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# Vegetable-derived bioactive nitrate and cardiovascular health

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ABSTRACT

Vegetable derived nitrate is now recognised as an important bioactive phytochemical with cardioprotective properties. Nitrate, through the recently described enterosalivary nitrate-nitrite-nitric oxide (NO) pathway, increases NO, a molecule pivotal for cardiovascular health. Clinical trials have observed that dietary nitrate has similar effects to NO when supplied exogenously. These effects include reduced blood pressure and improvements in other markers of vascular health such as endothelial function, arterial stiffness, ischemia reperfusion injury, blood flow, and platelet aggregation. Few observational studies, however, have examined dietary nitrate intake and long term cardiovascular health outcomes. This represents a significant gap in the literature. There is also a lingering concern about a possible carcinogenic effect of nitrate intake. Additionally, a number of potential factors that could impact nitrate to nitrite to NO reduction have been identified. This review will provide an overview of the evidence to date that nitrate, through its effects on endogenous NO and vascular health, is an important bioactive cardioprotective component of a diet rich in vegetables.



## 1. Introduction

Cardiovascular disease (CVD) is responsible for 17.5 million deaths per year despite major advances in the understanding of its pathophysiology and associated risk factors (Tzoulaki et al., 2016). Over 70% of deaths from CVD are attributable to modifiable risk factors. These include high blood pressure, high serum cholesterol, high body mass index, smoking, alcohol intake, physical inactivity and low intake of fruit and vegetables (Tzoulaki et al., 2016). A research priority, therefore, is to identify strategies to modulate these risk factors to prevent cardiovascular disease morbidity and mortality. One such strategy is to establish optimal diets and identify their cardioprotective components. This approach is supported by numerous studies which show that particular dietary patterns, such as a diet rich in fruit and vegetables, are associated with a reduced risk of cardiovascular disease (Appel et al., 1997; Joshipura et al., 2001). A fruit and vegetable rich diet may contain a number of bioactive components. A recently identified bioactive component is nitrate, found predominantly in green leafy vegetables and beetroot (Blekkenhorst et al., 2017b). Vegetable derived nitrate is now known to be a major exogenous source of nitric oxide (NO) and other bioactive nitrogen oxides. These molecules play a key role in cardiovascular function (Jin and Loscalzo, 2010). Interventions that protect the production and biological activity of NO and other nitrogen oxides may play an important role in maintaining a healthy vasculature into old age. It is the aim of this review to provide an overview of vegetable derived nitrate as a source of NO and other nitrogen oxides; to detail the observed vascular effects of dietary nitrate intake; to discuss the number of potential factors that could impact nitrate to nitrite to NO reduction as well as to discuss the controversy around the possible carcinogenic effects of nitrate.

## 2. Dietary nitrate as a source of NO

### 2.1. The nitrate-nitrite-NO pathway

The L-arginine-NO synthase pathway was originally assumed to be the sole source of NO (described in Fig. 1). Through this pathway

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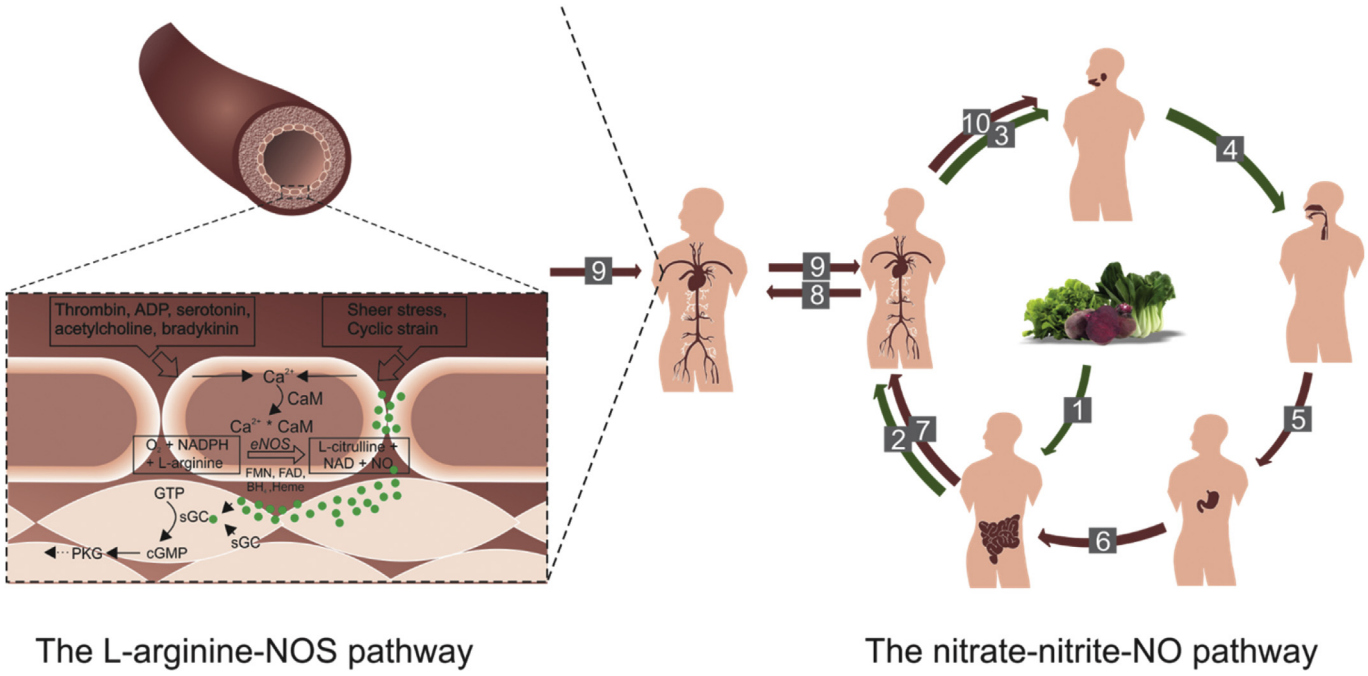


**Fig. 1. The L-arginine-nitric oxide synthase (NOS) pathway**: Endothelial NOS synthesis is increased in the endothelial cells lining the lumen of blood vessels in response to biochemical and mechanical stimuli. NO, once synthesised, diffuses rapidly across the endothelial cell membrane where it activates soluble guanyl cyclase (sGC) causing a rise in cyclic guanosine monophosphate (cGMP) concentrations mediating smooth muscle relaxation. ADP: adenosine diphosphate, Ca: calcium, CaM: calmodulin, $O_2$: oxygen, NADPH: nicotinamide adenine dinucleotide phosphate, NAD: nicotinamide adenine dinucleotide, FMN: flavin mononucleotide, FAD: flavin adenine dinucleotide, $BH_4$: tetrahydrobiopterin, GTP: guanosine triphosphate, PKG: protein kinase G. **The nitrate-nitrite-NO pathway**: Nitrate, 80% of which derives from vegetables, is absorbed in the small intestine (1) from where it enters the circulation (2). There is an active uptake of approximately 25% of plasma nitrate by the salivary glands (3). Nitrate is concentrated in the saliva and reduced to nitrite by oral bacteria found on the dorsal surface of the tongue (4). Once swallowed, some nitrite is converted to NO in the acidic environment of the stomach (5). The remaining nitrite is absorbed from the small intestine (6) into the circulation (7). In the circulation nitrite can be reduced to NO (8). Nitrate and nitrite are generated as end-products of NO metabolism (9) and are recycled through the nitrate-nitrite-NO pathway (10).

NO is continuously generated at low levels in the endothelium by the enzymatic action of endothelial nitric oxide synthase (eNOS) (Vallance and Chan, 2001). The discovery of the enterosalivary nitrate-nitrite-NO pathway (described in Fig. 1) through which nitrate and nitrite, oxidative end-products of endogenous NO metabolism, are recycled back into NO and other bioactive nitrogen oxides raised the possibility that dietary nitrate could also be an important source of NO (Lundberg and Govoni, 2004). It has now been established that dietary nitrate metabolism through this pathway is a nitric oxide synthase (NOS) - and oxygen - independent source of NO (Govoni et al., 2008; Petersson et al., 2009; Webb et al., 2008). The significance of this discovery pertains to the critical role NO has in a large number of physiological processes. Endothelium-derived NO controls vascular tone, influencing blood flow and blood pressure (Vallance and Chan, 2001). A reduction in the bioavailability and/or bioactivity of NO results in dysfunction to the vasculature which affects cardiovascular health. Indeed, increased arterial stiffness, higher blood pressure and attenuated blood flow are observed after inhibition of NO synthesis (Cayatte et al., 1994). NO also maintains vascular integrity by suppressing platelet aggregation, leukocyte migration, cellular adhesion to the endothelium, as well as vascular smooth muscle cell proliferation (Jin and Loscalzo, 2010).

### 2.2. Vegetable sources of dietary nitrate

Vegetable consumption accounts for approximately 80% of dietary nitrate intake. Some vegetables are naturally rich in nitrate, e.g. Chinese flat cabbage and arugula which contain >3000 mg/kg fresh weight (FW), while other vegetables contain nitrate at substantially lower concentrations, e.g. mushrooms and corn at <50 mg/kg FW (Blekkenhorst et al., 2017b). The level of nitrate within a particular vegetable can also vary considerably according to country of cultivation; growing method (organic vs conventional; undercover vs open air); cooking and preservation methods; month, season and year of cultivation; and the analytical procedure used to assess nitrate concentration (Blekkenhorst et al., 2017b).

### 2.3. Dietary nitrate intake in different populations

Dietary nitrate intake varies greatly between individuals and populations. Mean daily intakes of most populations are estimated to be between 31 and 185 mg (Gangolli et al., 1994) and actual individual intakes range from <20 mg to >400 mg (Petersen and Stoltze, 1999; Ysart et al., 1999). The European Food Safety Authority has set the Acceptable Daily Intake (ADI) for nitrate at 3.7 mg/kg (approximately 260 mg for a 70 kg adult). Individuals who follow the Dietary Approaches to Stop Hypertension (DASH) diet may consume as much as 1000 mg/d (Hord et al., 2009) but it is unknown if, or how much, of the blood pressure lowering effects of this diet are due to higher nitrate intake. There is also considerable variation among different populations, for example: low nitrate intake in Australia 79 mg/adult/day [4]; medium nitrate intake in Belgium 148 mg/adult/day (Dejonckheere et al., 1994); high nitrate intake in China 486 mg/adult/day (Zhong et al., 2002); and very high nitrate intake in Japan >1100 mg/adult/day (Sobko et al., 2010).

## 3. Vascular effects of dietary nitrate - human intervention studies

Dietary nitrate, now recognised as an important contributor to the reservoir of NO, has similar effects to NO when supplied exogenously. Effects on blood pressure and other markers of

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