrode. (C) Relation between DA concentration and Relative ECL Intensity using modified Pt electrode. (D) Relationship between logarithm of different DA levels and Relative ECL Intensity.

Figure 3 shows the response of the electrochemiluminescence method with the enzyme electrode and the bare electrode to DA under the same conditions. The response to DA is significantly increased after the double enzyme was modified on the electrode. After the modification of the Pt electrode with the enzyme, the minimum content of DA measured reached 10 pM, and the linear equation describing

the relationship between DA concentration and relative ECL intensity was y=1.00306 + 0.34021c. The relationship diagram is shown in Figure 3C. In all coreactant ECL systems toward light emission, four processes are generally involved: (a) redox reactions at the electrode, (b) homogenous chemical reactions, (c) excited state species formation and (d) light emission [44]. The results showed that the minimum detection limit of the Pt electrode response to DA after double enzyme fixation is 8.9 pM, and the linear range is 8.9 pM to 1.3 μM. The linear range measured in the enzyme solution is 0.89 nM to 1.3 μM, indicating that the modified biosensor is very sensitive. Figure 3D shows the relationship between the logarithm of DA level and relative ECL intensity.

Figure 4A shows the CV response of the enzyme-modified electrode to DA and the bare electrode to hydrogen peroxide at a potential range of -1.0 V to 1.0 V. The oxidation peak potential of the electrode in response to $H_2O_2$ is approximately -0.5 V, and the peak potential of the enzyme electrode in response to DA is also approximately -0.5 V, indicating that DA in the system reacts to generate $H_2O_2$ under the dual enzyme system.

The kinetics of enzymatic reactions, referred to as enzyme kinetics, mainly refers to the speed of enzymatic reactions and the corresponding factors. There are several methods to measure the $K_m$ and Vmax values in enzymatic reactions [45–47]. By fixing the enzyme concentration in a reaction and analysing the initial velocity at different substrate concentrations, Km and Vmax values can be obtained. However, it is difficult to determine the $K_m$ or $V_{max}$ value directly from a graph of the initial velocity against the substrate concentration because the curve gradually approaches $V_{max}$. A transformation of the mi equation is used to determine $K_m$ and $V_{max}$, such as the Lineweaver-Burk equation, also known as the double reciprocal equation. Therefore, we also used this method to find the milt constant of lactate dehydrogenase and the maximum reaction rate of the system.

In the analysis of enzyme kinetic properties, the mi constant Km is a basic characteristic constant of the enzyme, which includes the binding and dissociation properties of the enzyme and substrate. When the same enzyme can act on several different substrates, the mi constant Km can often reflect affinities between the enzyme and various substrates. The larger the Km value is, the weaker the affinity between the enzyme and substrate is. Conversely, the smaller the Km value is, the stronger the affinity between the enzyme and the substrate, and the substrate with the smallest Km value is the optimal substrate of the enzyme. Through experiments, we found that the measured value of the milt constant of lactate dehydrogenase was much smaller than that reported in the literature in the enzyme solution, which also indicated that the enzyme activity was significantly improved after modification by carbon nanotubes.

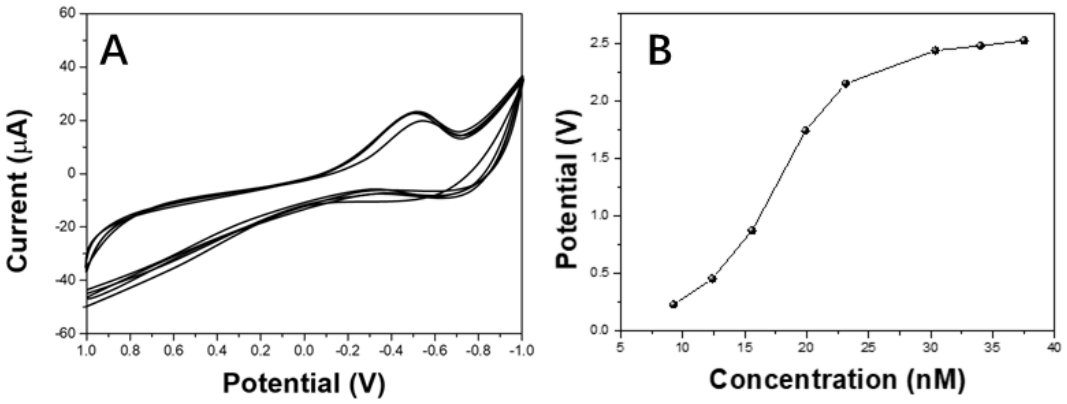


**Figure 4.** (A) CV response of enzyme electrode to DA and CV response of bare electrode to hydrogen peroxide. (B) Kinetic study of reaction promotion.

With the development of sports, sports science has gradually infiltrated into sports training. Among them, the determination of blood DA concentration has received increasing attention. It has been found that the DA concentration in blood after exercise not only reflects the functional status of athletes but also directly reflects the capacity of the glycolysis energy supply system at rest because blood DA is the product of glucose anaerobic metabolism. Therefore, the determination of blood DA can provide an objective basis for coaches to arrange training intensity, especially in track and field, swimming and other periodic events with more practical value. Table 1 shows the standard addition method for real serum sample detection of DA. Human serum samples were provided by a university hospital. The obtained stock solution of human serum was first diluted 10 times with a phosphate buffer (7.4) and then used. In this work, all biomaterials were placed at − 20 °C before use. The proposed method showed an excellent recovery rate and RSD.

**Table 1.** Determination of DA in serum by enzyme biosensor

| Detection (nM) | Addition (nM) | Detection (nM) | Recover rate (%) | RSD (%) |
|---|---|---|---|---|
| 20.4 | 20 | 40.7 | 100.74 | 3.21 |
| 21.1 | 20 | 41.6 | 100.97 | 3.11 |
| 20.6 | 20 | 39.8 | 98.03 | 1.09 |
| 22.2 | 20 | 41.6 | 98.28 | 1.27 |

The experiment was conducted with two athletes, A and B, where A was an untrained athlete and B was a trained athlete. The serum samples of the athletes were taken after each period of exercise, and one sample was taken after each intensive exercise. The collected samples were placed in a refrigerator for determination. Through a large amount of experimental data, we determined the relationship between the athletes' exercise intensity and DA content, as shown in Figure 5.

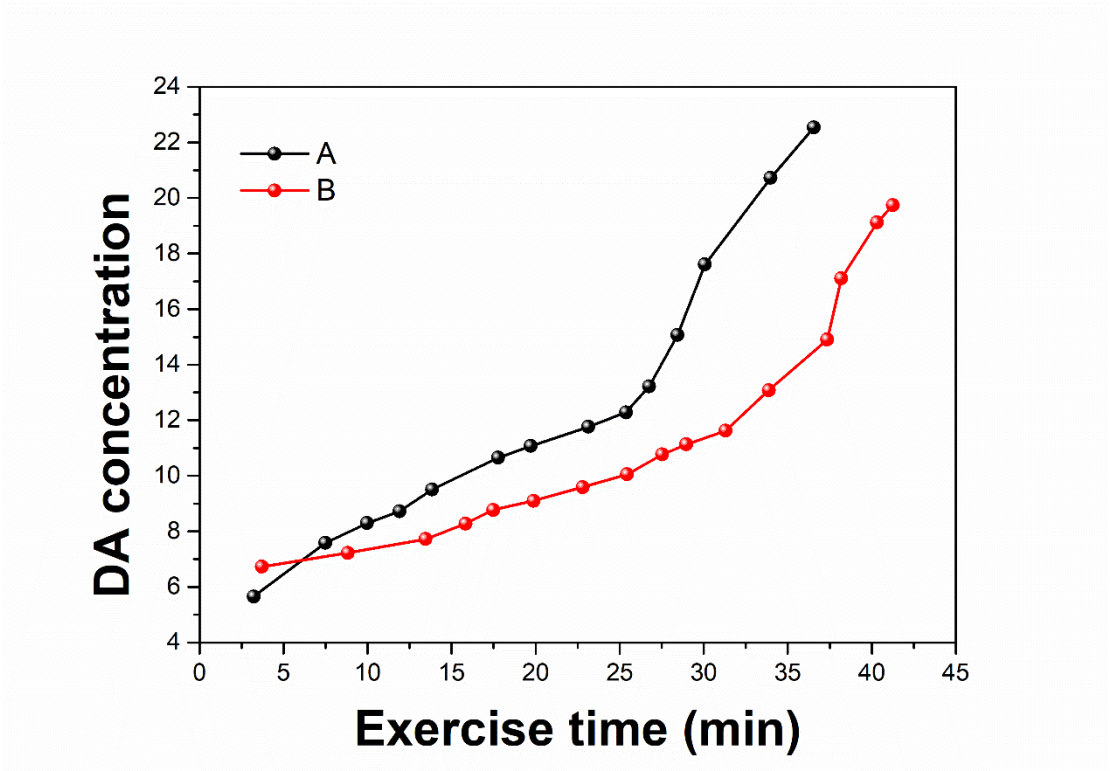


**Figure 5.** The relative relationship between dynamic intensity and DA content

The figure shows the results as time gradually increases with the athlete exercise intensity; starting at a low intensity, the increase in DA is not large, is relatively flat, and shows that when the body requires aerobic energy, it produces less DA. With the increase of the exercise intensity to a certain point, blood DA will increase drastically, as anaerobic glycolysis that participates in energy production also produces large amounts of DA. This sudden increase in DA is a critical point in the transition from aerobic to anaerobic metabolism, which corresponds to the anaerobic threshold level. However, due to individual differences, virtually everyone's anaerobic threshold is not the same. The value of the anaerobic threshold can reflect the quality of exercise ability. In elite athletes, the anaerobic threshold is large, and the curve of DA concentration is more curved. Athletes with poor athletic ability have low anaerobic thresholds. Additionally, DA levels rise faster and earlier when exercise intensity is gradually increased. The curve shows a small curvature and is steeper. Therefore, the anaerobic threshold of trained athlete B in the figure is greater than that of untrained athlete A, and the increased DA curve of B is less than that of A.

## 4. CONCLUSION

Carbon nanotubes were used to fix lactate dehydrogenase and pyruvate oxidase. In the presence of potassium ferricyanide, the enzyme electrode was used to detect the content of DA indirectly. It has high sensitivity and selectivity when detecting the corresponding target and can provide satisfactory reproducibility and stability. This dual enzyme biosensor can be used to measure blood DA to predict endurance training intensity, and we believe this enzyme biosensor will have further extensive applications in sports medicine and other fields in the future.

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