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TITLE

Nitric oxide boosting effects of the Mediterranean diet: A potential mechanism of action

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The traditional Mediterranean diet comprises a high intake of fruits, vegetables, olive oil, unrefined grains, legumes, and fish, a moderate intake of nuts and red wine, and a low intake of red meat and refined sugar. High adherence to this dietary pattern has been linked to positive health outcomes, including the prevention of cardiovascular disease (CVD) (1), type II diabetes (2), and cancer (3,4). Moreover, the Mediterranean diet has recently been shown to reduce age-related cognitive decline, suggesting potential applications in the prevention of neurodegenerative diseases (5,6). However, the mechanisms through which this dietary pattern exerts its beneficial effects are unclear.

In the *Journals of Gerontology: Biological Sciences*, the article "Health Benefits of the Mediterranean Diet: Metabolic and Molecular Mechanisms" by Tosti et al. (7) highlights five plausible mechanisms through which the Mediterranean diet may elicit its beneficial effects. These are: i) lipid lowering effect, ii) protection against oxidative stress, inflammation, and platelet aggregation, iii) modification of cancer-related hormones and growth factors, iv) inhibition of nutrient sensing pathways via restriction of specific amino acids, and v) gut microbiota-mediated production of metabolites influencing metabolic health. However, the potential nitric oxide (NO) 'boosting' effects of the Mediterranean diet which could complement these mechanisms was not discussed by Tosti et al. (7).

NO is a pleiotropic gasotransmitter implicated in multifarious physiological processes including blood pressure control, glucose homeostasis, neurotransmission, mitochondrial function, muscle contraction, and host defence (8). Decreased NO bioavailability has been associated with ageing and multiple pathological conditions including hypertension (9), congestive heart failure (10), hypercholesterolemia (11), type II diabetes (12), and the metabolic syndrome (13). Conversely, increasing NO bioavailability has been proposed as a

physiological target for nutritional approaches aiming to mitigate age-related cardiovascular, metabolic, and neurodegenerative diseases (14). The Mediterranean diet has considerable potential for enhancing NO bioavailability because it contains many foods rich in L-arginine and nitrate – two key substrates for endogenous NO generation. Moreover, this dietary pattern is rich in vitamin C, polyphenols, and the marine-derived long chain n-3 fatty acids, which can potentiate NO production and decrease NO degradation in the body (Figure 1).

The Mediterranean diet is abundant in nuts, legumes, unrefined grains, and fish, all of which are significant sources of L-arginine (15). This semi-essential amino acid is oxidised by the NO synthase enzymes to form NO and, consequently, elicits multiple potentially beneficial effects. Indeed, previous studies have reported cardiovascular benefits of supplementation with 4–24 g/d L-arginine – an amount attainable through consumption of L-arginine-rich foods – including reduced blood pressure (systolic: -5 mmHg; diastolic: -3 mmHg) (16), improved endothelial function (17), and decreased platelet aggregation (18). The Mediterranean diet is also rich in vegetables, many of which have a high inorganic nitrate content. This inorganic anion, via the recently elucidated nitrate-nitrite-NO pathway, can also serve as a NO precursor. Consumption of ~6–12 mmol/d inorganic nitrate produces similar reductions in blood pressure (systolic: -4 mmHg; diastolic: -1 mmHg) (19), improvements in endothelial function (20), and inhibition of platelet aggregation (21) as L-arginine supplementation. Additionally, inorganic nitrate ingestion has been reported to enhance cerebral blood flow and improve cognitive function (22) although this was not confirmed in a recent systematic review and meta-analysis (23). Since spinach, beetroot, lettuce, rocket, and celery have a nitrate content >4mmol/100g fresh-weight (24), intake of 6–12 mmol/d nitrate is easily achievable through a high-vegetable diet such as the Mediterranean diet (e.g. 150–300 g nitrate-rich vegetables) (25).

Vitamin C and polyphenols, available via fruits, vegetables, red wine, and olive oil, could also contribute towards the NO boosting effects of this dietary pattern, by potentiating both the L-arginine and nitrate-nitrite-NO pathways, and minimising superoxide scavenging of NO via antioxidant effects (26,27). In a sub-study of 200 participants within the PREDIMED intervention study (28), plasma biomarkers of NO availability (sum of plasma nitrate and nitrite) increased in participants consuming the Mediterranean diet supplemented with extra-virgin olive oil or nuts. The change in plasma nitrate plus nitrite was associated with lower systolic and diastolic blood pressure, and there was a positive correlation between urinary total polyphenol excretion – a biomarker of polyphenol intake – and the change in these NO biomarkers. Furthermore, a Mediterranean diet, where fish is typically consumed 2-4 times per week, will be rich in the long chain n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Consumption of EPA and DHA has been shown to induce vasodilation and increase circulating NO metabolite concentrations (29,30). The incubation of EPA+DHA-rich lipoproteins from human plasma following a fish oil-rich meal increased eNOS and decreased NADPH oxidase gene expression in endothelial cells compared with lipoproteins isolated following a control meal (29), suggesting increased production and decreased degradation may have contributed to an overall increase in NO bioavailability.

In summary, many components of the Mediterranean diet have the potential to enhance NO bioavailability. A classic Mediterranean meal of fish and salad sprinkled with nuts and drizzled with olive oil could contain sufficient L-arginine, nitrate, polyphenols, vitamin C, and EPA plus DHA to provide a significant NO ‘boost’ with attendant beneficial physiological consequences. Given the myriad of health benefits previously associated with dietary augmentation of NO bioavailability, we propose the potential NO enhancing effects of the

Mediterranean diet as a further, complementary mechanism through which this dietary pattern may elicit beneficial health outcomes.

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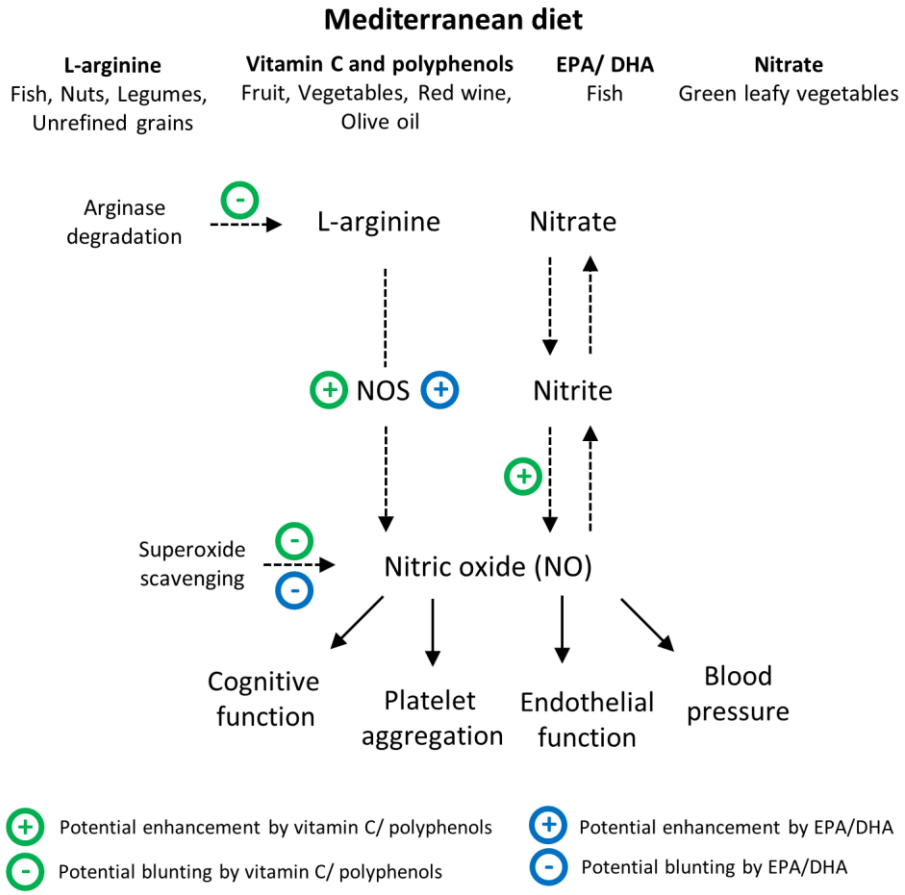

Figure 1 A schematic representation of pathways for nitric oxide (NO) generation in the human body. The Mediterranean diet provides L-arginine (fish, nuts, legumes, and unrefined grains) and nitrate (green leafy vegetables), which may serve as NO precursors in the body. Additionally, it is rich in vitamin C and polyphenols (fruit, vegetables, red wine, and olive oil), and the long chain n-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (fish) which may serve to enhance both pathways for NO production, and minimise superoxide scavenging of NO, thus prolonging the activity of this gasotransmitter.