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Higher endogenous nitrite levels are associated with superior exercise capacity in highly trained athletes

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ABSTRACT

Factors improving exercise capacity in highly trained individuals are of major interest. Recent studies suggest that the dietary intake of inorganic nitrate may enhance athletic performance. This has been related to the stepwise *in vivo* bioactivation of nitrate to nitrite and nitric oxide (NO) with the modulation of mitochondrial function. Here we show that higher baseline levels of nitrite are associated with a superior exercise capacity in highly trained athletes independent of endothelial function. Eleven male athletes were enrolled in this investigation and each participant reported twice to the testing facility (total of $n = 22$ observations). Venous blood was obtained to determine the levels of circulating plasma nitrite and nitrate. Endothelial function was assessed by measuring flow-mediated vasodilation (FMD). Hereafter, participants completed a stepwise bicycle exercise test until exhaustion. Blood was drawn from the ear lope to determine the levels of lactate. Lactate anaerobic thresholds (LAT) in relation to heart rate were calculated using non-linear regression models. Baseline plasma nitrite levels correlated with LATs ($r = 0.65$; $p = 0.001$, $n = 22$) and with endothelial function as assessed by FMD ($r = 0.71$; $p = 0.0002$). Correlation coefficients from both testing days did not differ. Multiple linear regressions showed that baseline plasma nitrite level but not endothelial function was an independent predictor of exercise capacity. No such correlations were determined for plasma nitrate levels.

Introduction

Exercise capacity is determined by a coordinated interplay between the cardiovascular, respiratory and nervous system, blood and muscles [1]. There are still controversies regarding possible factors that limit exercise performance and whether these factors can be measured and modulated. One central theory supports the notion that the overall ability of an individual to remain in an aerobic state during exercise reflects exercise capacity [2]. Increasing workloads require the muscles to produce adequately higher amounts of energy substrates, namely ATP. However, with more intense exercise, myocytes will not be able to maintain this process using aerobic respiration. Quite consequently, the exercising muscle will utilize anaerobic glycolysis, which produces ATP more rapidly at the cost of increased lactate generation. Once the bodily capacities of lactate elimination are exhausted, lactate is accumulated in the circulation. This in turn has a negative and limiting impact on muscle function and has been termed anaerobic threshold. Endurance exercise training aims to increase the capability of an athlete to maintain aerobic metabolism, and changes in lactate anaerobic threshold are one of the most important measures to evaluate the effectiveness of training routines [2]. However, mechanisms that increase the ability to exercise below anaerobic thresholds are incompletely understood.

Nitric oxide (NO) is a gaseous signaling molecule that contributes to the regulation of a wide variety of processes in the cardiovascular, nervous and immune system [3–5]. NO is enzymatically generated through three NO synthase isoforms. Particularly for the maintenance of cardiovascular function endothelial NO synthase (eNOS) activity is essential. Nitrite as the main oxidation product in human plasma sensitively reflects eNOS-dependent NO production under certain conditions [6]. We recently demonstrated that impaired eNOS function, either through its pharmacological inhibition [7] or in patients with endothelial dysfunction

Abbreviations: NO, nitric oxide; eNOS, endothelial NO synthase; FMD, flow-mediated dilation; LAT, lactate anaerobic threshold.

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[8,9], significantly impairs NO bioavailability, deteriorates vascular function and finally leads to a much-reduced exercise capacity.

Under physiological hypoxia, e.g. in the exercising muscle, generation of NO by NOS may be impaired due to a lack of oxygen as essential cofactor. Whilst previously regarded to be an inert product of NO metabolism, nitrite is now suggested to provide an alternative source of bioactive NO. Along the physiological oxygen gradient, nitrite may be reduced back to NO involving a variety of different mechanism [10–16]. Apart from NOS dependent nitrite generation, bodily nitrite levels also derive from nutritional sources. After the intake of inorganic nitrate, as highly concentrated in green leafy vegetables, oral cavity resident bacteria reduce inorganic nitrate to nitrite, which is continuously swallowed and absorbed [17,18]. Exogenous supplementation of nitrate has recently been demonstrated to improve endurance exercise capacity mainly by decreasing the amount of oxygen cost [19–26]. These studies demonstrated that intake of nitrate increased circulating nitrite levels and reduced the uptake of oxygen needed for delivering comparable amounts of work. This has been attributed to an optimization of mitochondrial respiration with increased rates of ATP formed in relation to otherwise constant oxygen consumption (P/O gradients) [19]. The reduction of dietary nitrate is therefore regarded to be an alternative pathway for NO generation that may modulate the ability to exercise in aerobic states. While dietary nitrate increased plasma nitrite levels approximately 2–4-fold leading to the documented benefits for exercise capacity, the role of endogenous nitrite levels on exercise capacity is not known. It therefore remains elusive whether baseline endogenous nitrite levels correlate with exercise capacity as measured by anaerobic thresholds, and whether this may provide advantages to those individuals with higher levels.

Although the mentioned studies suggest that nitrate treatment optimizes skeletal muscle metabolism and respiration, it has also been demonstrated that elevation of circulating nitrite affects other components relevant for exercise, e.g. endothelial function. Nitrite levels correlate with endothelial function, which can be measured by means of ultrasound-guided assessment of endothelium dependent maximum dilation using FMD technique [27,28]. Improved endothelial and thus vascular function and the ability to circulate oxygen to the exercising muscle may therefore be another contributor to enhanced athlete's performance with higher nitrite levels.

We here show that baseline plasma nitrite levels correlate with exercise capacity and vascular function in a selected collective of young, healthy male athletes. Using multivariate linear regression models we furthermore demonstrate that baseline nitrite predicts exercise capacity independent of an improvement of vascular function.

Materials and methods

Study subjects

11 male athletes were enrolled in this study applying the specific in- and exclusion criteria as listed in Fig. 1. All participants were either competitive cyclists or triathletes and thus familiar with the testing devices. Baseline participants' characteristics including training details are shown in Table 1. All subjects were asked to remain on their regular exercise routine and daily diet. Nitrate supplements were not allowed during the entire study. Subjects were asked to fasten 12 h before both testing days. All tests were conducted between 7.00 and 11.00 am. Upon arrival at the testing laboratory, subjects received a standardized breakfast on both testing days consisting of 500 ml mineral water and one cheese bun. The participants were hereafter asked to rest in a supine position before a blood draw from the cubital vein was conducted. 15 min later FMD was assessed on the contralateral arm using ultrasound technique [7,8,29,30]. This was followed by measuring blood pressure. Upon completion of this protocol step, subjects were challenged with a step-wise ergometric testing on a stationary bicycle ergometer [31]. In order to corroborate the results of day 1 and to obtain an independent correlation on two consecutive occasions, 7 days later each participant repeated the procedures providing a total of $n = 22$ observations. All participants gave written consent to the study procedures as well as to the data handling prior to the first testing. The responsible ethics committee at the Medical Faculty, Heinrich-Heine-University, Düsseldorf, Germany, had approved all procedures.

Table 1
Subjects characteristics and blood parameters.

	Means ± S.D.
Age (a)	24 ± 2
Height (cm)	185 + 5
Weight (kg)	79 ± 5
Body mass index	23.1 ± 1.2
Training units/week	5 ± 1
Watt maximum (W/kg)	4.2 ± 0.6
Chemistry Panel	
Sodium (mmol/l)	139 ± 3
Calcium (mmol/l)	2.3 ± 0.1
Chloride (mmol/l)	103 ± 1
Creatinine (mg/dl)	1.0 ± 0.1
Uric acid mg/dl)	5.5 ± 0.6
Total cholesterol (mg)	174 ± 29
Triglycerides (mg/dl)	117 ± 79
Total protein (g/dl)	7.1 ± 0.2
C-reactive protein (mg/dl)	<0.03
HbA1c (%)	5.3 ± 0.2
Blood Count	
Leucocytes ($\times 10^4/\mu l$)	5.1 ± 1.8
Red blood cells ($x10^6/\mu l$)	5.2 ± 0.3
Hemoglobin (g/dl)	15.4 ± 0.9
Hematocrit (%)	45 ± 3
Platelets (/μl)	217 ± 44

Blood parameters

Blood samples were taken from the left anticubital vein for the determination of clinical parameters and for the assessment of the circulating NO pool (nitrite and nitrate).

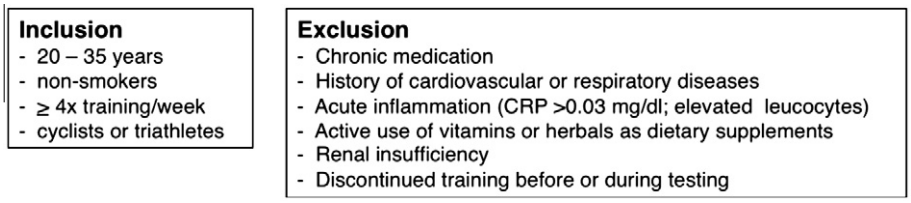

Fig. 1. In- and exclusion criteria. Subjects ($n = 11$) were selected based on their training status and whether they were accustomed to the experimental set up including stationary bicycle ergometers. Accordingly, only cyclists and triathletes were included if all further criteria were met.