Nitrate Supplementation, Exercise, and Kidney Function: Are There Detrimental Effects?

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ABSTRACT

CARPENTIER, A., S. STRAGIER, C. BRÉJEON, and J. R. POORTMANS. Nitrate Supplementation, Exercise, and Kidney Function: Are There Detrimental Effects? *Med. Sci. Sports Exerc.*, Vol. 47, No. 7, pp. 1519–1522, 2015. **Purpose**: Recently, dietary supplementation with inorganic nitrate (NO_3^-) has been proposed to endurance athletes to increase their performance. However, it has been suggested that an excess of NO_3^- might be harmful. The present study analyzed the effect of NO_3^- supplementation on kidney function. **Methods**: Thirteen young male subjects performed a 20-min cycling exercise at 85% of the maximal oxygen capacity. Seven days before exercise, the subjects ingested either a placebo (Pl) or 450 mg of potassium nitrate (PN) per day. Venous blood samples and urine collections were collected before and immediately after exercise and after 60 min of recovery. Glomerular filtration rates (GFR) and clearances (Cl) were calculated from serum content and urine output for creatinine (Crn), albumin (Alb), and urea. **Results**: Under resting conditions, GFR and all clearance measures did not differ between Pl and PN. Immediately after exercise, GFR remained stable in both Pl and PN, whereas Cl-urea decreased significantly ($P < 0.05$) in Pl (−44%) and PN (−49%). Alb urine outputs were enhanced by 18- to 20-fold in Pl and PN, respectively ($P < 0.05$). After the recovery period, GFR remained enhanced under Pl conditions, whereas Cl-urea returned to initial values in placebo and nitrate supplementation. Alb output and Cl-Alb remained enhanced under PN conditions. **Conclusion**: These results mainly indicate that dietary nitrate supplementation over a week does not induce any specific kidney function modifications either at rest or during sustained submaximal exercise as compared with Pl. **Key Words:** NITRATE SUPPLEMENTATION, GLOMERULAR MEMBRANE PERMEABILITY, GLOMERULAR FILTRATION RATE, TUBULAR REABSORPTION

Dietary nitrate, which determines reactive nitrogen oxide species in the stomach, has emerged as an effective host defense against gastrointestinal pathogens that could have an important therapeutic role to play in humans (15). Quite recently in physiology, nitric oxide (NO) has received much interest as an ergogenic aid based on the evidence that NO is an important modulator of blood flow and mitochondrial respiration during exercise performance (4). In humans, L-arginine is taken up by most cells and oxidized in the presence of molecular oxygen and nicotinamide adenine dinucleotide phosphate to NO (18). NO synthases synthesize NO from L-arginine (10). It has been shown in several studies that L-arginine supplements might lead, under certain conditions, to an improvement on exercise performance (see Ref. [2,4]). In addition, nitrate and nitrite have been generally considered to be end products of NO metabolism that comes from endogenous as well as dietary sources (13,14). Regular exercise increases the NO synthase expression and activity (8), which results in higher circulating levels of nitrate. The cardioprotective effects of NO and β-adrenergic receptors have also been suggested in patient population (5). Eventually, beetroot juice, as a source of nitrate, enhances the cardiovascular and exercise performance in young adults (27).

However, several publications suggest caution as opposed to potential benefit induced by food sources of nitrates and nitrites (10,13,14). Indeed, in human subjects, the major supply of nitrate and nitrite in our bodies comes from our everyday diet (12). The acceptable daily intake for humans averages 3.7 $mg \cdot kg^{-1}$ body weight for nitrate and 0.07 $mg \cdot kg^{-1}$ body weight for nitrite (3,16). The European Food Safety Authority stressed consumers that nitrate is an important component of vegetables because of its potential for accumulation. Nitrate *per se* is relatively nontoxic, but its metabolites and reaction products, e.g., nitrite, NO, and *N*-nitroso compounds, have raised concern because of implication for adverse health effects such as carcinogenesis when given at very high doses. This is the reason researchers exercise caution while using supplementation with nitrate and nitrite salts (6,11,12).

Moreover, a few publications pointed out that nitrate and nitric excess exposure (3,5) is associated with increased risk of renal cell carcinoma (26) and acute kidney injury (17). More data are required to clarify the effect of nitrate supplementation

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on exercise performance and to elucidate the optimal way to implement ideal effective supplementation (9). Thus, we decided to investigate in healthy young subjects the effects of nitrate ingestion upon renal function evaluated under sustained submaximal exercise protocol.

MATERIALS AND METHODS

Study population. Thirteen white young male students (age, 21.9 ± 0.7 yr; weight, 76.2 ± 3.3 kg; height, 1.80 ± 0.02 m; body mass index, 23.3 ± 0.8 kg·m^{-2} (mean ± SE)) were enrolled in this study. All subjects had no signs of abnormal kidney function. The informed written consent of the subjects was recorded after full explanation of the whole investigation. This study was approved by the medical ethical committee of the academic hospital, and the conduct of this project was in accordance with the guidelines of the Declaration of Helsinki of 1975 as revised in 1976.

At one occasion, each subject drank either a placebo solution or a nitrate supplement (1 g·L^{-1}) three times per day (breakfast, midday, evening meal). Each solution (150 mL) contained 80 g·L^{-1} of sucrose, green lemon (1 mL), and potassium nitrate (150 mg for the nitrate experiment only). Placebo and nitrate supplementation doses, randomly distributed, were double blinded for the subjects and the research team.

The subjects were asked to maintain placebo or nitrate supplements during 6 d. A 7-d period was implemented between the placebo and nitrate supplements to ensure no carryover effect (27). Subjects were also asked to maintain their usual food intake and to refrain from consumption of high-level nitrate food (beetroot, rocket, celery, lettuce, cabbage, leek, etc.). Fifty percent of the subjects started the experimental protocol on submaximal exercise with nitrate supplementation, whereas others were under placebo condition.

Exercise protocol. Before the analysis of renal function, the subjects were first tested on a cycle ergometer for the determination of their maximal oxygen consumption ($\dot{V}O_{2peak}$) by stepwise increase of power (25 W; 60 rpm) every 1 min, starting at 25 W, until stable oxygen consumption with the usual criteria (RQ, ventilation, HR) was achieved. Pulse rate was recorded throughout the exercise protocol (Table 1). The exercise protocol was applied in the morning, in the placebo and nitrate experiment, 2 h after the subjects ingested the appropriate supplement, according to the pharmacokinetic protocol elaborated by Wylie et al. (27). One or two days after, the subjects came back to the laboratory in the morning after having voided their bladder about 2 h before the test (Fig. 1).

They noted precisely the time of voiding. Before starting the exercise protocol, the subjects emptied their bladder and

TABLE 1. Physiological characteristics at rest and during exercise (mean ± SEM).

	Maximal Exercise	85% $\dot{V}O_{2max}$
Charge (W)	282 ± 4	250 ± 3
$\dot{V}O_2$ (mL·min^{-1}·kg^{-1})	46.8 ± 1.1	39.9 ± 0.9
HR (bpm)	184 ± 4.5	178 ± 3.0
Ventilation (L·min^{-1})	134.0 ± 5.0	98.0 ± 2.8
Respiratory quotient	1.2 ± 0.1	1.1 ± 0.1

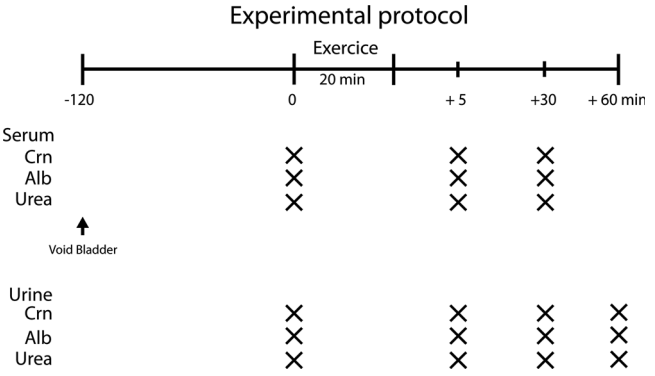

FIGURE 1—Experimental protocol for rest, exercise and recovery. Crn, creatinine; alb, albumin.

total urine volume was measured. A venous blood sample was taken from the forearm, and the subjects drank 250 mL of plain water. According to previous experiments and publications, we applied a constant 85% of the maximal intensity during a medium time of 20 min to induce an optimal postexercise proteinuria and reduction of glomerular filtration rate (GFR) (19–22,24). The exercise workload on cycle ergometer was adjusted to reach 85% of the $\dot{V}O_{2peak}$ within 5 min. The required power was regulated during another 15 min, and during this period, a steady-state HR was obtained. At the end of the 20-min run, the load was reduced to 25 W during 3 min and a second blood sample was collected. Immediately after the exercise, the subjects were given 250 mL of plain water while maintaining a seated posture at rest. Thirty minutes after stopping the exercise, a third blood sample was collected and the bladder was completely voided. A third urine collection was obtained 60 min after stopping the exercise. The blood and urine samples collected before exercise were used for rest analysis. The blood and urine samples collected immediately and 30 min after exercise, respectively, were used for postexercise analysis. Finally, the blood and urine samples collected 30 and 60 min after exercise, respectively, were used for recovery analysis (Fig. 1).

Biochemical analyses. Blood investigations have been undertaken on serum samples. Urea measurements were performed on the Roche Modular Analytics P automated clinical chemistry analyzer from Roche Diagnostics (COBAS) using commercially available methods from the same company (Roche Diagnostics, Mannheim, Germany). This analyzer also measured creatinine using a rate-blanked creatinine/Jaffé compensated kinetic method and serum albumin with a bromocresol green dye binging method. The interassay coefficients of variation for the measurements of all components were under 3%.

Renal clearance determinations. The renal clearances were determined using the usual definition of kidney clearance, namely, urine concentration/serum concentration ratio multiplied by the urine output, for the three appropriate serum and urine samples (albumin, urea, and creatinine).

Albumin clearance (Cl-Alb) is used as an indirect evaluation of glomerular membrane permeability (19), whereas creatinine clearance (Cl-Crn) and urea clearance (Cl-urea) are utilized as indirect calculations of GFR and kidney tubular uptake.

TABLE 2. Data at rest and after exercise on serum and urine samples (mean ± SEM).

Values	Rest Placebo	Rest Nitrate	Exercise Placebo	Exercise Nitrate	Recovery Placebo	Recovery Nitrate
Serum						
Albumin ($g \cdot L^{-1}$)	45.5 ± 1.2	45.5 ± 1.6	45.7 ± 1.2	49.0 ± 1.0	46.5 ± 0.9	45.0 ± 1.0
Creatinine ($g \cdot L^{-1}$)	9.2 ± 0.5	9.4 ± 0.5	10.5 ± 0.5*	11.1 ± 0.5*	10.2 ± 0.4*	10.3 ± 0.4*
Urea ($mg \cdot L^{-1}$)	284 ± 17	259 ± 15***	284 ± 18	282 ± 16*	290 ± 17	275 ± 15*
Urine						
Albumin ($\mu g \cdot min^{-1}$)	6.7 ± 1.5	6.0 ± 1.4	122.6 ± 36.8*	117.8 ± 45.4*	56.0 ± 18.6	118.8 ± 37.4*
Creatinine ($mg \cdot min^{-1}$)	1.2 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.5 ± 0.2**	1.5 ± 0.2*,**
Urea ($mg \cdot min^{-1}$)	14.9 ± 2.6	13.6 ± 1.8	8.6 ± 1.2	7.9 ± 1.1	11.5 ± 1.7	14.1 ± 3.1

*$P < 0.05$ between exercise—recovery versus rest.
**$P < 0.05$ between exercise and recovery.
***$P < 0.05$ between placebo and nitrate.

Statistical analyses. Conventional statistical methods were used to calculate means, SD, and SE of the mean. Before comparing each dependent variable, the normality of the data was confirmed with the Kolmogorov–Smirnov test. The serum values of Crn, urea, and albumin before (rest) and after exercise (postexercise) and after the recovery period (recovery) were analyzed using a two-way ANOVA repeated measures test and by a Tukey *post hoc* test to estimate significant differences between the means at rest and after specific events (1,7). Changes in GFR determined by Cl-Crn, glomerular membrane permeability (Cl-Alb), and kidney tubular uptake (Cl-urea) between rest, postexercise, and recovery periods were also tested. A probability of $P \leq 0.05$ was considered as significant for all conditions.

RESULTS

By submaximal exercise (85% of $\dot{V}O_{2max}$), the power output attained 178 ± 15 W with an HR reaching 174 ± 1 bpm under placebo condition and 179 ± 15 W with an HR of 176 ± 1 bpm under nitrate supplementation. The serum and urine values of albumin, Crn, and urea obtained before and after exercise are included in Table 2. The immediate postexercise serum values increased to 14% and 18% for Crn as compared with rest ($P < 0.05$) under placebo and nitrate conditions, respectively. However, serum values of urea are increased after exercise under nitrate condition.

At rest, the mean values of albumin, Crn, and urea output were within a normal population under both conditions. The postexercise output of albumin revealed a very high value (18- to 20-fold as compared with the resting level) under both ingestion (placebo and nitrate). Nevertheless, urine output of urea and Crn remained stable under exercise condition. The 1-h postexercise values of urine output remain stable under both Pl and PN conditions, whereas albumin excretion rate was still higher (19-fold) as compared with resting values ($P < 0.05$) under nitrate condition. Sixty minutes after stopping the exercise, the creatinine excretion rate remained higher (+50%, $P < 0.05$) under nitrate ingestion as compared with resting condition.

The clearance values emphasized the effect of exercise on kidney functions. Postexercise Cl-Alb revealed a higher glomerular membrane permeability (17-fold, $P < 0.05$) (Fig. 2) under the two conditions, whereas Cl-urea showed a major reduction of tubular reabsorption after stopping the exercise (44%–49%, $P < 0.05$) under both ingestion. GFR, evidenced by Cl-Crn, remained stable under placebo and nitrate conditions. Sixty minutes after stopping the exercise, clearance values of Crn were reaching the normal resting level, whereas Cl-Alb still showed a statistical higher value (20-fold, $P < 0.05$) under nitrate ingestion as compared with preexercise condition. Tubular reabsorption rate during recovery phase was reaching the resting level faster under nitrate exposure as compared with placebo condition.

DISCUSSION

The present study demonstrates, for the first time, the effect of regular nitrate supplementation upon kidney functions, both under resting and submaximal endurance exercise conditions. There are some concerns raised by some scientists advising athletes to refrain from nitrate and nitrite salts dietary supplements

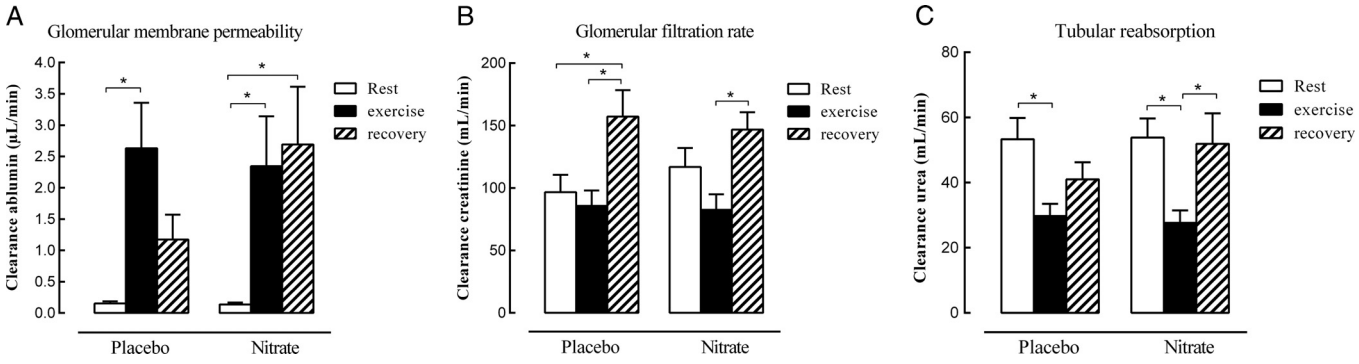

FIGURE 2—Glomerular membrane permeability (a), GFR (b), and tubular reabsorption rate (c). Clearances were measured at rest, by the end of exercise, and in the recovery period. *Significant differences with initial resting or exercise values ($P < 0.05$).

(6,11,12). Indeed, nitrite-formed dietary nitrate might react with dietary amines to form carcinogenic nitrosamines (11,12) and the risk–benefit of excess nitrate uptake needs to be evaluated in healthy population (25).

Our results collected on healthy young subjects revealed that normal kidney function responses to strenuous sustained exercise were obtained with placebo and nitrate supplementation (19,21,23). There is a major reduction of renal clearance of creatinine (GFR) and urea (tubular reabsorption rate) due to a reduction of blood flow through the kidney induced by the steady increase of exercise intensity. Moreover, when the exercise intensity is over 50%–70% of maximal oxygen uptake, the glomerular membrane permeability shows an increase of albumin urine output and clearance (22–24). Comparing the placebo and nitrate trials under resting condition did not reveal any physiological difference when comparing serum and urine values (clearances included). Moreover, as observed in other publications, the submaximal intensity exercise induced a reduction of urea clearances and a major increase of albumin clearance and excretion (15,19,25). However, during the recovery period (+60 min), nitrate supplementation induced a slower recovery as compared with the still reduced state observed under placebo condition. Thus, we could suggest that nitrate salts do not have a higher detrimental consequence on glomerular membrane permeability, nor on the GFR and tubular reabsorption rate. However, in the recovery period, glomerular membrane permeability (Cl-Alb) remained higher as compared with placebo condition.

In summary, our results suggested that nitrate supplementation for 1 wk does not have a specific negative impact on kidney physiology under resting or strenuous exercise conditions. Nevertheless, as stressed by Lundberg et al. (12), athletes need to refrain from uncontrolled use of nitrate salts as dietary supplements. One should also take into consideration the daily intake of nitrate by vegetables; usually 400 $g \cdot d^{-1}$ per person (3) remains under a safety nutritional condition.

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