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Short Communication

Exercise is a potent stimulus for enhancing circulating DNase activity

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ABSTRACT

Objectives: To elucidate cell free DNA (cfDNA) clearance kinetics following an acute bout of high intensity exercise by measuring circulating DNase activity reduction (AR).

Design and methods: Serum cfDNA concentration and DNase-AR were measured prior to and post (immediately post, 7 and 30 min post) an acute bout of rowing exercise until exhaustion.

Results: Serum cfDNA concentration was significantly ($P \leq .001$) elevated immediately post (2.5-fold) and 7 min post exercise (2.3-fold) with a return close to baseline at 30 min post exercise (1.5-fold). The rise in cfDNA was accompanied by a concomitant, significant ($P \leq .001$) decrease in serum DNase-AR from 15.1% prior to exercise to 3.1% AR at cessation of the exercise test and 7 min post exercise (3.9% AR). DNase-AR returned close to baseline at 30 min post exercise (5.2% AR).

Conclusions: A single bout of high intensity exercise is a potent stimulus for enhancing circulating DNase activity in healthy people. Acute exercise may therefore be considered as a non-pharmacological stimulus to trigger DNase activity.

This finding may be relevant for pathological conditions associated with increased cfDNA concentrations like cystic fibrosis, where pharmacological recombinant human DNase (rhDNase) treatment has been successfully used to improve patients' health and physical function.

Introduction

DNase activity in tissues and body fluids clears the system from endogenous DNA of apoptotic and necrotic cells along with foreign DNA which originates from food digestion and potential pathogens. Circulating cell free DNA (cfDNA) in blood is hydrolyzed by DNase and cleared predominantly via the liver and to a lesser extent (<2%) by the kidney [1]. DNase activity varies between body fluids, with twice the activity levels observed in human serum compared to saliva [2].

In healthy conditions cfDNA concentrations have been shown to increase transiently for minutes in response to acute exercise [3,4] or prolonged for 96 h in response to long-lasting high resistance exercise associated with sustained inflammation [5].

In pathological conditions like sepsis, cancer, autoimmune diseases, trauma, myocardial infarction, and pregnancy-associated complications [6] cfDNA concentrations are elevated as well. Extracellular DNA in lung secretions of cystic fibrosis (CF) patients, released from disintegrated immune cells and bacterial DNA, contributes to the increased viscosity and reduced effectiveness of antibiotic treatment [7]. CF sputum viscosity is greatly reduced when lung secretions are incubated with rhDNase in-vitro [7], suggesting that endogenous cfDNA clearance and degradation mechanisms are impaired or inadequate to cope with pathological cfDNA levels. This claim is supported by findings showing that DNase activity is reduced in several diseases such as cancer, which are associated with increased levels of cfDNA [8]. Consequently rhDNase was approved for medication two decades ago and is still one of the most widely used drugs for treating CF [9].

Since intense exercise induces transient increases in plasma and serum cfDNA [3,4,10,11], we hypothesized that exercise stimulates circulating DNase activity concomitant to the rise in cfDNA. Here we show that the reagibility of DNase activity is induced by an acute bout of high intensity ergometer rowing.

Methods

Study participants and exercise test

Ten highly trained male rowers, all qualified for the German 2013 Junior National team (Table 1) performed a step-wise incremental exercise trial until exhaustion on a rowing ergometer (Concept 2 Type D, Morrisville). The exercise test started at 200 W, and the increments amounted to 50 W/4 min. During a break of 30 s between each stage and immediately after the last stage, 20 μl of capillary blood was taken

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Table. 1
Anthropometric data of study participants, maximal (max) power output (P), max blood lactate (La) concentrations, heart rate (HR), total exercise duration, P and La at the individual anaerobic threshold (IAT), and fold-increase of max P and La versus IAT measured after cessation of the incremental rowing test to exhaustion.

	Age [years]	Height [cm]	Body mass [kg]	BMI [kg/m^2]	P max [W]	La max [mmol/l]	HR max [min^{-1}]	Total duration [s]	P IAT [W]	La IAT [mmol/l]	P max/ P IAT	La max/ La IAT
Mean	17	190	85.3	23.5	422.4	13.8	202	1260	300	2.9	1.41	4.76
SD	0.5	4	5.1	1.6	29.9	3.4	7	125	14	0.4	0.08	0.91
CV [%]	2.9	2.1	6	6.8	7.1	24.7	3.4	9.9	4.6	15.1	5.6	19.2
Min	17	182	77.3	21	374.9	9.1	193	1053	283	2.2	1.3	3.67
Max	18	197	91.1	26	468.7	20.4	217	1477	320	3.6	1.54	6.83

from the hyperemized ear lobe and analyzed for blood lactate (Biosen S-Line, EKF; Barsleben, Germany). Prior to and post (immediately post, 7 and 30 min post) exercise larger volume capillary blood samples (300 μl) were drawn from the ear lobe using a Microvette CB 300 (Sarstedt, Germany). All participants were asked to keep physical activity at a minimum until the last blood collection. Blood samples were allowed to clot for 15 min followed by centrifugation at 10,000 *g* for 10 min at room temperature. Serum was stored at −80 °C for subsequent analysis. Time points were chosen based on studies showing that cfDNA levels return to baseline between 30 min and 1 h post exercise [4,11].

The Human Ethics Committee of Ulm University approved the experimental protocol and each individual gave written informed consent to the study.

cfDNA concentration assay

The cfDNA concentrations were directly analyzed with a fluorescent nuclear stain (SYBR Gold) in serum samples without prior DNA extraction and amplification [13]. Briefly, 40 μl of SYBR Gold (1:10,000 dilution in PBS) was added to 10 μl of serum in 96-well plates. Fluorescence was recorded using a spectrofluorometer (SAFAS monaco flx Xenius, Monaco) with an excitation wavelength of 485 nm, and emission wavelength of 535 nm. A standard curve was generated by serial dilution of commercial salmon sperm DNA (Sigma-Aldrich).

This SybrGold assay was shown to exhibit a high correlation with the conventional quantitative polymerase chain reaction assay of beta-globin ($R^2 = 0.9987$, $P < .001$) [13].

As a control for specificity of fluorescence signals concurrent pretreatment of serum samples with recombinant DNase I resulted in complete extinction of detectable cfDNA.

DNase assay

DNase activity was measured using a commercial solid phase enzyme immunoassay (ELISA) ORG590 (ORGENTEC Diagnostica, Mainz, Germany) according to the manufacturer's protocol. Test principle in brief: serum DNase reacts with immobilized DNase substrate which results in a color change. The color intensity is measured photometrically on a microplate photometer (Thermo Scientific Multiscan FC, Fisher Scientific, Germany) at 450 nm with a correction wavelength of 620 nm.

Statistics

Measurements of cfDNA and DNase-AR were performed in duplicate. Data is expressed as means and SD. The coefficient of determination (R^2) for linearity of cfDNA standard curve was ≥0.97. GraphPad Prism 5 (La Jolla, CA, USA) was used for statistical analysis and graphical presentation. D'Agostino & Pearson omnibus K2 test was used to check for normal distribution, Friedman's test and Dunn's Multiple Comparison Test to assess matching among groups. Differences of $P < .05$ were considered statistically significant. For the DNase activity assay a 4-Parameter-Fit with lin–log coordinates for optical density and concentration was employed.

Results

All athletes performed the exercise trial until exhaustion and finished with mean lactate concentrations of 13.8 mmol/l and a mean power max 422.4 W (Table 1). Exercise intensity was high in all athletes, because maximal values of blood lactate and power at end of the experiment were 4.8-fold and 1.4-fold higher compared to values observed at the individual anaerobic thresholds [12]. Mean heart rate at test cessation was 202 beats/min with a mean exercise duration of 21 min. Instructions to keep physical activity at a minimum until the last blood collection were obeyed by all participants with one exception.

The kinetics of cfDNA is shown in Fig. 1A with a highly significant 2.5-fold increase from 117 ng/ml cfDNA prior to exercise to an average of 290 ng/ml at exhaustion. After termination of exercise, excessive cfDNA is gradually removed from the circulation, declining to 2.3-fold above resting levels after 7 min and reaching near baseline levels at 30 min post exercise. While all athletes showed the same basic kinetics in response to acute exercise, individual levels differed with regard to min/max values and fold changes (Fig. 1D). Noteworthy, 7 min after cessation of exercise, 5 athletes showed diminished and 5 athletes increased amounts of cfDNA compared to immediately post exercise. Another 23 min later 9 of 10 athletes had levels close to baseline with only one rower showing increased levels compared to post +7 min (Fig. 1D; dotted line). Apart from the possibility of a measurement inaccuracy this athlete's deviant kinetics might be caused by a noncompliant cool down behavior. Interestingly, the cfDNA-elevation was also accompanied by prolonged increase in DNase activity indicating the validity of data acquisition.

DNase activity reduction (AR) was inversely proportional to the kinetics of cfDNA as revealed in Fig. 1B. DNase AR was significantly reduced ($P < .001$) from baseline to post exercise 0 min (4.9-fold) and 7 min (3.9-fold) with a trend to return to baseline at 30 min (2.9-fold), albeit with some delay compared to the kinetics of cfDNA (Fig. 1C). The latter was reduced to one third of its peak value after 30 min, while DNase-AR was still about half of its maximum value after 30 min (Fig. 1C).

Discussion

Exercise of sufficient intensity has been consistently shown to induce rapid, transient increases in circulating cfDNA similar to concentrations observed after trauma or sepsis [3,4,10]. Our fluorescent assay for detection of cfDNA confirmed data acquired by PCR methods [13] and served previously as a prognostic tool of death and disease in colorectal cancer patients that was even superior to pathologic staging [14].

While the precise origin, triggers and regulation patterns of cfDNA in athletes exercising at high intensities are still a topic of debate and subject of ongoing research, we show for the first time that significantly elevated levels of cfDNA in healthy trained subjects are efficiently reduced by adaptations of endogenously expressed DNase activity to regain homeostasis.

Although the precise mechanism underlying the exercise induced DNase activity alterations remains to be investigated, our findings raise the question if exercise serves as a stimulus to evoke enhanced

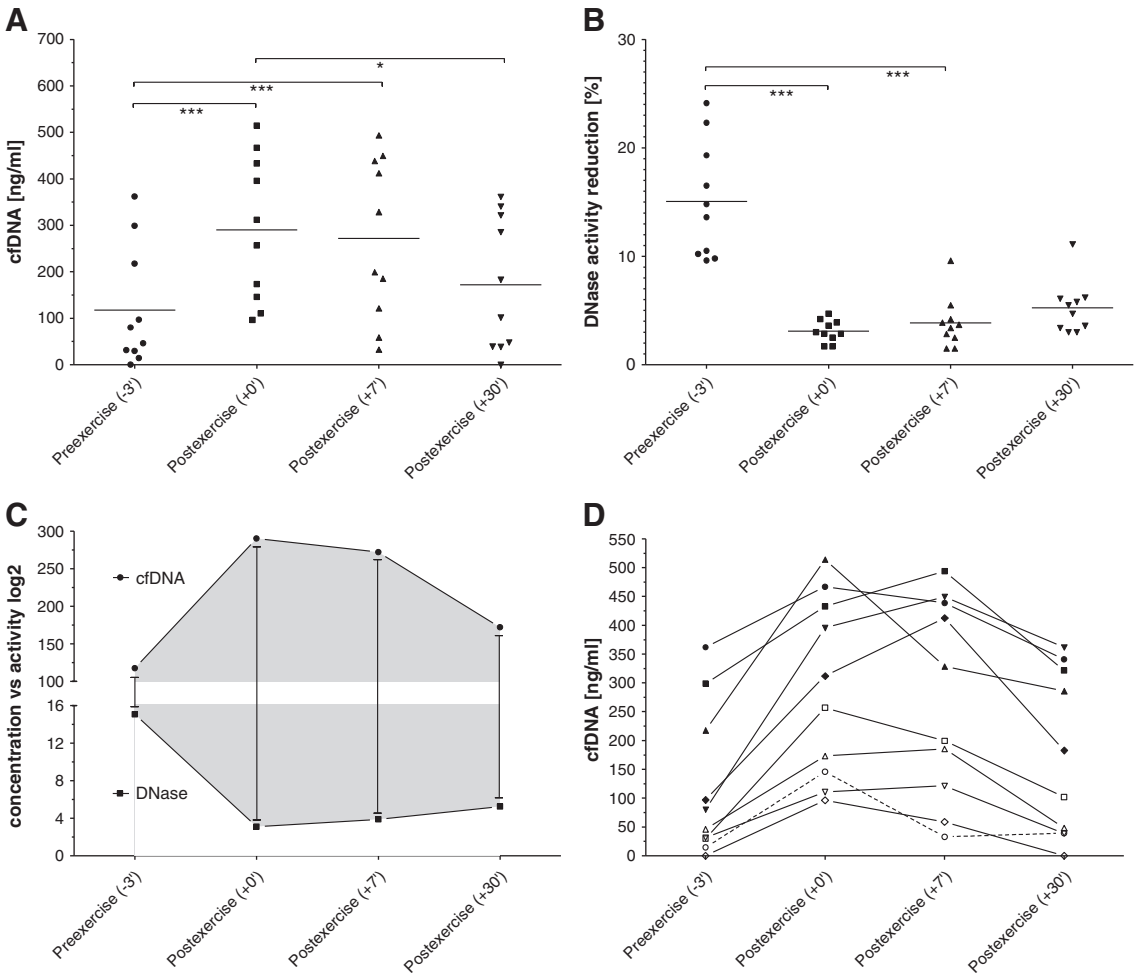

Fig. 1. Kinetic time course of cfDNA (A) and DNase activity reduction (B). (C) shows the relationship between cfDNA concentration and DNase-AR during the exercise test. Individual time courses of cfDNA are presented in (D) with one athlete showing differing kinetics at 30 min post exercise (dotted line). Significance levels are as follows, $^{*}P < .05$, $^{***}P < .0001$.

DNase activity also in patients taking rhDNase as a medication. The enzymatic processing of sticky cfDNA into shorter chains by DNase reduces the viscosity in sputum of CF patients [15] and clinical studies with CF patients have repeatedly proven that regular inhaling of rhDNase improves lung function and thereby ameliorates the disease progress. Considering that chronic inflammation in CF patients is not comparable to the acute and transient inflammation in healthy elite athletes in response to an acute bout of intense exercise, we performed a similar experiment to exhaustion with male and female recreational athletes to approximate our findings to the average population before premature transfer of results to patients. Kinetics of cfDNA and DNAse activity resembled those of trained athletes in this study (own unpublished results).

Despite differences between healthy and diseased individuals, temporal alterations and severity of etiopathology, it is common consent that the general health benefits of exercise activities also apply to CF patients. This comprises also high intensity exercise programs around the anaerobic thresholds given that patients' lung function is sufficient and medical guidance is provided [16].

While our study and data from another exercise study suggests that the exercise induced kinetics of cfDNA correlates with the production and release of lactate [4], further studies are required to test whether exercise protocols consisting of lower intensities and durations, which may not lead to a significant rise in systemic cfDNA concentrations, are also able to stimulate circulating DNase activity in healthy and diseased people.

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