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Review

Vascular effects of the Mediterranean diet Part I: Anti-hypertensive and anti-thrombotic effects

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ABSTRACT

This review summarizes available evidence on the beneficial effects of inorganic nitrates and the monounsaturated fatty acid (MUFA) oleic acid, largely contained in Mediterranean diet, on blood pressure and coagulation activity.

Inorganic nitrate. Normal vascular function requires NO production from the L-arginine–NO synthase (NOS) pathway. This process is defective in conditions of local hypoxia, and here nitrite can substitute for L-arginine–NOS derived NO. In this context, NO generation from the nitrate–nitrite–NO pathway mostly derived from green leafy vegetables appears to be an alternative source for NOS-dependent NO production, ensuring NO bioavailability also in situations when the endogenous L-arginine/NO synthase pathway is dysfunctional or physiologically reduced in local hypoxic conditions.

Olive oil and oleic acid. In addition to effects on lipoprotein metabolism and oxidation, the beneficial effects of oleic acid occur also on coagulation activity, namely on coagulation factor VII (FVII). Normally, a substantial increase of FVII coagulant activity (FVIIc) occurs within 2–3 h after a fatty meal and persists for several hours thereafter. When a background diet high in MUFA is consumed, a lower post-prandial increase of FVIIc takes place.

Contents

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1. Introduction

The Mediterranean diet (MeD) refers to a dietary profile commonly available in the early 1960s in the Mediterranean regions. It may be considered not one specific diet, but rather a collection of eating habits (hence the term "Mediterranean diets" preferred by some authors) traditionally followed by people bordering the Mediterranean sea, and consisting in a plant-centered diet with high intakes of vegetables and fruit, whole-grain cereals, extra-virgin olive oil, nuts, a moderate consumption of fish and poultry, a low intake of dairy products, red meat, and sweets, and a moderate consumption of red wine [1,2].

A persuasive body of evidence from observational studies [3–5] and secondary prevention trials [6,7] has now documented that adherence to a Mediterranean-style diet is consistently beneficial, compared with other dietary patterns, with respect to the risk of cardiovascular disease, cancer, Alzheimer's disease, and Parkinson disease, as well as of death from cardiovascular disease or cancer and even premature death overall.

A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease [8] has recently ranked the MeD as the most reliable dietary model to provide protection primarily from coronary heart disease. The Scientific Advisory Committee of the American Heart Association stated that the Mediterranean-style diet has impressive effects on cardiovascular disease [9].

The healthful properties of MeD have been mainly attributed to the additive or synergistic interaction of its various constituents as a whole. Despite an awareness of the high complexity of the MeD nutrient composition, basic researchers are still nowadays concentrating their efforts on single food items with the hope that such single food-focused research might eventually be exploited for specific appropriate dietary advice. Some MeD constituents have indeed been found to exert specific actions on the cardiovascular system. Such are inorganic nitrate, n−9 and n−3 fatty acids, antioxidants and polyphenols, which have been shown to exert effects particularly on blood pressure, coagulation activity and endothelial functions.

In two separate reviews we will review the effects of specific MeD constituents. In the present one we will deal with the role of MeD inorganic nitrate and saturated/monounsaturated fatty acids as to their effects on hypertension and thrombosis respectively. A second, parallel review will deal with the effects of n−3 fatty acids and polyphenols.

2. Leafy vegetables, inorganic nitrate and blood pressure: a rational basis for the anti-hypertensive effects of the Mediterranean diet

Many trials have shown that diets rich in fruits and vegetables reduce blood pressure [10,11] and the risk of cerebro- and cardiovascular events [12–15]. These benefits have previously been attributed to MeD constituents as a whole, including vitamins, minerals, fibers, and the so-called non-nutritive substances, such as flavonoids and glucosinolates as a whole. At present, other factors are clearly emerging as alternative candidates.

According to recent epidemiologic trials, the greatest protection against coronary heart disease is associated with the consumption of green leafy vegetables (e.g., spinach, lettuce, collard greens, beetroots) [14,15]. Such vegetables have a high content of nitrate (NO_3^-), and actually are the richest known source of dietary inorganic nitrate. They account for 80–85% of daily dietary nitrate exposure in the average population [16,17].

This dietary inorganic nitrate has been shown to be an important source of nitric oxide (NO). There is a consensus that dietary nitrate is essentially inert, but acquires biological activity after reduction to nitrite [16]. In particular, the reduction of dietary nitrate to nitrite is necessary for nitrate to serve as a substrate for NO production. Most if not all the beneficial effects of dietary nitrate are considered to be mediated by its reduction to nitrite and then to NO, a critical regulator of vascular homeostasis [18–23] (Fig. 1).

Dietary nitrate (NO_3^-) undergoes reduction to nitrite (NO_2^-) and then to NO through a nitrate–nitrite pathway alternative to the classical L-arginine–NO synthase (NOS) pathway for NO production in the body [24–27]. Even though 80% of the basal plasma nitrite levels derive from the oxidation of NO [28], reduction of nitrate also significantly contributes to increasing nitrite levels and, eventually, to the synthesis of NO [29,30] (Fig. 1).

2.1. NO production by eNOS in the vascular bed in physiologic conditions

The normal functioning of the human vasculature requires the presence of nitrite and NO along with the biochemical machinery necessary to respond to these important signaling molecules [25,26]. The generation of up to 70% of systemic NO is accomplished by endothelial cells through the action of the endothelial nitric oxide synthase (eNOS), one of the 3 members of the NOS family of enzymes [31]. NOS synthesizes NO from the amino acid L-arginine and molecular oxygen (Fig. 1). In the vasculature, this results in vasodilation and blood pressure regulation. The production of NO by endothelial cells is stimulated by laminar shear stress, part of the tangential shear forces generated by the flowing blood on the endothelial surface [32].

2.2. Alternative NO production by nitrate in tissues

Recently, part of NO synthesis in healthy tissues has been shown to occur independent of the L-arginine–NOS pathway [20]. In fact, at variance from the provision of eNOS-derived NO to the endothelium to regulate the vasomotor tone, NO production also occurs in other tissues, and the dietary provision of nitrate and nitrite may account for approximately half the steady state NO concentration [33].

The reduction of dietary nitrate to NO in the body involves its initial reduction to nitrite, and then to NO [24–27] (Fig. 1). There are 2 systems of reducing dietary nitrate to nitrite in mammals.

1. The first system involves the action of commensal Gram-negative bacteria on the tongue (Fig. 2). After swallowing and the absorption through the stomach wall, about 25% of consumed nitrate (NO_3^-) enters the entero-salivary circulation. The absorbed nitrate (NO_3^-) is then concentrated 10 fold in the salivary glands, and reduced to nitrite (NO_2^-) by bacterial nitrate reductases contained in facultative anaerobes present on the dorsal surface of the tongue [24,29,34]. Once swallowed into the stomach, nitrite is partly converted to NO by the local acidic conditions (Fig. 2). Nitrite and NO then diffuse into the portal circulation, and NO is oxidized to nitrite, which in turn is transported in the arterial circulation to resistance vessels, where it lowers O_2 tension and favors the reduction of nitrite to NO, which in turn causes vasodilatation and the consequent lowering of blood pressure.
2. There are also several different mammalian enzymes and metalloproteins that have nitrate reductase activity, a function previously

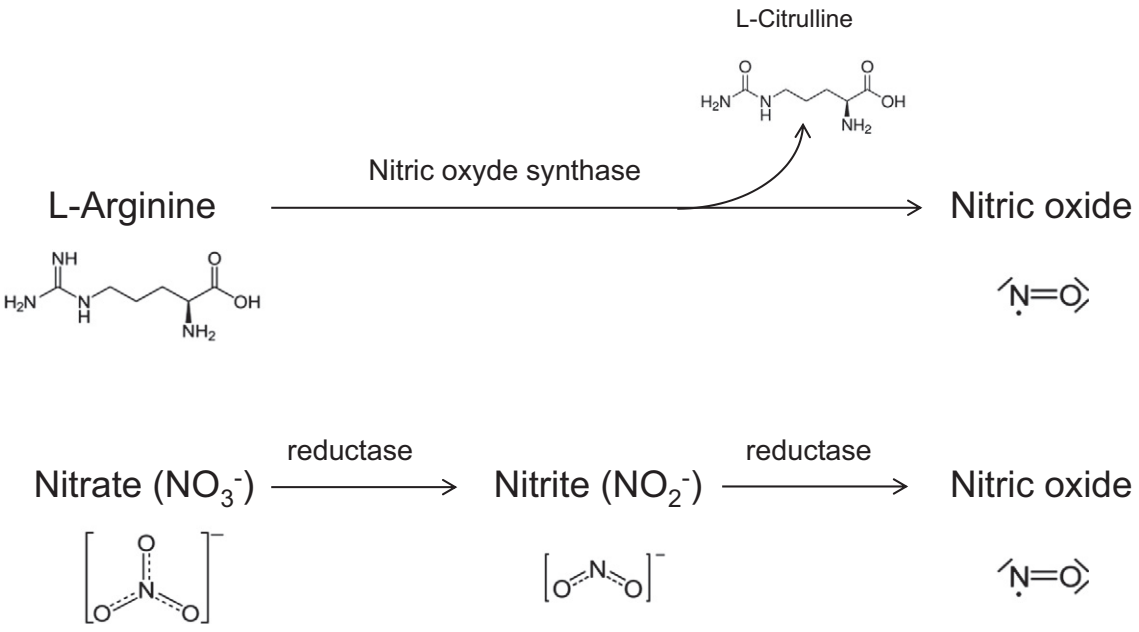

Fig. 1. The two-ways synthesis of nitric oxide (NO). NO production by eNOS in the vascular bed in physiologic conditions (top) and the alternative NO production by nitrate in tissues (bottom).

thought to be carried out only by bacteria [34]. These include xanthine oxidoreductase, aldehyde oxidase, heme proteins, and mitochondria [20,35]. Once nitrate (NO_3^-) has been reduced to nitrite (NO_2^-) by the above mentioned mammalian enzymes, the nitrite reduction to NO is carried out by numerous metalloproteins and compounds with redox potential, including hemoglobin, deoxyhemoglobin,

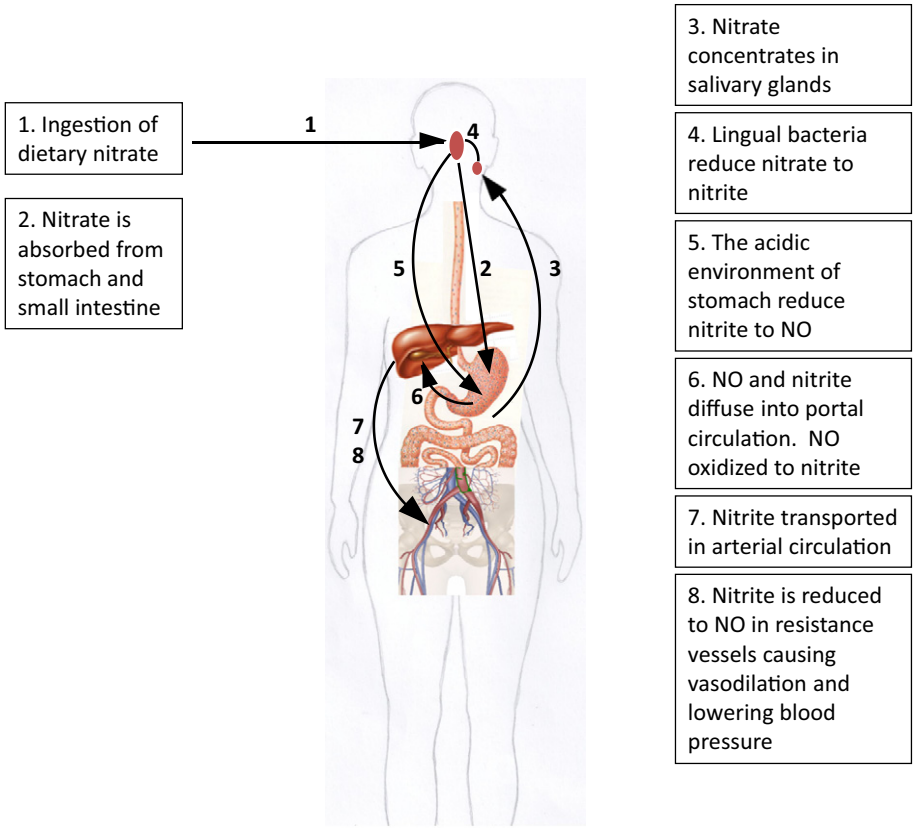

Fig. 2. The fate of dietary nitrate derived from consuming leafy vegetables. After absorption through the stomach wall, ~25% of consumed nitrate enters the entero-salivary circulation and is concentrated 10 fold in the salivary glands, where it is reduced to nitrite by bacterial nitrate reductase from facultative anaerobes present on the dorsal surface of the tongue. Subsequently, nitrite is swallowed and, in the acidic environment of the stomach, is reduced to nitric oxide (NO) or re-enters the circulation as nitrite, then diffusing into the portal circulation and thus providing a source of systemically available nitrite/NO. Nitrite is then transported in the arterial circulation to resistance vessels, where a lower O_2 tension favors the reduction of nitrite to NO, causing vasodilation and the consequent blood pressure lowering.

deoxymyoglobin, xanthine oxidoreductase, vitamin C, and polyphenols [20,36].

Importantly, unlike NO production from eNOS, the activity of which is oxygen-dependent (meaning that endothelial NO-driven processes decline with progressive depletion of oxygen levels), NO production from the nitrate–nitrite–NO pathway increases with diminished oxygen tension [37–41]. In this context, NO generation from the nitrate–nitrite–NO pathway is viewed as an alternative source to NOS-dependent NO production in the body, a sort of backup system to ensure NO-like bioactivity also in situations when the endogenous L-arginine/NO synthase pathway is dysfunctional [37–41]. Inorganic nitrite, therefore, appears to be a circulating storage pool of NO that selectively donates NO to hypoxic vascular beds [25], and dietary nitrate can be therefore considered a large reservoir of substrates for the eventual NO production. Dietary nitrate has been shown to be involved in blood pressure regulation and in ischemia/reperfusion injury prevention.

2.3. Dietary nitrate and blood pressure

The MeD is a diet rich in fruit and vegetables. The dietary nitrate, contained in most vegetables (Table 1), but particularly in green leafy vegetables and beetroots, appears to be a major contributor to vascular effects. Nitrite, the reduced form of nitrate, is an intrinsic signaling molecule [16,26,42], and its ability to form NO under hypoxic conditions has transformed the perception of this molecule from a once inert anion into a critical molecule in maintaining NO and nitroso homeostasis throughout the entire physiological oxygen gradient in vivo. Nitrate and nitrite in vegetable-rich diets have been demonstrated to have significant blood pressure lowering effects and underpin a cardioprotective effect of vegetables [43]. Of interest, the blood pressure response to a high fruit and vegetable diet appears to be much greater in hypertensive compared to normotensive subjects [10]. It is therefore likely that the blood pressure lowering effects of dietary nitrate are particularly prominent in hypertensives.

The MeD appears to provide greater nitrate supplementation than a regular Western diet. It has been reported [44] that a typical Mediterranean meal contains approximately 325 mg of combined nitrate/nitrite per serving, significantly (approximately 10-fold) higher in comparison with Western diet (only ~20 mg/serving).

In peripheral resistance vessels, the dietary nitrate-derived nitrite is reduced to NO, the main function of which is to regulate the tone of vascular smooth muscle cells. The endothelium of blood vessels uses NO to signal relaxation in the surrounding smooth muscle cells, thus resulting in vasodilation and increased blood flow. The reduction of blood pressure through NO-dependent vasorelaxation is mediated via nitrosylation of endothelial soluble guanylate cyclase [31].

In humans, the vascular effects of dietary nitrate are consistent with those observed in animals, with effects particularly on blood pressure [21,22]. Larsen et al. [22] showed that a 3-day dietary supplementation with sodium nitrate at a dose corresponding to the amount normally found in 150–250 g of nitrate-rich vegetables, such as spinach, beetroots, or lettuce, did not change systolic blood pressure and heart rate, but significantly reduced diastolic and mean arterial blood pressure. The authors concluded that, similarly to other previous studies [10,11], a diet rich in vegetables can reduce diastolic blood pressure. In a study conducted in healthy volunteers, Webb et al. [23] demonstrated that the ingestion of a dietary nitrate load (500 mL beetroot juice; mean nitrate concentration 45 mmol/L) significantly reduced systolic blood pressure by 10.4 ± 3 mm Hg 2.5 h (h) after the ingestion, an effect that correlated with peak increases in plasma nitrite concentration. The peak differences in diastolic blood pressure were seen at 3 h after ingestion, with a drop of 8.1 ± 2.1 mm Hg. The dietary nitrate load also prevented endothelial dysfunction induced by an acute ischemic insult in the forearm, and significantly attenuated ex vivo platelet aggregation in response to collagen and ADP [23].

The in vivo half-life of nitrite (~1.5 h) in these studies was much longer than the ex vivo half-life of 2 min, suggesting that in the in vivo conditions nitrite was continuously produced from nitrate (which has a long half-life of ~8 h) via the entero-salivary circulation [23,25].

2.4. Dietary nitrate and myocardial ischemia/reperfusion injury

Dietary nitrate has been shown to have a significant influence also on the pathophysiology of myocardial ischemia/reperfusion (I/R) injury [45]. Mice fed a diet deficient in nitrate and nitrite for 7 days exhibit significantly reduced plasma and heart levels of nitrite and NO metabolites. Supplementation of nitrite in drinking water for 7 days reverses the effects of nitrite deficiency. These data show the significant influence of dietary nitrate and nitrite intake in the maintenance of steady-state tissue nitrite/NO metabolite levels [18].

While nitrite is normally an inert end-product of NO oxidation, during ischemic conditions sufficient acidosis develops, permitting NO generation from the endogenously stored nitrite [46]. This potential for nitrite-derived NO production has been clearly demonstrated both in vitro and in animal models of I/R injury in various organs [18,47,48].

Mice fed with low nitrate and nitrite diets show exacerbated myocardial injury and post-myocardial mortality rates compared with a control group. On the contrary, nitrate supplementation (1 g/L in drinking water) affords significant protection against myocardial ischemic injury in mice fed with standard diet or low nitrite and nitrate diet [18]. A time-course study on ischemia–reperfusion has revealed that nitrite is consumed during the ischemic phase, with an increase in nitroso/nitrosyl products in the heart [18]. In this respect, it should be emphasized that nitrite (NO_2^-) in normoxic and hypoxic conditions transiently forms two classes of compounds:

1) the nitroso products, i.e. organic compounds which have the NO group attached to the organic moiety (S-nitrosothiols and N-nitrosoproteins); the protein S-nitrosation represents a form of posttranslational modification of proteins that is rapid and reversible (Fig. 3). S-nitrosated proteins exist mainly in the mitochondria

Table 1
Classification of vegetables according to nitrate content (Hord NG et al. Am J Clin Nutr 2009; 90: 1–10).

Nitrate content (mg/100 g fresh weight)	Vegetable varieties
Very low, <20	Artichoke, asparagus, broad bean, eggplant, garlic, onion, green bean, mushroom, pea, pepper, potato, summer squash, sweet potato, tomato, watermelon
Low, 20 to <50	Broccoli, carrot, cauliflower, cucumber, pumpkin, chicory
Middle, 50 to <100	Cabbage, dill, turnip, savoy cabbage
High, 100 to <250	Celeriac, Chinese cabbage, endive, fennel, kohlrabi, leek, parsley
Very high, >250	Celery, cress, chervil, lettuce, red beetroot, spinach, rocket (rucola)

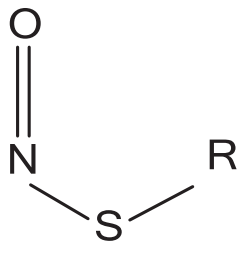

Fig. 3. An organic nitroso compound with the NO group attached to the organic moiety (R).

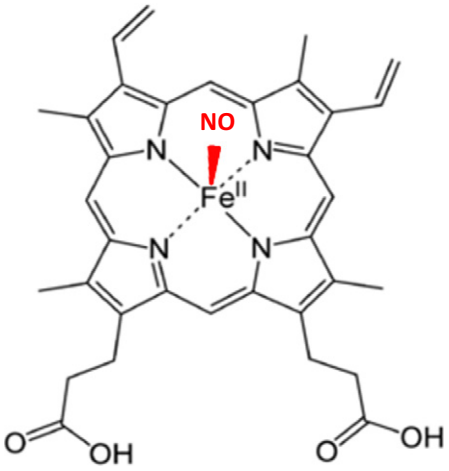

Fig. 4. A nitrosyl-heme compound containing the NO group directly bound to a metal (Fe) giving a metal-NO moiety.

and peri-mitochondrial compartment of endothelial cells, and their half-life is 1 h.

2) nitrosyl-heme products, i.e. non-organic compounds containing the NO group directly bound to a metal giving a metal-NO moiety (i.e. NO bound directly to Fe of the eme-group, i.e. nitrosyl-heme) (Fig. 4).

Mice supplemented with nitrite in their drinking water have shown significantly higher heart levels of nitrite, nitroso, and nitrosyl-heme, which were associated with a 44% significant attenuation of the experimental myocardial infarct size [18]. These data reveal that increasing nitrite dietary intake affects steady-state concentrations of cardiac nitrite, nitroso-modified proteins, and nitrosyl-heme products and provides significant cardioprotection against I/R injury [18]. During the ischemic phase of I/R injury the cardiac nitrite level is reduced [18] . This reduction has been attributed to its consumption and bioconversion to NO, nitroso and nitrosyl products. During reperfusion, cardiac nitroso (nitrosothiols) and nitrosyl-heme products decay over time and reach near starting concentrations by 30 min of reperfusion [18].

Because both NO and nitrosothiols have been demonstrated to be protective in the setting of I/R [47,49], nitrite has become a critical molecule in that it can form both NO and nitrosothiols. Based on these data, it has been proposed [18] that nitrite serves two functions in the setting of I/R. First, it serves as a NOS-independent source of NO by which nitrite is reduced to NO during ischemia when NOS is inactive. Second, nitrite reacts with critical thiols to form nitrosothiols. This nitroso modification very probably acts as a reversible protective shield that prevents irreversible oxidation of proteins and lipids during the oxidative burst of reperfusion, or it may alter protein or enzymatic function and thereby modulate protective signaling pathways. Aside from thiol modification, it has been proposed that the nitroso products can also release NO or the NO^+ moiety during the reperfusion phase and act as a redox-sensitive NO donor [50].

NO has been also shown to effectively improve the endothelial dysfunction induced by acute ischemia–reperfusion injury [51,52]. The primary target for damage in such conditions is the mitochondrial bioenergetics machinery [53], with damage promoted by the excessive generation of reactive oxygen species (ROS) and Ca^{2+} overload [54]. It has been reported that the nitrate–nitrite–NO system preserves mitochondrial functions during I/R injury through a mechanism of reversible inhibition of mitochondrial respiration [55]. This reversible inhibition appears to facilitate the slow restoration of the electron flow through the respiratory chain, thus preventing the burst of ROS, and also appears to delay the full restoration of the membrane potential, a driving force for Ca^{2+} uptake, thus preventing ischemia/reperfusion [56].

2.5. Dietary nitrate and metabolic syndrome

Dietary nitrate has been shown also to be able to prevent the features of metabolic syndrome. In eNOS-deficient mice that develop the metabolic syndrome, supplementation with sodium nitrate at a dose that is readily achievable through diet reversed several features of the metabolic syndrome, with a reduction of visceral fat accumulation, blood pressure and circulating levels of triglycerides, as well as improving glucose homeostasis [19]. Overall, studies in animals have shown that dietary nitrate exposure may reverse or improve pathological changes that are secondary to the loss of endothelium-derived NO bioavailability, leading to improved cardiovascular parameters [57].

2.6. Dietary nitrate and cancer concern

A major health concern with dietary nitrate is the risk of cancer development, because of its proposed association with the in vivo formation of N-nitrosamines, a class of carcinogenic substances [58]. Indeed, in 2003, a Joint Food and Agriculture Organization (FAO)/World Health Organization (WHO) Expert Committee on Food Additives reviewed studies investigating possible association between nitrate intake and cancer risk, and concluded that there was no evidence that nitrate was carcinogenic to humans [59]. Importantly, epidemiological evidence shows that abundant consumption of fruit and vegetables reduces the risk of cancer [60,61]. Collectively, these studies suggest that dietary nitrate does not exert carcinogenic activity in humans and would not be harmful to human health via this mechanism.

3. Monounsaturated fatty acids and factor VII: anti-thrombotic effects of the Mediterranean diet

In persons at high risk of cardiovascular disease a chronic activation of the hemostatic system occurs, resulting in a prothrombotic environment [62]. A prothrombotic condition normally takes place in the postprandial phase, due to a postprandial increase in coagulation factor VII (FVII). This postprandial prothrombotic condition is heightened in type 2 diabetic patients [62], obese individuals [62], and men at high risk of myocardial infarction [63].

Several dietary intervention studies have shown that FVII is influenced by the diet [64–67], and total dietary fat intake appears to be the main determinant of postprandial FVII coagulant activity (FVIIc) [68]. One effect of olive oil, a fundamental component of MeD, is on FVII, a key protein in thrombosis and an important risk factor for ischemic heart disease [69–73]. The monounsaturated fatty acid (MUFA) oleic acid, which represents 75% of olive oil fatty acids, has been shown to significantly attenuate the prothrombotic state occurring in the postprandial phase.

3.1. Coagulation factor VII

FVII, an enzyme of the serine protease family, is one of the vitamin-K dependent coagulation factors, synthesized mainly in the liver and secreted as a single-chain glycoprotein zymogen circulating at an average concentration of about 450 ng/mL [74]. The active enzyme, FVIIa, is generated by limited proteolysis of FVII to produce a two-chain active form circulating at a concentration of about 4 ng/mL [74]. FVIIa forms a complex with tissue factor (TF) and calcium, to activate factor X (FX) (Fig. 5). The main role of FVII, therefore, is to initiate the coagulation process in conjunction with TF. TF, in turn, is a protein present in the sub-endothelial tissue and in leukocytes, and is necessary to promote thrombin formation from the zymogen prothrombin. When sub-endothelial cells are damaged, TF is exposed to FVII in the blood, the formation of TF-FVII complex occurs, and this converts FX to FXa, initiating the extrinsic blood coagulation cascade (Fig. 3).

An increase in FVII coagulant activity (FVIIc) in middle-aged men at high risk of myocardial infarction has been described since 1980 [63].

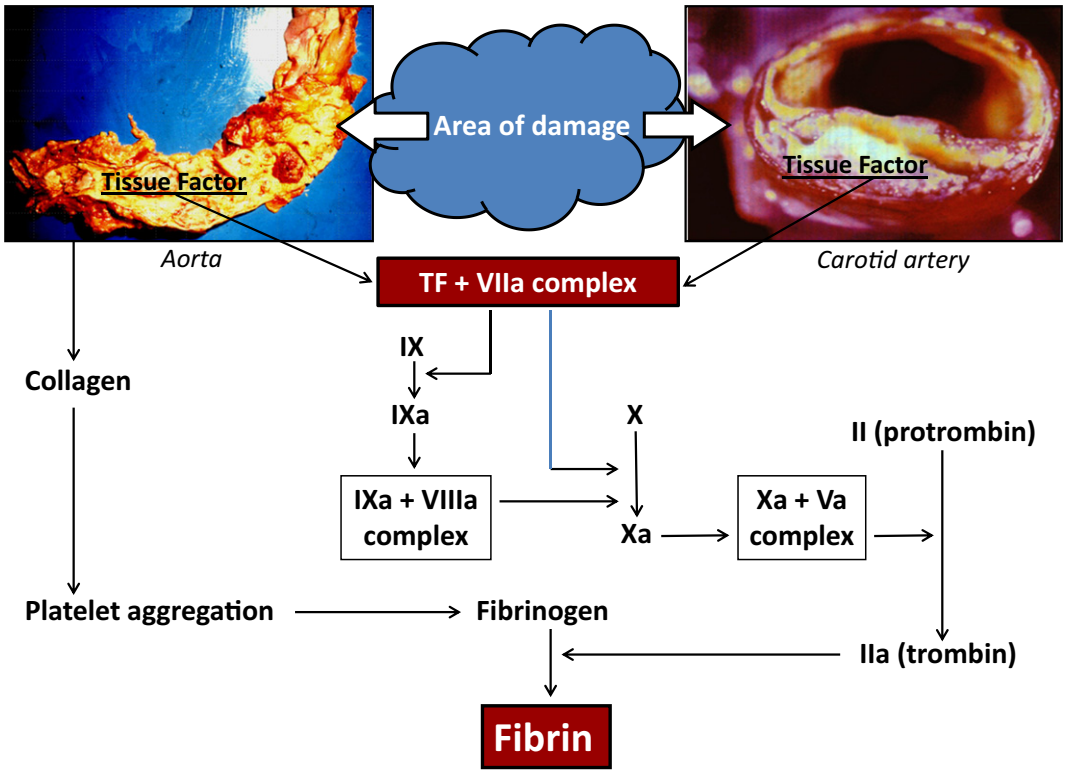

Fig. 5. A schematic representation of the extrinsic pathway of blood coagulation. The extrinsic pathway is activated when blood comes in contact with cell membranes with exposed tissue factor (TF). The monomer factor (F) VII is activated to the active dimer FVIIa in the presence of FXIIa, FXa, FIXa and thrombin. These sequential proteolytic activations take place on cell membrane surfaces and are dependent on phospholipids, mostly provided by activated platelets, but also by endothelial cells or leukocytes. Platelets are attracted to the vessel wall by collagen. Once platelets are activated, they release several of their constituents, including fibrinogen, causing thrombosis in concert with activated coagulation and fibrin formation.

The increase in FVIIc is due to an increase of activated FVII (FVIIa), the active dimeric form of the inactive FVII monomer [75]. Because FVIIa initiates the thrombotic response to rupture of an atheromatous plaque, raised post-prandial levels of FVIIc produce a transient increase in the likelihood of a clinically significant coronary thrombosis.

3.2. The post-prandial increase in FVII

FVIIc increases in the post-prandial phase. This increase has been shown to be associated with post-prandial triglyceride levels, and was first described by Miller [75]. This increase in FVIIc takes place within 2–3 h after the intake of a fatty meal, and persists for several hours thereafter [76]. The maximum activation of FVIIa takes place 8 h post-prandially [77]. Fat intake—rather than dietary energy intake—has been shown to be the primary determinant of the post-prandial increase in FVIIc [68].

Post-prandial activation of FVIIa is mainly driven by usual diets rich in long-chain saturated fatty acids (SFAs). A single fat-rich meal, irrespective if rich in SFAs or polyunsaturated fatty acids (PUFAs), indeed induces an increase in FVIIa when taken in individuals with a background diet rich in long-chain SFAs, but not when the usual diet is rich in unsaturated fatty acids [68].

Post-prandial hypertriglyceridemia has been associated with the post-prandial increase of FVIIa and with endothelial dysfunction, including changes of the vasorelaxing, antiplatelet, and anticoagulant properties of the endothelium towards a more atherogenic profile [78] . In a study with a single high-fat meal, the observed increase in serum triglycerides from 94 to 147 mg/dL was associated with a decrease of flow-dependent vasodilation from 21 ± 5% to 10 ± 3% at 4 h after the high-fat meal, confirming that a single high-fat meal transiently impairs many endothelial functions [79]. These findings identify a potential process by which a high-fat diet may be atherogenic, independent of changes induced in cholesterol levels.

3.3. Factor VIIa increases and genetic polymorphisms

The extent of post-prandial FVIIa increase associated with hypertriglyceridemia is in part linked to the R353Q gene polymorphism. In the gene coding for FVII the R353Q polymorphism is the result of a single base change in the codon for amino acid 353, and leads to the replacement of an arginine (R) with a glutamine (Q). The presence of the Q allele is associated with lower concentrations of post-prandial FVIIa. Compared with subjects with the R allele (Arg_{353}), subjects with one or two Q alleles (Gln_{353}) had levels of FVIIc that were 20% and 78% lower, respectively [74,80].

3.4. Factor VIIa, high-fat and high-carbohydrate diets

A study that compared the effects of a high-fat meal with those of a high-carbohydrate meal in healthy subjects showed that both meals increased post-prandial level of FVII and impaired the antiplatelet functions of the endothelium [78]. However the post-prandial absolute increase in FVIIa was significantly lower after the high-carbohydrate meal than after the high-fat meal. In the high-carbohydrate meal, FVIIa returned to normal values after an L-arginine intravenous infusion increased NO bioavailability, at variance from the high-fat meal, in which case the normalizing effect of L-arginine did not occur [78]. This indicates that the increased NO availability from L-arginine or other substrates, such as vegetable inorganic nitrate, resets the activated hemostasis. This putative effect appears to be deeply impaired after a high-fat meal.

In summary, data from studies investigating associations between dietary fat and concentrations of FVII have shown that an increased intake in total fat results in a concomitant increase in FVIIc and FVIIa concentrations. The observed relationship between total dietary fat and FVIIc is apparent when chronic (habitual) or acute (single-meal)

effects of diet are investigated. When the habitual diet is high in saturated fat the accompanying increase in FVIIc is mainly due to a rise in FVII antigen (FVIIAg). Conversely, the transient increase of FVIIc after a single fat-rich meal is entirely due to a rise in the concentration of FVIIa [81].

3.5. Factor VII and dietary oleic acid

Although the data on post-prandial FVIIc after MUFA- and PUFA-enriched meals are contrasting [82,83], a recent report demonstrated a lower post-prandial increase of FVIIa in subjects consuming background diets high in MUFAs, mostly oleic acid [84]. In particular, a significantly lower post-prandial FVIIa and FVIIAg concentrations were found after approximately 40% of dietary SFAs were replaced with MUFAs in an 8-week crossover dietary intervention study [84].

The beneficial effect of MUFA-rich diets was confirmed in another study where the effects of two diets, one rich in MUFAs and one in PUFAs, were compared [85]. Here diets rich in olive oil, rapeseed oil and sunflower oil were administered to 18 healthy young individuals for 3 weeks. The olive oil-rich diet was associated with a lower post-prandial mean and peak concentrations of FVIIa in comparison with the sunflower oil and the rapeseed oil diets. These data are by-and-large in agreement with those of a cross-cultural study involving northern and southern European males [86]. Although no significant effect of test meal composition (SFAs vs MUFAs) was found, FVIIc was significantly higher 8 h post-prandially in northern than in southern Europeans. If these findings are indeed an effect of a chronic MUFA diet, they suggest the interesting hypothesis that the lower rate of coronary heart disease in Mediterranean countries may result—at least in part—from the antithrombotic effects of MUFA-rich diets of Mediterranean populations [86].

The role of a MUFA-rich diet on FVII has been further investigated in a randomized single-blind parallel-design study conducted on 51 healthy volunteers [87]. All participants here consumed a reference diet rich in SFAs (37% of total calories from fat; 15.6% SFAs; 11.8% MUFAs; 5.8% PUFAs; 0.7% *trans*-fatty acids) for 8 weeks, followed by a MUFA-enriched diet for a further 16 weeks, aimed at replacing 24% of dietary SFAs with MUFAs while maintaining total fat, PUFAs and *trans*-fatty acids constant in the two diets. At the end of each dietary period, the subjects were requested to consume the test meal, which provided 45 g fats, 93 g carbohydrates and 33 g proteins, with a total energy of 3720 kJ (890 kcal). No significant differences in plasma triglyceride levels or in peak triglyceride concentrations were observed between the reference and the MUFA-diet periods. No difference in fasting FVIIc was observed between the two diets. However, the mean post-prandial FVIIc value after the MUFA intervention was significantly lower than that after the reference (SFA) diet. Also the peak of post-prandial FVIIc was lower in the MUFA diet compared to the reference (SFA) diet. FVIIa was significantly increased in response to the 45 g fat loads administered at the end of the reference and MUFA dietary intervention periods. Although there was no difference in fasting FVIIa concentrations on any post-prandial study day, the post-prandial peak concentration achieved after the MUFA diet (122 mU/mL) was less pronounced than that after the reference diet (142 mU/mL). Moreover, the post-prandial increase (difference between fasting and peak values) in FVIIa after the MUFA intervention (52 mU/mL) was significantly lower than that after the reference diet (68 mU/mL). In conclusion [87], a lower post-prandial activation of FVII takes place following a high-fat test meal in subjects chronically consuming a high-MUFA diet. These findings suggest potentially beneficial effects on hemostasis for diets habitually rich in MUFAs. Moreover, these data show that the tendency of a fat-rich meal to be pro-thrombotic is substantially attenuated if background diets rich in MUFAs are consumed.

The mechanisms underlying the post-prandial activation of FVII are not completely understood, making it difficult to explain why the MUFA-rich diet results in less activation of FVII in comparison with the reference SFA-rich diet. In the above-mentioned study [87] no differences between dietary treatments in the pattern of response in plasma triglycerides levels were found. However, the substitution of dietary SFAs with equal amounts of MUFAs, without changes in other fatty acids or total fat intake, reduced postprandial and fasting plasma apo B-48 concentrations by 30–40%. Since apo B-48 is considered the unique marker of chylomicron particles, the authors concluded that the number of chylomicron particles in postprandial and fasting plasma was significantly reduced—despite being much larger in size—when the background fatty acid composition was changed to a MUFA-rich diet. Hence, the MUFA diet leads to the production of less chylomicrons which are larger in size. It has been hypothesized that the size of chylomicrons influences the ability of triglyceride-rich lipoproteins to promote the cleavage of the inactive single-chain zymogen FVII to the active dimer FVIIa, possibly because larger particles provide less contact sites for the activation of FVII [88].

4. The concept of convergent-synergistic effects of Mediterranean diet components

Numerous data suggest that cardiovascular effects of the MeD are the results of multiple actions of single components, but also of convergent synergistic effects.

An example of the diverse effects of single components is given by the multiple biological actions of NO, causing vasodilation and anti-hypertensive effects, but also inhibiting platelet aggregation and platelet adhesion to endothelium, suppressing vascular smooth muscle cell proliferation, and reducing the adhesion and migration of leukocytes/monocytes into the arterial wall [31,57]. An example of a convergent synergistic action is that of the polyphenolic flavonoid quercetin, and of vitamin C. These two compounds (discussed in Part II of this review), quite abundant in MeD, besides having their well-known antioxidant activities, actively participate in reducing nitrite to NO [89,90]. Other antioxidants, e.g., resveratrol of red wine and caffeic acid, have also been shown to stimulate the bioconversion of nitrite to NO [90,91].

In conclusion, the effects of several MeD components appear to be complex. Several single components, besides exerting well-identified specific actions on single targets, also participate in numerous other processes, altogether resulting in the final cardioprotective effect of the MeD as a whole.

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