Research Paper

Effect of chronic nitrate and citrulline supplementation on vascular function and exercise performance in older individuals

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ABSTRACT

Increased nitric oxide (NO) bioavailability may improve exercise performance and vascular function. It remains unclear whether older adults who experience a decreased NO bioavailability may benefit from chronic NO precursor supplementation. This randomised, double-blind, trial aims to assess the effect of chronic NO precursor intake on vascular function and exercise performance in older adults (60-70 years old). Twenty-four healthy older adults (12 females) performed vascular function assessment and both local (knee extensions) and whole-body (incremental cycling) exercise tests to exhaustion before and after one month of daily intake of a placebo (PLA) or a nitrate-rich salad and citrulline (N+C, 520mg nitrate and 6g citrulline) drink. Arterial blood pressure (BP) and stiffness, post-ischemic, hypercapnic and hypoxic vascular responses were evaluated. Prefrontal cortex and quadriceps oxygenation was monitored by near-infrared spectroscopy. N+C supplementation reduced mean BP (-3.3mmHg; p=0.047) without altering other parameters of vascular function and oxygenation kinetics. N+C supplementation reduced heart rate and oxygen consumption during submaximal cycling and increased maximal power output by 5.2% (p<0.05), but had no effect on knee extension exercise performance. These results suggest that chronic NO precursor supplementation in healthy older individuals can reduce resting BP and increase cycling performance by improving cardiorespiratory responses.

INTRODUCTION

Nitric oxide (NO) is a gaseous signalling molecule involved in a variety of physiological functions throughout the body [1]. The first pathway for NO production is endogenous via the citrulline-arginine-NO pathway requiring the activity of the nitric oxide synthase (NOS) enzymes. The second pathway is partially exogenous since it uses nitrate and nitrite brought by water and food to produce NO based on the simple one-electron reduction of nitrite. Systemic NO bioavailability can be enhanced by NO precursor supplementation such as arginine [2] and nitrate [3]. Interestingly, it has been shown that oral citrulline supplementation increases the circulating [4,5] and tissue [6] arginine concentration more efficiently than an equivalent dose of arginine, suggesting that exogenous citrulline administration might represent an interesting option to increase the amount of arginine to be converted by NOS in NO.

In the peripheral vessels, NO regulates vascular tone by activating soluble guanylate cyclase in the vascular smooth muscle. During physical activity, NO bioavailability is important to match blood flow to oxygen demand in the brain and contracting muscles. During intermittent handgrip exercise for instance, NOS inhibition via NG-monomethyl-Arginine reduces muscle

blood flow [7] and total vasodilator responses to muscle contraction [8]. NO is also an important neurotransmitter and neuromodulator (chemical messenger, [9]). It is involved in cerebral blood flow autoregulation [10] and neurovascular coupling [11,12].

A reduction in NO bioavailability has been singled out as the main cause of endothelial dysfunction [13]. The latter is recognized as an important predictive factor for several cardiovascular disorders and has been implicated in the pathogenesis of hypertension, atherosclerosis, arterial thrombosis [14–16]. Advanced age is associated with endothelium dysfunction due to impairments in NO signalling pathways. Several possible mechanisms may underlie this impairment in NO metabolism, including limited substrate (arginine, [17]) and cofactor bioavailability (e.g. tetrahydrobiopterin, [18]) and reduced abundance or activity of NOS. In addition to vascular function, cardiorespiratory exercise responses are also considered as a reliable predictive factor for cardiovascular diseases [19,20]. Hence, some recent studies investigated the potential benefits of NO precursor supplementation on vascular function and exercise performance in the older population. Contrasting effects of chronic NO precursor (i.e. arginine, citrulline, nitrate) intake on exercise performances have been reported in older adults [for a review, see [21,22]]. While some studies found a positive effect of nitrate intake on exercise time to exhaustion [23,24] and oxygen consumption (VO_2) response time [25], other authors showed no significant effect on exercise performance [26]. Some studies have also shown positive vascular effects in older adults following acute and chronic nitrate intake, including reduced blood pressure (BP) [27,28], improved regional brain perfusion [29] and improvements in several parameters of vascular function [30]. However, Miller et al. [31] showed no effect of nitrate supplementation on blood pressure (BP) despite increased plasma nitrate and nitrite. Regarding citrulline intake, while chronic supple-mentation has been shown to reduce BP [32], acute ingestion showed no effect on vascular function in older adults with heart failure [33]. These contrasting results may be due to different types of supplementation (i.e. NOS-independent or NOS-dependent supplementation), dosage or duration of supplementation, and health status of participants, making the potential interest and optimal strategy for NO precursor supplementation in older individuals still unclear.

Thus, this study aims to assess the effect of chronic NO precursor supplementation on vascular function, muscle and cerebral oxygenation and performance during both local and whole-body exercise in healthy older adults. To enhance NO bioavailability, nitrate and citrulline supplementation (N+C) were used in order to supplement both NOS-independent and NOS-dependent pathways, since ageing may impair NO bioavailability due to both an impairment in NOS activity and a lack of NOS substrate. We hypothesized that chronic NO precursor intake would improve vascular function and cerebral and muscular responses to exercise, leading to increased exercise performances.

RESULTS

Vascular function

Resting vascular function parameters are provided in Table 1. There were no significant difference between groups for baseline systolic (SBP), diastolic (DBP), and mean (MBP) blood pressure (all $p > 0.05$). After one month of supplementation, systolic (SBP) and diastolic (DBP) blood pressure did not change significantly although the PRE-POST difference in SBP tended to be larger in the N+C group compared to placebo (PLA) (N+C versus PLA t-test p value = 0.058, Cohen's d = 0.660). As shown in Figure 1, the N+C group showed a significantly greater reduction in MBP compared to PLA ($p = 0.047$, $d = 0.71$).

PRE-POST changes in PWV did not differ significantly between groups (all $p > 0.05$). Similarly, there was no significant difference between groups for post-ischemia Δmax/min HbO_2 and Δmax/min HbTot (all $p > 0.05$).

Hypercapnic and hypoxic responses

As shown in Table 2, there was no significant difference between groups at baseline and no effect of NO precursor on hypercapnic responses at rest (all $p > 0.05$). As shown in Table 3, there was also no significant difference between groups at baseline and no effect of NO precursor supplementation on hypoxic responses, neither at rest nor during submaximal cycling exercise (all $p > 0.05$).

Knee extension exercise performance

There was no significant difference between groups for TSI (Table 4) and all other NIRS parameters (results not shown; all $p > 0.05$) during knee extensions. There was also no significant difference between groups regarding PRE-POST changes in MVC and total number of contractions during the knee extension exercise test ($p > 0.05$, Table 5).

Incremental cycling exercise test

There was no significant difference between groups for baseline maximal power output and VO_2 (all $p > 0.05$). The increase in maximal power output between PRE and POST was significantly larger in the N+C group

compared to PLA ($p < 0.05$, Table 5 and Figure 3). Figure 2 shows heart rate and VO_2 kinetics during the cycling exercise. There was a significant ANOVA main group effect on PRE-POST changes for heart rate and VO_2 during cycling (at 25%, 50%, 75%, and 100% of the first test duration, i.e. at isowatt). The reduction in heart rate and VO_2 was significantly larger in the N+C g group compared to PLA. However, there was no effect on maximal heart rate and maximal VO_2 (all $p > 0.05$; Figure 2, Table 5) nor on submaximal and maximal minute ventilation (results not shown; all $p > 0.05$). There was no significant difference between groups for TSI (Table 4) and all other NIRS parameters (results not shown; all $p > 0.05$) during cycling.

Table 1. Vascular function before and after one month of NO precursor supplementation.

		PRE	POST	ΔPRE/POST	pΔ	dΔ
SBP (mmHg)	**N+C**	123.2 ± 13.9	115.7 ± 12.3	-7.5 ± 6.5	0.058	0.660
	PLA	117.8 ± 7.2	114.3 ± 8.9	-3.4 ± 5.7		
DBP (mmHg)	**N+C**	78.2 ± 6.5	71.9 ± 5.8	-6.2 ± 5.1	0.130	0.460
	PLA	76.0 ± 9.8	72.1 ± 6.9	-3.9 ± 4.6		
PWV ($m \cdot s^{-1}$)	**N+C**	9.2 ± 5.9	7.0 ± 2.8	-2.2 ± 5.3	0.220	0.550
	PLA	6.7 ± 2.7	6.7 ± 3.1	0.0 ± 2.1		
Reperfusion (mmol of HbO_2)	**N+C**	15.9 ± 11.2	14.4 ± 10.3	-1.5 ± 3.1	0.250	0.200
	PLA	12.7 ± 6.7	12.6 ± 5.3	-0.7 ± 4.9		
Reperfusion (mmol of Hbtot)	**N+C**	10.8 ± 4.2	10.5 ± 6.3	-0.2 ± 2.9	0.710	0.130
	PLA	9.2 ± 4.9	8.4 ± 2.3	-0.7 ± 4.3		

Data are presented as mean ± SD, n = 24. SBP, systolic blood pressure; DBP, diastolic blood pressure; PWV, carotid-femoral pulse wave velocity; Reperfusion, difference between the value reached at the end of the ischemic phase and the maximal value reached during the reperfusion phase in the ischemia-reperfusion test; HbO_2, oxyhaemoglobin; HbTot, total haemoglobin. PRE, measure before the supplementation period; POST, measure after the supplementation period. N+C, nitrate + citrulline, PLA, placebo; ΔPRE/POST, difference between PRE and POST measures; pΔ, p value for ΔPRE/POST group comparison; dΔ, Cohen's d effect size of N+C supplementation on ΔPRE/POST.

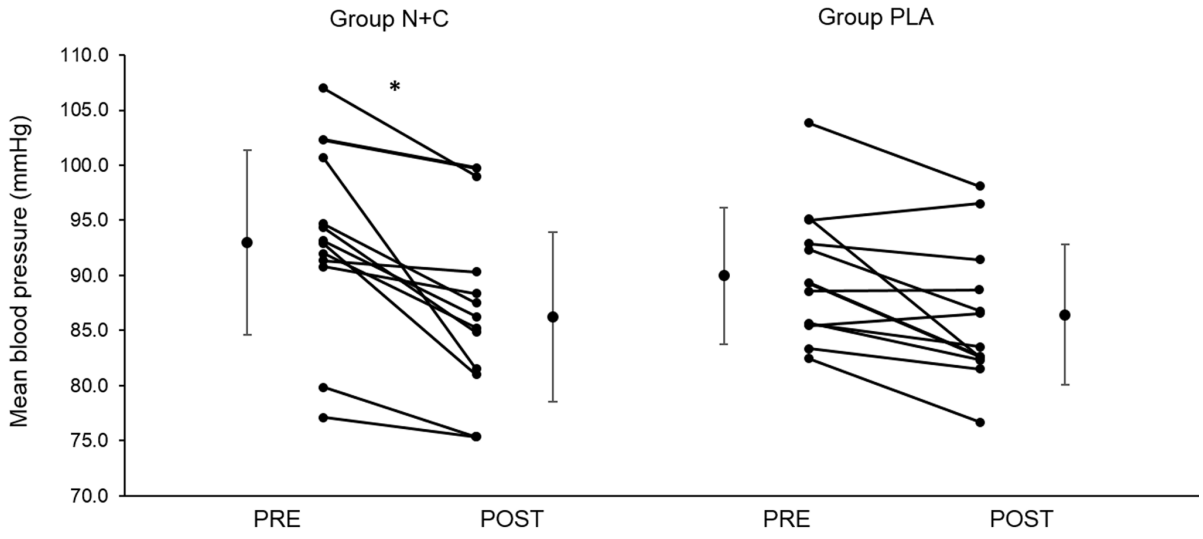

Figure 1. Individual and group mean changes in mean arterial blood pressure before and after one month of placebo or nitrate and citrulline intake in older adults. N+C, nitrate + citrulline; PLA, placebo; PRE, measure before the supplementation period; POST, measure after the supplementation period; * significant difference between PRE and POST; n=24.