Review

Olive oil and the haemostatic system

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Interest in the Mediterranean diet (MD) has grown worldwide due to its link with greater longevity and lower cardiovascular disease rate, cancer and age cognitive decline. Despite the high complexity of its nutrients composition, olive oil emerges as its principal food, since it provides the higher percent of energy and a lot of bioactive compounds. In this review we will discuss the benefits of diets enriched in virgin olive oil, whose effects are probably due not only to its oleic acid content but also to its other potentially health-promoting components. Traditionally, the benefits of MD were linked to its effect on lipoprotein metabolism but today we realise that there exists a whole sheaf of other benefits, including the components of haemostasis: platelet function, thrombogenesis and fibrinolysis. A diet enriched in virgin olive oil can reduce the sensitivity of platelets to aggregation, decreasing von Willebrand and tromboxane B_2 plasma levels. Moreover a particular interest has arisen about its capacity to decrease fasting Factor VII plasma levels and to avoid or modulate its postprandial activation. Also Tissue Factor expression in mononuclear cells could be reduced with the chronic intake of virgin olive oil and finally, studies performed in different experimental situation have shown that it could also increase fibrinolytic activity, reducing plasma concentration of Plasma Activator Inhibitor type-1.

Keywords: Atherosclerosis / Haemostasis / Mediterranean diet / Olive oil / Thrombosis

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1 Introduction

Haemostasis is a fundamental process in the maintenance of circulation, and is the result of a complex equilibrium between coagulation and fibrinolysis. At present, we know that the procoagulant state is capable of producing acute episodes of vascular thrombosis and is a key factor in the initiation and development of arteriosclerotic platelets [1, 2].

Atherosclerosis is a complex pathogenic process. In recent years we have seen important developments in the study of the role of thrombotic phenomena in the biology of atheroma plaque.The current concept of atherothrombosis comprises the two main processes involved in coronary disease: atherosclerosis (which studies the role of thrombotic phenomena during the chronic phase of formation and growth of atheroma plaque) and thrombosis (which studies the acute flow dysruption mechanism due to thrombus formation in a vulnerable plaque, and its consequences), and is becoming generally accepted as the term which most accurately denotes the biological reality of the disease [3].

Endothelial dysfunction plays a prominent role in the pathogenesis and progression of these phenomena, as it is the first pathological symptom of anatomical lesions. This dysfunction implies a breakdown in the defence mechanism of the endothelial wall which in turn induces a prothrombotic state, activates the inflammatory process and alters the vasomotor regulation of the vascular wall. This is why the endothelium and the factors which can damage it are now considered so important, and it is also the reason why the traditional approach to atherosclerosis, which used to focus prevention and treatment on attacking risk factors, has been broadened to a more global approach. The new approach aims to treat the biological conditions which activate or advance the vascular lesion: preventing LDL oxida-

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Abbreviations: **ADP**, adenosine diphosphate; **FVII,** Factor VII; **FVIIa,** activated FVII; **FVIIag**, antigenic FVII; **FVIIc,** coagulant activity of FVII; **MD,** Mediterranean diet; **PAI-1,** plasminogen activator inhibitor-1; **SAFA**, saturated fatty acids; **TBX,** thromboxanes; **TF,** tissue factor; **TFPI**, tissue factor pathway inhibitor; **vWF**, von Willebrand factor

tion, reducing the inflammatory process, increasing plaque stability and preventing thrombus formation [4]. The traditional dietary approach, which sought to reduce the presence of risk factors, has changed direction. Recent studies show that diet may have an effect on atherogenesis mechanisms, because it could be a potential factor related not only to cholesterol, but also to thrombosis and the endothelial inflammation and dysfunction [5, 6]. These studies have also shown that diet can lower cardiovascular risk to an even greater extent than drugs.

Interest in the Mediterranean diet (MD) has grown worldwide in the course of the past decade, even among nutritionists outside of the Mediterranean area. This is largely due to the fact that the consumption of the MD has been linked to greater longevity, improved quality of life and lower incidences of cardiovascular disease, cancer and age cognitive decline, in spite of being a dietary model with a high fat content, unlike the diets recommended for several decades by many experts in nutrition in other geographical areas [7]. However, most of the fat content of the MD is derived from a single component of the diet, namely olive oil, which means that the diet is low in saturated fats and high in MUFA, particularly in oleic acid. Moreover, the gastronomic characteristics of this dietary component encourage a higher level of consumption of plant products such as fruit, vegetables, pulses, and cereals, all of which are foods that contain a high proportion of low glycemic index carbohydrates and that have important potential for promoting good health [8]. In the course of the past few years, thanks to modern technology, other types of oil with similar fat composition have become available to human nutrition. These include oils obtained from certain types of seed, some of whose varieties are high in oleic acid, such as high oleic sunflower, soya and rapeseed oils. This situation has generated a new concept of MD, according to which dietary oleic acid is not necessarily derived predominantly from olive oil but rather, from seeds [9, 10]. However, these oils must be refined before its use, losing during the process many non-fat microcomponents, much of them with several biological effects, including the phenols (tyrosol and hydroxytyrosol), secoroids (oleuropein and its conjugate forms) and lignans. Virgin olive oil, on the contrary, posseses all these microcomponents, because it's obtained exposing olives only to physical pressure [11]. This is important, because in this review we will discuss the benefits of diets rich in virgin olive oil, the genuine MD, whose effects are due not only to its oleic acid content but also to its other potentially health-promoting components.

Traditionally, the benefits of MUFA-rich diets were linked to its effect on lipoprotein metabolism and, to a lesser extent, to other risk factors, but today we realise that there exists a whole sheaf of other benefits, including the components of haemostasis [12 – 18]. The interest that this has aroused in the course of the past few years is perfectly understandable when taken into account that, in persons at high risk of cardiovascular disease, there may well be present a chronic activation of the mechanisms of thrombosis, resulting in what might be called a prothrombotic environment [16]. Thrombogenesis is a very complex process, which involves the platelets activation (primary haemostasis), the mechanisms of coagulation (secondary haemostasis) and fibrinolysis, a system that has been implicated in the reabsorption of recently formed fibrin thrombus.

This paper reviews the current state of our knowledge of the effects of olive oil on haemostasis, bearing in mind that some of these effects are derived from their fatty acid profile and others from their minor components. Although the component of olive oil that has been most thoroughly studied is oleic acid, the principal representative of the MUFA, other minor products such as the phenols are just as interesting for the sake of their potential effects on health. The quantity and variety of these additional components of olive oil combine with the effects of oleic acid to make up one of the most functional ingredients of our diets.

The MUFA are a large group of fatty acids, the most important of which in the human diet is oleic acid (18:1n-9), which represents 95% of our ingestion of MUFA and is the most important component of olive oil (70 – 85% of its content). Although a number of positive effects on health have been attributed to MUFA, in this paper we concentrate on its action on the essential components of coagulation.

2 Platelet aggregation

Some of the factors that affect the physiology of the platelets, such as the thromboxanes (TBX) and von Willebrand Factor (vWF), will be individually reviewed later in this paper. As far as the influence of MUFA on platelet aggregation is concerned, some studies have used oils other than olive oil; these include high-oleic sunflower oil and rapeseed oil (which, although it contains oleic acid, is higher in linoleic and linolenic acids). In a study carried out in 2003, the administration of a diet rich in MUFA derived from these oils reduced the aggregation response of platelets to adenosine diphosphate (ADP) in healthy subjects, compared to a saturated fatty acid (SAFA)-rich diet. Furthermore, this phenomenon appears to have a graded effect, in that the benefits obtained by replacing 16 g/day of SAFA with MUFA increased when the amount of fat substituted was increased to 32 g/day [19]. However, the differential effects of MUFA versus PUFA, were less clear. Misikangas *et al.* found no differences in platelet aggregation or in protein kinase C (an important platelet regulator) induced by a diet high in linoleic acid but low in vegetables, as against one that was high in oleic acid and also vegetable-rich, although there is a certain complicating factor in interpreting this study due to the mixing of the potential effects of the fatty acids with those of the vegetables. Furthermore, the authors did not identify the origin of the oleic acid they employed [20].

Studies that have utilised olive oil directly as a source of MUFA also indicate that their consumption is associated with reduced platelet aggregation. The first study that offered evidence of this association [21] was published more than 20 years ago; in a Greek sample, four weeks of a diet consumed in a fast-food restaurant and based on sandwiches and salads dressed with extra virgin olive oil produced a reduction in platelet aggregation induced by ADP and arachidonic acid as compared with a traditional diet [22]. However, Vicario *et al.* found no effects of a dietary supplement of oleic acid derived from olive oil on the thrombogenic response to ADP [23]. In order to compare the effects of different sources of oleic acid, Karantonis *et al.* measured platelet aggregation following the consumption of olive, soya, maize, sunflower and sesame oils, obtaining a lower degree of activation after the olive oil [24]. This distinctive effect suggests that some of the properties encountered are not due to oleic acid itself but rather to the minor components of olive oil, as will be discussed below.

On the basis of the above-mentioned studies, it seems clear that MUFA are capable of playing an anti-thrombogenic role via their abilty to partially inhibit platelet aggregation. The studies that utilised olive oil reproduced the results found using other sources of oleic acid.

2.1 Factor VII

Factor VII (FVII) initiates the extrinsic cascade process of coagulation. Once it has combined with tissue factor (TF), it is activated and converts Factor F into Xa, which finally generates thrombine and triggers the formation of the thrombosis. There exists two ways of quantifying this process; one of them is in terms of activated FVII (FVIIa), while the other is to measure the coagulant activity of FVII (FVIIc), which is the combination of FVIIa and antigenic FVII (FVIIag). In the fasting state, FVIIc correlates with serum cholesterol, triglycerides and the amount of fat in the diet [25], primarily via variations in FVIIag [26 – 29]. FVIIc also rises postprandially, particularly if the amount of fat ingested is greater than 70 – 90 g [30], although in such cases this is a result of the rise in FVIIa [31].

Although a number of authors consider that dietary variations in FVII are due exclusively to the amount of fat consumed [27, 32 – 34], it now seems clear that such variations are a qualitative effect of the individual fatty acids.

The inclusion of SAFA in the diet on a chronic basis, for example, raises concentrations of FVIIc [28], while the influence of MUFA is more controversial. Some studies show a rise in fasting levels of FVIIc following diets rich in MUFA, which raise levels of FVIIc more than other types of fat [35], including saturated fats [36]. However, other studies have found falls in FVIIc levels in comparison with SAFA and n-6 PUFA [37, 38]. For example, Junker compared the effects on various coagulation factors of a chronic intervention rich in oleic acid derived from olive oil, which demonstrated an improvement in factors XIIa and XIIc in common with PUFA from sunflower oil; while the sunflower oil also reduced factor IXc, the olive oil was unique in lowering both Xc and FVIIc [39]. Another study demonstrated lower concentrations of FVII after 90 days on a diet described as Mediterranean, as opposed to another rich in SAFA [40].

In order to study this topic in more detail, our group performed a study that compared the effects of four weeks of an olive oil-rich MD with those of a low-fat diet and of one that was rich in SAFA (western diet) on plasma levels of FVIIa in 16 healthy normolipaemic men. The transition from the western diet to the MD produced an significant fall in FVIIa levels (101.5 ± 19.2 *vs.* 34.6 ± 15.3, $p < 0.05$), which confirmed the superiority of MUFA over SAFA in reducing FVIIa levels [39].

However, in addition to influencing fasting levels, the diet may also modify postprandial levels, although there are different effects with different types of fat [27, 41] and according to the type of diet that had been consumed during the previous weeks. Some studies thus show a larger rise in postprandial FVIIa after MUFA than following SAFA, after an isolated overload [42 – 44], although when a homogenisation diet is utilised previous to the overload, the results are different [45]. In a study carried out by Larsen *et al*., the subjects ate a diet rich in oil, sunflower or rapeseed oil for three weeks, and FVII levels were then studied both in fasting and following a fat-laden meal rich in rapeseed oil, reducing the postprandial peak in FVII after the olive oil-rich meal by 18% versus sunflower oil and by 15% versus rapeseed oil [46]. These data have been consistently reproduced when compared to SAFA, thus demonstrating that a basic diet rich in MUFA protects against the postprandial rise in FVIIc [26, 31]. In general, we may say that these data suggest that the postprandial rise in FVII is less after MUFA than after SAFA [19], although more evidence is still required. This protection even appears to be dose-dependent, in that consumption of a diet moderate in MUFA reduces postprandial increases in FVIIc and FVIIa by 18 and 17% respectively, while a MUFA-rich diet reduces them by 50 and 29% [47].

2.2 TF

TF is a transmembrane glycoprotein that unites with FVII to transform it into its active form. The activation of expression of TF in macrophages increases the coagulatory activity of the lesion, and favours the appearance of acute coronary syndrome. The quantity of fatty acids in the diet proportionately regulate the expression of lipopolysaccharide-induced TF in monocytes. PUFA inhibits this expression [48, 49]. Saturated fats raise levels of TF both post-prandially (by up to 56%) and in their basal values [50], while low-fat diets, like the MUFA-rich MD, lower the activity of TF in circulating monocytes [51].

Tissue Factor Pathway Inhibitor (TFPI) is an activated factor X-dependent inhibitor of TF-induced coagulation. The principal role of TFPI appears to be to inhibit small amounts of TF, which are probably essential for the maintenance of normal haemostatic balance. A previous study has shown that TFPI in the plasma of crab-eating monkeys increases markedly in response to a high-cholesterol diet [52]. The effects of diets enriched with fats from various sources were explored by Larsen *et al*. in a randomised crossover study that compared three dietary periods based on an olive oil-enriched diet, a sunflower oil diet and a rapeseed oil diet respectively [46]. The study revealed no influence of dietary intervention on plasma TFPI levels. In contrast, we have recently shown that the isocaloric replacement of a palm oil-enriched diet or a low-fat diet by a MD had the effect of reducing plasma TFPI [53]. Another, more recent, intervention study [54] compared oleic acid, docosahexaenoic acid/eicosapentaenoic acid and alpha linolenic acid. The second diet reduced TFPI on slightly compared to alpha linolenic acid, while there were no differences *vis-à-vis* the oleic acid consumption phase [54]. Although the decrease in plasma TFPI levels is difficult to interpret, it has previously been suggested that it may reflect an increase in the protease on the endothelial surface, which would have a regulatory effect on thrombogenesis. The decrease in plasma TFPI levels would therefore be interpreted as a change in the protective effect against thrombogenesis.

2.3 Fibrinogen

Only a few studies have explored the effects of MUFA on fibrinogen levels in isolation. Freese did not find any differences between basal levels and those that he obtained after 2.5 and 5 h when he compared three types of oil (rapeseed, sunflower and butter) in healthy female subjects [32]. Nor were there any differences in the postprandial figures in healthy men following different overloads of SAFA, MUFA and PUFA [55, 56]. It may be that the amount of fat administered influenced these negative results, since Sanders observed a postprandial increase in 29 young men after 60 – 90 g, and Kozima after 100 g, of butter [57].

The influence of the type of fatty acid on fasting fibrinogen levels was explored by Mutanen. Eighty men were administered a diet rich in SAFA derived from dairy products and were subsequently randomly assigned to groups that received either *trans* fatty acids (derived from partially hydrogenated vegetable oil) or stearic acid (replacing 8.8 and 9.3% respectively of the daily caloric intake with respect to the basal diet). The stearic acid-rich diet significantly increased the level of fibrinogen in comparison with the *trans* fatty acid-rich diet. Among the saturated fats, different fatty acids may have different effects [58]. In a study carried out by Baer *et al.,* 50 men consumed six diets, each of them rich in a different SAFA and oleic acid. The period during which they were given stearic acid produced higher levels of fibrinogen than any of the other periods, including that on oleic acid [59]. In a study performed on Chilean students, allocation to a diet characterised as Mediterranean, which included 32 mL of olive oil, rather than to another rich in saturated fat [40], lowered basal fibrinogen levels, although the results may have been affected to a certain extent by the fact that they consumed a larger amount of fish in their MD model. These studies contrast with another recently published investigation, in which 7% of the total daily energy of 45 subjects (27 men and 18 women) was provided by different diets (stearic, oleic and linoleic acids) but no differences in basal fibrinogen levels were found after five weeks on the diets [60]. In fact, there is still a certain controversy regarding whether the consumption of oleic acid-rich diets lowers basal fibrinogen levels more than do diets rich in saturated fats.

2.4 Plasminogen activator inhibitor-1 (PAI-1)

In the stabilisation and progress of thrombus, fibrinolysis plays an important role as a mechanism that is regulated by the equilibrium between tissue plasminogen activator and its strongest natural inhibitor, PAI-1. Few studies have attempted to determine the influence of dietary factors on fibrinolysis, and most of these utilised omega-3 fatty acids.

Studies that have been carried out to date using MUFA support findings of a fall in PAI-1 following their ingestion [7, 15]. Our own group has demonstrated a decline in levels of PAI-1 of MUFA compared with SAFA [53], and *vis-à-vis* other low-fat diets [53, 61]. In a rural Italian population a decline in levels of PAI-1 was observed following a change from a western to a Mediterranean diet. Moreover, this sample returned to its original PAI-1 levels on resuming the western diet [62]. Another study of 44 hypertension patients compared the influence of two diets (one of them rich in olive oil and the other in soya oil) on arterial tension and haemostatic markers. Both diets produced a fall in systolic pressure and diminished PAI-1 in comparison with the SAT-rich control diet, although the effects of the olive oil-enriched diet were more evident [63]. Mezzano found a decrease of around 20% in PAI-1 on administering two diets, one of them containing MUFA and the other SAFA, without observing any difference between them. However, both levels rose when the diets were supplemented with red wine [40]. In spite of these results, these results have not otherwise been confirmed in subjects with altered glucose tolerance, since no differences were found between a SAFA diet, one that was low in fat or a high-MUFA diet [64].

We may therefore conclude that the oleic acid-rich diet lowers basal levels of PAI-1 compared to SAFA. However, it has not been demonstrated that these results would be reproduced with PUFA [37], or postprandially [43]. Finally, a recent study performed in normal subjects showed that the administration of olive oil induced a lower postprandial

response in PAI-1 and TF than the ingestion of high-palmitic sunflower oil and butter [65] (Table 1).

2.5 TBX

The TBX are cyclic derivatives of arachidonic acid, like the prostaglandins and leucotriens. They are vasoconstrictors, bronchoconstrictors and platelet aggregation inductors. Sirtori noted a fall in the production of platelet TBX_2 following an olive oil-rich diet, compared with a basal SAFA-rich diet and another diet that was rich in maize oil [21].This fall was subsequently reproduced in an animal model [66]. In support of this effect, urinary TBX metabolites fall after a MUFA-rich diet as opposed to n-6 [67]. In short, the avail-

Table 1. Summary of studies testing the effects of olive oil on PAI-1 concentration

Author	Population	Intervention	Conclusion
Lopez Segura *et al* [61]	21 healthy males	– two olive oil rich diets containing 115 & 280 mg cholesterol/1000 calories – two low fat diets with the same cholesterol amounts	The two olive oil rich diets decreased PAI-1 versus the low fat diets
Perez Jimenez *et al* [53]	25 healthy males	– Palmitic acid rich diet – Olive oil rich diet – Low fat diet	The isocaloric replacement of palmitic acid rich diet by the other two diets lowered the PAI-1 (higher decrease after olive oil rich one)
Avellone et al [107]	– 40 rural subjects – 40 urban subjects	– High cholesterol, fat diet rich in SAFA (Urban diet) for 8 weeks – MD (rich in olive oil) 8 weeks – Crossover design with return to baseline diet	MD lowered PAI-1 concentration. Return to higher PAI-1 levels when returning to the Urban diet
Trifiletti *et al* [63]	44 subjects with hypertension	– Olive oil rich diet – Soya oil rich diet – SAT-rich control diet	Decrease of PAI-1 with the two first diets versus the SAT diet. The decrease was larger after the olive oil diet
Mezzano *et al* [40]	42 healthy males	– MD (high in vegetables and fish. Average of 32 mL olive oil/day). – High fat diet (saturated). Each diet lasting 90 days. During the 30 to the 60 days of the study, 240 mL red wine were added	A reduction of 20% versus the baseline values was found irrespectively of the diet. Red wine induced a increase of PAI-1
Niskanen *et al* [64]	– 28 subjects with impaired glucose tolerance: 17 men and 11 women	– SAT rich control diet for 3 weeks – 12 received subsequently a monounsaturated rich diet for 8 weeks (low erucic acid rapeseed oil and high-oleic acid sunflower oil as monounsaturated sources). – 18 received NCEP diet for 8 weeks	No differences between the groups
Turpeinen *et al* [37]	38 healthy persons (20 men, 18 women)	– Baseline SAT diet for 4 weeks. The participants received after one of the following two diets for another 4 weeks: – linoleic acid rich diet (PUFA). – oleic acid rich diet (MUFA).	No differences between groups
Oakley *et al* [43]	12 healthy men	– Three isolated fat meals containing high oleate (high- oleic acid sunflower oil), butter or high oleate plus medium chain triacylglycerols and a low fat meal (isoenergetic to the other three)	No differential effects of the isolated meals
Pacheco *et al* [65]	14 healthy males	– 1 week on NCEP step1 adaptation period – Postprandial state evaluation after two isolated fat meals containing extra-virgin or refined olive oil in a cross-over design	Bigger decrease (-92.6 ± 11.4 VS -76.1 ± 15.2) in the incremental area under curve after the extra-virgin olive oil meal compared with the refined oil meal.

able data suggest that MUFA have the effect of reducing the concentration and metabolism of TBX. However, the influence of minor components of olive oil on TBX appears to be clearer than those of oleic acid, as we shall see below.

2.6 vWF

vWF is a fundamental component of platelet adhesion and aggregration processes. This prothrombotic product is reduced in diabetic patients who are fed a MUFA-rich diet rather than a carbohydrate-rich [68] or a high-PUFA diet [69]. Our group carried out a study of 25 healthy males, based on three periods of dietary intervention (low-fat, MUFA and SAFA), each period having a duration of 28 days. At the end of each period we measured serum markers of endothelial function, including vWF. Levels of vWF fell following the MUFA-rich diet, as did those of PAI-1 and TFPI [53]. However, Mezzano found no differences when he gave two groups of 21 young healthy males a MUFA-rich diet followed by a SAFA-rich diet after 30 days of the first [40, 70]. These results agree with those of another study carried out by Turpeinen (1999), which compared the effects of a chronic intervention with oleic acid or linoleic acid on 38 persons who had previously been given a SAFA-rich diet [37]. In short, intervention study data indicate that MUFA-rich diets are capable of lowering fasting vWF levels, although in view of the wide differences in the results, more studies are still needed.

3 Other minor components of olive oil

Olive oil consists of 90 – 99% fat and an insaponifiable fraction of 0.4 – 5%. Of the latter set of components, great importance has been ascribed to phenolic compounds during the past ten years due to their wide range of biological properties. This category includes simple compounds such as phenolic acids (caffeinic, vanillic, gallic and coumaric acid), tyrosol and hydroxytyrosol, and other more complex substances such as secoiridoids (oleuropein and ligstroside), and lignans (1-acetoxypinoresinol and pinoresinol). In the use of the nomenclature of this group of substances, it is worth remembering that the first group of these (the simple phenols) contain a single phenolic ring, which means that it is not legitimate to call them "polyphenols". For this reason we use the term "phenols" rather than "polyphenols" in referring to the characteristics of this group.

It is important to point out that refined oils do not contain significant proportions of phenols, which are destroyed during the refining process. Moreover, there are wide variations in the concentration of phenols in different olive oils, depending on such factors as the variety of olive, the climate in which it grows and the ripeness of the fruit when it is harvested. Oleuropein, for example, is the most important phenol in unripe olives, and it hydrolyses into simple phenols as the fruits ripen, being present in much lower concentrations in the oil [71 – 73]. Although phenols are the most widely studied of the minor components of olive oil, there are others which cannot be classified as belonging to this family, and which may possess additional properties of interest.

3.1 Phenols

The study of these components has intensified in the course of the past ten years, and their presence in certain vegetable products such as tea, grape juice, wine and red grapes has been related to antioxidant and antiinflammatory properties [18, 74], and to improvements in fasting endothelial function [75 – 77].

Our group has published a study that demonstrated a protective effect of phenols from virgin olive oil on postprandial endothelial function in hypercholesterolaemic subjects [78].

With regard to their influence on haemostasis, there is evidence to suggest that these compounds affect platelet aggregation, according to data from observation-based studies, in which the consumption of phenol-enriched foods was linked to a fall in cardiovascular disease. However, there exist few clinical studies directly related to the action of phenols on haemostasis, and most of them have been done using fruit juice rather than olive oil [79 – 82]. The results of these studies vary, but generally speaking, they suggest that there is an antithrombotic effect. For example, a phenol-rich diet (purple grape juice, 5 – 7.5 mL/kg/day) administered to ten subjects lowered platelet aggregation in response to collagen by 77% [83].

In vitro studies have shown that phenols can lower platelet aggregation in response to ADP or collagen, as well as the production of TBX_2 [84]. Leger demonstrated a fall of 46% in this last after administering 25 mg of hydroxytyrosol obtained from olive oil to healthy volunteer subjects for three days [85], while Visioli found a reduction of 20% in TXB_2 in hyperlipaemic subjects following chronic consumption of an olive oil rich in phenols compared with a similar oil that lacked these substances [86]. Other data in support of this effect are that virgin olive oil reduces the production of TBX_2 more than high-oleic sunflower oil, which contains a higher percentage of oleic acid than olive oil itself, for which reason the fall may be attributable to its minor components [87].

In addition to their action during fasting, tyrosol and hydroxytyrosol also lower postprandial production of of TBX_2, as was shown by Bogani, compared with refined olive oil or maize oil [88].

As well as tyrosol and hydroxytyrosol, other phenols found in olive oil may be responsible for its properties. For example, quercetin, a flavonoid found in virgin olive oil, inhibits platelet aggregation both *in vitro* [89] and *ex vivo* [90, 91], although its activity *in vivo* has yet to be confirmed.

Rechner submitted platelets to a series of procedures in order to increase their activation, aggregation and expression of prothrombotic molecules such as P-selectin and CD63 markers (membrane glycoproteins that appear with the expression of lysosomes). Incubating them with a mixture of antocyanines and colonic phenol derivatives reduced both thrombin-dependent aggregation (but not that induced by collagen) and platelet activation (the latter measured in terms of P-selectin production and the expression of the pro-oxidant CD63 marker) [92]. Other authors have demonstrated the biological activity of various metabolites of hydroxytyrosol. Togno studied the activity of two such hydroxytyrosol derivatives known as isochromans by combining them with compounds containing carbonyl groups also present in the oil. These products are more efficient than hydroxytyrosol itself as inhibitors of collagen- and arachidonate-dependent platelet aggregation, but not of aggregation produced by ADP [93].

3.2 Minor non-phenolic components

Olive oil contains thousands of other components. A recent study demonstrated a fall in TBX_2 produced by normal human umbilical vein endothelial cells following incubation with postprandial triacylglycerol-rich lipoprotein derived from an olive oil whose concentration of insaponifiable particles had been doubled, in comparison with a virgin olive oil and sunflower oil [94]. The authors suggested that, besides the phenolic compounds themselves, other substances might be responsible for part of these results since, because of their hydrophilic nature, the phenols were not incorporated into these postprandial triacylglycerol-rich lipoprotein. Furthermore, olive oil phenols do not affect cyclooxygenase activity in rats, a finding that contradicts their potential effect on the TBX. In addition, a recent study performed in rabbits, showed that in animals fed olive oil or olive oil polar lipid extract blood platelet-activating factor-acetylhydrolase acetylhydrolase increased, platelet aggregation was attenuated, less oxidation occurred in plasma, lesion thickness was reduced and vessel walls retained elasticity. Most of these beneficial changes were not seen in animals fed olive oil neutral lipid extract although blood platelet-activating factor and plasma oxidation were lower[95]. These same authors found a decrease in ADP-induced platelet agreggation and platelet-activation factor when healthy and patients with type 2 Diabetes Mellitus followed a diet rich in traditional Greek meals with high anti-aggregating activity for a period of 28 days[96].

The identity of these other substances has yet to be clarified. B-sitosterol, tocopherols and triterpenoids have all been suggested, due to their inhibitory action on the production of prostaglandin E2 by macrophages, a circumstance that correlates with the activity of cyclooxygenase. Others, like glycerinetheric glycolipid contain high platelet-activation factor antagonists, which reduces directly platelet aggregation. Many other compounds, as we have said before, are being tested, as possible additional healthy micropounds of virgin olive oil.

4 Relationship between thrombosis and the endothelium

The initial alteration that precedes the development of arteriosclerosis is the activation of the endothelium, through which the cells that line the vessel walls express, on the surface of their lumen, a combination of molecules that encourage the adhesion and migration of the circulating mononuclear cells to the sub endothelial space. This forms the initiation of the inflammatory process, one of the consequences of which is the loss of endothelial functions. Among these functions are the well-known vasodilatory response that is dependent on the production of nitric oxide, and its capacity to reduce thrombogenesis, which is the subject of this review. The cellular mechanism, which mediates the expression of the genes involved in the inflammatory response, both in the endothelium and in other cells that contribute to the inflammation of the vascular walls, depends on the cytoplasmic expression of the transcription factors. Among them, NF-κB is particularly interesting as the mediator sensitive to oxidative changes, and as that which induces the activation of the genes that are involved in the synthesis of the adhesion molecules. Of special interest in this respect is the demonstration that oleic acid buffers the inflammatory process that leads to endothelial dysfunction [97 – 99]. Although we now find ourselves in fairly speculative field, where we do not even know what initiates the initial phenomenon of wall lesions, the anti-inflammatory effect of olive oil needs to be considered in the context of the interaction produced during the ingestion of high-energy diets that are capable of promoting the overproduction of reactive species of oxygen and inducing changes in fraction 3 of the complement [100, 101]. Data exist that suggest that fats with antioxidative or membrane-stabilising capacity might be capable of protecting endothelial cells [102]. In this context, olive oil has such an ability, both through its high content of MUFA [103 – 105] and via the antioxidative effect of its microcomponents, particularly of phenolic compounds, among which it is typical the oleuropein, an aglycone the hydrolysis of which generates tyrosol and hydroxytyrosol, which in both their free, secoroid and conjugated forms make up some 80% of the phenolic compounds of virgin olive oil. These products are absorbed by the intestine in human beings, as has been demonstrated by experimental studies, possess antioxidative and anti-inflammatory properties, and are also capable of modifying haemostasis, inhibiting platelet aggregation and displaying antithrombotic properties [106].

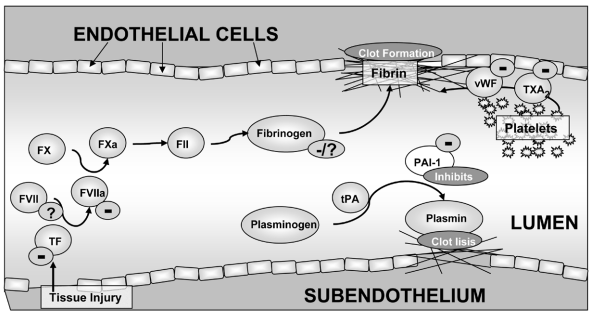

Figure 1. Effect of olive oil on coagulation. Coagulation factors influenced by olive oil components:
⊖: Inhibition. ?: Further information needed.

5 Conclusions

We may conclude that MUFA have positive effects on a number of factors that are responsible for haemostasis (Fig. 1), such as platelet aggregation and FVII. Although there is less evidence for the following claims, data do exist that suggest that MUFA also lower fibrinogen, PAI-1 and vWF.

Apart from MUFA, other minor components of olive oil also influence markers of haemostasis. Current evidence indicates that phenols reduce platelet aggregation, probably by reducing TBX, while other effects of these and other minor components are also under study.

Finally, it is important to emphasise that olive oil is the basic, though not the only, component that produces the health-promoting effects of the Mediterranean diet. The combination of high consumption of fruit, vegetable, cereals and fish with low consumption of saturated fats, adds up to a dietary model with cardioprotective properties, to which we may add other potential benefits, which will need to be studied in greater depth in the future, such as a reduction in the risk of suffering certain types of cancer and a better quality of life in the course of ageing.

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6 References

[1] Nylaende, M., Kroese, A., Stranden, E., Morken, B., *et al.*, Prothrombotic activity is associated with the anatomical as well as the functional severity of peripheral arterial occlusive disease. *Thromb. Haemost.* 2006, *95*, 702 – 707.

[2] Libby, P., Theroux, P., Pathophysiology of coronary artery disease. *Circulation* 2005, *111*, 3481 – 3488.

[3] Fuster, V., Badimon, L., Badimon, J. J., Chesebro, J. H., The pathogenesis of coronary artery disease and the acute coronary syndromes (1), *N. Engl. J. Med.* 1992, *326*, 242 – 250.

[4] Badimon, J. J., Fuster, V., Chesebro, J. H., Badimon, L., Coronary atherosclerosis. A multifactorial disease. *Circulation* 1993, *87*, 3 – 16.

[5] Kris-Etherton, P. M., A new role for diet in reducing the incidence of cardiovascular disease: evidence from recent studies. *Curr. Atheroscler. Rep.* 1999, *1*, 185 – 187.

[6] Mustad, V. A., Kris-Etherton, P. M., Beyond cholesterol lowering: deciphering the benefits of dietary intervention on cardiovascular diseases. *Curr. Atheroscler. Rep.* 2000, *2*, 461 – 466.

[7] Perez-Jimenez, F., International conference on the healthy effect of virgin olive oil. *Eur. J. Clin. Invest.* 2005, *35*, 421 – 424.

[8] Serra-Majem, L., Ngo de la Cruz, J., Ribas, L., Tur, J. A., Olive oil and the Mediterranean diet: beyond the rhetoric. *Eur. J. Clin. Nutr.* 2003, *57*, S2 – S7.

[9] de Lorgeril, M., Renaud, S., Mamelle, N., Salen, P., *et al.*, Mediterranean alpha-linolenic acid-rich diet in secondary prevention of coronary heart disease. *Lancet* 1994, *343*, 1454 – 1459.

[10] Singh, R. B., Dubnov, G., Niaz, M. A., Ghosh, S., *et al.*, Effect of an Indo-Mediterranean diet on progression of coronary artery disease in high risk patients (Indo-Mediterranean Diet Heart Study): a randomised single-blind trial. *Lancet* 2002, *360*, 1455 – 1461.

[11] Owen, R. W., Giacosa, A., Hull, W. E., Haubner, R., *et al.*, Olive-oil consumption and health: the possible role of antioxidants. *Lancet Oncol.* 2000, *1*, 107 – 112.

[12] Keys, A., Coronary heart disease in seven countries. *Circulation* 1970, *41*, 1 – 211.

[13] Mensink, R. P., Zock, P. L., Kester, A. D., Katan, M. B., Effects of dietary fatty acids and carbohydrates on the ratio of serum total to HDL cholesterol and on serum lipids and apolipoproteins: a meta-analysis of 60 controlled trials. *Am. J. Clin. Nutr.* 2003, *77*, 1146 – 1155.

[14] Perez Jimenez, F., Fuentes, F., Fernandez de la Puebla, R. A., Lopez-Miranda, J., Efectos pleiotrópicos de la grasa de la dieta sobre el riesgo cardiovascular. *Clin. Invest. Arterioscler.* 2003, *15*, 27.

[15] Perez-Jimenez, F., Lopez-Miranda, J., Mata, P., Protective effect of dietary monounsaturated fat on arteriosclerosis: beyond cholesterol. *Atherosclerosis* 2002, *163*, 385 – 398.

[16] Mutanen, M., Freese, R., Fats, lipids and blood coagulation. *Curr. Opin. Lipidol.* 2001, *12*, 25 – 29.

[17] Moreno, J. J., Mitjavila, M. T., The degree of unsaturation of dietary fatty acids and the development of atherosclerosis (Review). *J. Nutr. Biochem.* 2003, *14*, 182 – 195.

[18] Visioli, F., Bogani, P., Grande, S., Galli, C., Mediterranean food and health: building human evidence. *J. Physiol. Pharmacol.* 2005, *56*, 37 – 49.

[19] Smith, R. D., Kelly, C. N., Fielding, B. A., Hauton, D., *et al.*, Long-term monounsaturated fatty acid diets reduce platelet aggregation in healthy young subjects. *Br. J. Nutr.* 2003, *90*, 597 – 606.

[20] Misikangas, M., Freese, R., Turpeinen, A. M., Mutanen, M., High linoleic acid, low vegetable, and high oleic acid, high vegetable diets affect platelet activation similarly in healthy women and men. *J. Nutr.* 2001, *131*, 1700 – 1705.

[21] Sirtori, C. R., Tremoli, E., Gatti, E., Montanari, G., *et al.*, Controlled evaluation of fat intake in the Mediterranean diet: comparative activities of olive oil and corn oil on plasma lipids and platelets in high-risk patients. *Am. J. Clin. Nutr.* 1986, *44*, 635 – 642.

[22] Karantonis, H. C., Fragopoulou, E., Antonopoulou, S., Rementzis, J., *et al.*, Effect of fast-food Mediterranean-type diet on type 2 diabetics and healthy human subjects' platelet aggregation. *Diabetes Res. Clin. Pract.* 2006, *72*, 33 – 41.

[23] Vicario, I. M., Malkova, D., Lund, E. K., Johnson, I. T., Olive oil supplementation in healthy adults: effects in cell membrane fatty acid composition and platelet function. *Ann. Nutr. Metab.* 1998, *42*, 160 – 169.

[24] Karantonis, H. C., Antonopoulou, S., Demopoulos, C. A., Antithrombotic lipid minor constituents from vegetable oils. Comparison between olive oils and others. *J. Agric. Food Chem.* 2002, *50*, 1150 – 1160.

[25] Marckmann, P., Sandstrom, B., Jespersen, J., Effects of total fat content and fatty acid composition in diet on factor VII coagulant activity and blood lipids. *Atherosclerosis* 1990, *80*, 227 – 233.

[26] Kelly, C. M., Smith, R. D., Williams, C. M., Dietary monounsaturated fatty acids and haemostasis. *Proc. Nutr. Soc.* 2001, *60*, 161 – 170.

[27] Larsen, L. F., Bladbjerg, E. M., Jespersen, J., Marckmann, P., Effects of dietary fat quality and quantity on postprandial activation of blood coagulation factor VII. *Arterioscler. Thromb. Vasc. Biol.* 1997, *17*, 2904 – 2909.

[28] Mennen, L. I., de Maat, M. P., Schouten, E. G., Kluft, C., *et al.*, Dietary effects on coagulation factor VII vary across genotypes of the R/Q353 polymorphism in elderly people. *J. Nutr.* 1998, *128*, 870 – 874.

[29] Miller, G. L., Birzgalis, R., Carboxymethylcellulase and cellopentase activities of electrophoretic fractions of cellulase. *Arch. Biochem. Biophys.* 1961, *95*, 19 – 24.

[30] Poppitt, S., Postprandial Lipaemia, Haemostasis, Inflammatory Response and other Emerging Risk Factors for Cardiovascular Disease: The Influence of Fatty Meals. *Curr. Nutr.-Food Sci.* 2005, *1*, 23 – 34.

[31] Williams, C. M., Beneficial nutritional properties of olive oil: implications for postprandial lipoproteins and factor VII. *Nutr. Metab. Cardiovasc. Dis.* 2001, *11*, 51 – 56.

[32] Freese, R., Mutanen, M., Postprandial changes in platelet function and coagulation factors after high-fat meals with different fatty acid compositions. *Eur. J. Clin. Nutr.* 1995, *49*, 658 – 664.

[33] Hunter, K. A., Crosbie, L. C., Horgan, G. W., Miller, G. J., Dutta-Roy, A. K., Effect of diets rich in oleic acid, stearic acid and linoleic acid on postprandial haemostatic factors in young healthy men. *Br. J. Nutr.* 2001, *86*, 207 – 215.

[34] Sanders, T. A., Oakley, F. R., Crook, D., Cooper, J. A., Miller, G. J., High intakes of trans monounsaturated fatty acids taken for 2 weeks do not influence procoagulant and fibrinolytic risk markers for CHD in young healthy men. *Br. J. Nutr.* 2003, *89*, 767 – 776.

[35] Sanders, T. A., Oakley, F. R., Cooper, J. A., Miller, G. J., Influence of a stearic acid-rich structured triacylglycerol on postprandial lipemia, factor VII concentrations, and fibrinolytic activity in healthy subjects. *Am. J. Clin. Nutr.* 2001, *73*, 715 – 721.

[36] Tholstrup, T., Marckmann, P., Hermansen, J., Holmer, G., Sandstrom, B., Effect of modified dairy fat on fasting and postprandial haemostatic variables in healthy young men. *Br. J. Nutr.* 1999, *82*, 105 – 113.

[37] Turpeinen, A. M., Mutanen, M., Similar effects of diets high in oleic or linoleic acids on coagulation and fibrinolytic factors in healthy humans. *Nutr. Metab. Cardiovasc. Dis.* 1999, *9*, 65 – 72.

[38] Temme, E. H., Mensink, R. P., Hornstra, G., Effects of diets enriched in lauric, palmitic or oleic acids on blood coagulation and fibrinolysis. *Thromb. Haemost.* 1999, *81*, 259 – 263.

[39] Junker, R., Kratz, M., Neufeld, M., Erren, M., *et al.*, Effects of diets containing olive oil, sunflower oil, or rapeseed oil on the hemostatic system. *Thromb. Haemost.* 2001, *85*, 280 – 286.

[40] Mezzano, D., Leighton, F., Haemostatic cardiovascular risk factors: differential effects of red wine and diet on healthy young. *Pathophysiol. Haemost. Thromb.* 2003, *33*, 472 – 478.

[41] Roche, H. M., Gibney, M. J., Postprandial coagulation factor VII activity: the effect of monounsaturated fatty acids. *Br. J. Nutr.* 1997, *77*, 537 – 549.

[42] Sanders, T. A., de Grassi, T., Miller, G. J., Humphries, S. E., Dietary oleic and palmitic acids and postprandial factor VII in middle-aged men heterozygous and homozygous for factor VII R353Q polymorphism. *Am. J. Clin. Nutr.* 1999, *69*, 220 – 225.

[43] Oakley, F. R., Sanders, T. A., Miller, G. J., Postprandial effects of an oleic acid-rich oil compared with butter on clotting factor VII and fibrinolysis in healthy men. *Am. J. Clin. Nutr.* 1998, *68*, 1202 – 1207.

[44] Tholstrup, T., Miller, G. J., Bysted, A., Sandstrom, B., Effect of individual dietary fatty acids on postprandial activation of blood coagulation factor VII and fibrinolysis in healthy young men. *Am. J. Clin. Nutr.* 2003, *77*, 1125 – 1132.

[45] Roche, H. M., Zampelas, A., Knapper, J. M., Webb, D., *et al.*, Effect of long-term olive oil dietary intervention on postprandial triacylglycerol and factor VII metabolism. *Am. J. Clin. Nutr.* 1998, *68*, 552 – 560.

[46] Larsen, L. F., Jespersen, J., Marckmann, P., Are olive oil diets antithrombotic? Diets enriched with olive, rapeseed, or sunflower oil affect postprandial factor VII differently. *Am. J. Clin. Nutr.* 1999, *70*, 976 – 982.

[47] Silva, K. D., Kelly, C. N., Jones, A. E., Smith, R. D., *et al.*, Chylomicron particle size and number, factor VII activation and dietary monounsaturated fatty acids. *Atherosclerosis* 2003, *166*, 73 – 84.

[48] Tremoli, E., Eligini, S., Colli, S., Maderna, P., *et al.*, Effects of omega 3 fatty acid ethyl esters on monocyte tissue factor expression. *World Rev. Nutr. Diet.* 1994, *76*, 55 – 59.

[49] Tremoli, E., Eligini, S., Colli, S., Maderna, P., *et al.*, n-3 fatty acid ethyl ester administration to healthy subjects and to hypertriglyceridemic patients reduces tissue factor activity in adherent monocytes. *Arterioscler. Thromb.* 1994, *14*, 1600 – 1608.

[50] Motton, D. D., Mackman, N., Tilley, R. E., Rutledge, J. C., Postprandial elevation of tissue factor antigen in the blood of healthy adults. *Thromb. Haemost.* 2005, *94*, 504 – 509.

[51] Bravo-Herrera, M. D., Lopez-Miranda, J., Marin, C., Gomez, P. *et al.*, Tissue factor expression is decreased in monocytes obtained from blood during Mediterranean or high carbohydrate diets. *Nutr. Metab. Cardiovasc. Dis.* 2004, *14*, 128 – 132.

[52] Abumiya, T., Nakamura, S., Takenaka, A., Takenaka, O., *et al.*, Response of plasma tissue factor pathway inhibitor to diet-induced hypercholesterolemia in crab-eating monkeys. *Arterioscler. Thromb.* 1994, *14*, 483 – 488.

[53] Perez-Jimenez, F., Castro, P., Lopez-Miranda, J., Paz-Rojas, E., *et al.*, Circulating levels of endothelial function are modulated by dietary monounsaturated fat. *Atherosclerosis* 1999, *145*, 351 – 358.

[54] Goyens, P. L., Mensink, R. P., Effects of alpha-linolenic acid versus those of EPA/DHA on cardiovascular risk markers in healthy elderly subjects. *Eur. J. Clin. Nutr.* 2006, *60*, 978 – 984.

[55] Zahedi, R. G., Summers, L. K., Lumb, P., Chik, G., Crook, M. A., The response of serum sialic acid and other acute phase reactants to an oral fat load in healthy humans. *Eur. J. Intern. Med.* 2001, *12*, 510 – 514.

[56] Poppitt, S. D., Keogh, G. F., Mulvey, T. B., Phillips, A., *et al.*, Effect of moderate changes in dietary fatty acid profile on postprandial lipaemia, haemostatic and related CVD risk factors in healthy men. *Eur. J. Clin. Nutr.* 2004, *58*, 819 – 827.

[57] Kozima, Y., Urano, T., Serizawa, K., Takada, Y., Takada, A., Impaired fibrinolytic activity induced by ingestion of butter: effect of increased plasma lipids on the fibrinolytic activity. *Thromb. Res.* 1993, *70*, 191 – 202.

[58] Bladbjerg, E. M., Tholstrup, T., Marckmann, P., Sandstrom, B., Jespersen, J., Dietary changes in fasting levels of factor VII coagulant activity (FVII:C) are accompanied by changes in factor VII protein and other vitamin K-dependent proteins. *Thromb. Haemost.* 1995, *73*, 239 – 242.

[59] Baer, D. J., Judd, J. T., Clevidence, B. A., Tracy, R. P., Dietary fatty acids affect plasma markers of inflammation in healthy men fed controlled diets: a randomized crossover study. *Am. J. Clin. Nutr.* 2004, *79*, 969 – 973.

[60] Thijssen, M. A., Hornstra, G., Mensink, R. P., Stearic, oleic, and linoleic acids have comparable effects on markers of thrombotic tendency in healthy human subjects. *J. Nutr.* 2005, *135*, 2805 – 2811.

[61] Lopez-Segura, F., Velasco, F., Lopez-Miranda, J., Castro, P., *et al.*, Monounsaturated fatty acid-enriched diet decreases plasma plasminogen activator inhibitor type 1. *Arterioscler. Thromb. Vasc. Biol.* 1996, *16*, 82 – 88.

[62] Avellone, G., Cordova, R., Scalffidi, L., Bompiani, G., Effects of Mediterranean diet on lipid, coagulative and fibrinolytic parameters in two randomly selected population samples in Western Sicily. *Nutr. Metab. Cardiovasc. Dis.* 1998, *8*, 287 – 296.

[63] Trifiletti, A., Scamardi, R., Gaudio, A., Lasco, A., Frisina, N., Hemostatic effects of diets containing olive or soy oil in hypertensive patients. *J. Thromb. Haemost.* 2005, *3*, 179 – 180.

[64] Niskanen, L., Schwab, U. S., Sarkkinen, E. S., Krusius, T., *et al.*, Effects of dietary fat modification on fibrinogen, factor VII, and plasminogen activator inhibitor-1 activity in subjects with impaired glucose tolerance. *Metabolism* 1997, *46*, 666 – 672.

[65] Pacheco, Y. M., Lopez, S., Bermudez, B., Abia, R., Muriana, F. J., Extra-virgin vs. refined olive oil on postprandial hemostatic markers in healthy subjects. *J. Thromb. Haemost.* 2006, *4*, 1421 – 1422.

[66] Navarro, M. D., Hortelano, P., Periago, J. L., Pita, M. L., Effect of dietary olive and sunflower oils on the lipid composition of the aorta and platelets and on blood eicosanoids in rats. *Arterioscler. Thromb.* 1992, *12*, 830 – 835.

[67] Lahoz, C., Alonso, R., Ordovas, J. M., Lopez-Farre, A., *et al.*, Effects of dietary fat saturation on eicosanoid production, platelet aggregation and blood pressure. *Eur. J. Clin. Invest.* 1997, *27*, 780 – 787.

[68] Rasmussen, O., Thomsen, C., Ingerslev, J., Hermansen, K., Decrease in von Willebrand factor levels after a high-monounsaturated-fat diet in non-insulin-dependent diabetic subjects. *Metabolism* 1994, *43*, 1406 – 1409.

[69] Thomsen, C., Rasmussen, O. W., Ingerslev, J., Hermansen, K., Plasma levels of von Willebrand factor in non-insulin-dependent diabetes mellitus are influenced by dietary monounsaturated fatty acids. *Thromb. Res.* 1995, *77*, 347 – 356.

[70] Mezzano, D., Leighton, F., Strobel, P., Martinez, C., *et al.*, Mediterranean diet, but not red wine, is associated with beneficial changes in primary haemostasis. *Eur. J. Clin. Nutr.* 2003, *57*, 439 – 446.

[71] Visioli, F., Galli, C., The effect of minor constituents of olive oil on cardiovascular disease: New findings. *Nutr. Rev.* 1998, *56*, 142 – 147.

[72] Esti, M., Cinquanta, L., La Notte, E., Phenolic Compounds in Different Olive Varieties. *J. Agric. Food Chem.* 1998, *46*, 32 – 35.

[73] Tripoli, E., Giammanco, M., Tabacchi, G., Di Majo, D., *et al.*, The phenolic compounds of olive oil: structure, biological activity and beneficial effects on human health. *Nutr. Res. Rev.* 2005, *18*, 98 – 112.

[74] Visioli, F., Poli, A., Gall, C., Antioxidant and other biological activities of phenols from olives and olive oil. *Med. Res. Rev.* 2002, *22*, 65 – 75.

[75] Duffy, S. J., Keaney, J. F., Jr., Holbrook, M., Gokce, N., *et al.*, Short- and long-term black tea consumption reverses endothelial dysfunction in patients with coronary artery disease. *Circulation* 2001, *104*, 151 – 156.

[76] Stein, J. H., Keevil, J. G., Wiebe, D. A., Aeschlimann, S., Folts, J. D., Purple grape juice improves endothelial function and reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery disease. *Circulation* 1999, *100*, 1050 – 1055.

[77] Chou, E. J., Keevil, J. G., Aeschlimann, S., Wiebe, D. A., *et al.*, Effect of ingestion of purple grape juice on endothelial function in patients with coronary heart disease. *Am. J. Cardiol.* 2001, *88*, 553 – 555.

[78] Ruano, J., Lopez-Miranda, J., Fuentes, F., Moreno, J. A., *et al.*, Phenolic content of virgin olive oil improves ischemic reactive hyperemia in hypercholesterolemic patients. *J. Am. Coll. Cardiol.* 2005, *46*, 1864 – 1868.

[79] Freedman, J. E., Parker, C., 3rd, Li, L., Perlman, J. A., *et al.*, Select flavonoids and whole juice from purple grapes inhibit platelet function and enhance nitric oxide release. *Circulation* 2001, *103*, 2792 – 2798.

[80] Rein, D., Paglieroni, T. G., Wun, T., Pearson, D. A., *et al.*, Cocoa inhibits platelet activation and function. *Am. J. Clin. Nutr.* 2000, *72*, 30 – 35.

[81] Murphy, K. J., Chronopoulos, A. K., Singh, I., Francis, M. A., *et al.*, Dietary flavanols and procyanidin oligomers from cocoa (Theobroma cacao) inhibit platelet function. *Am. J. Clin. Nutr.* 2003, *77*, 1466 – 1473.

[82] Vita, J. A., Polyphenols and cardiovascular disease: effects on endothelial and platelet function. *Am. J. Clin. Nutr.* 2005, *81*, 292S – 297S.

[83] Keevil, J. G., Osman, H. E., Reed, J. D., Folts, J. D., Grape juice, but not orange juice or grapefruit juice, inhibits human platelet aggregation. *J. Nutr.* 2000, *130*, 53 – 56.

[84] Petroni, A., Blasevich, M., Salami, M., Papini, N., *et al.*, Inhibition of platelet aggregation and eicosanoid production by phenolic components of olive oil. *Thromb. Res.* 1995, *78*, 151 – 160.

[85] Leger, C. L., Carbonneau, M. A., Michel, F., Mas, E., *et al.*, A thromboxane effect of a hydroxytyrosol-rich olive oil wastewater extract in patients with uncomplicated type I diabetes. *Eur. J. Clin. Nutr.* 2005, *59*, 727 – 730.

[86] Visioli, F., Caruso, D., Grande, S., Bosisio, R., *et al.*, Virgin Olive Oil Study (VOLOS): vasoprotective potential of extra virgin olive oil in mildly dyslipidemic patients. *Eur. J. Nutr.* 2005, *44*, 121 – 127.

[87] Oubina, P., Sanchez-Muniz, F. J., Rodenas, S., Cuesta, C., Eicosanoid production, thrombogenic ratio, and serum and LDL peroxides in normo- and hypercholesterolaemic postmenopausal women consuming two oleic acid-rich diets with different content of minor components. *Br. J. Nutr.* 2001, *85*, 41 – 47.

[88] Bogani, P., Galli, C., Villa, M., Visioli, F., Postprandial anti-inflammatory and antioxidant effects of extra virgin olive oil. *Atherosclerosis* 2007, *190*, 181 – 186.

[89] Hubbard, G. P., Wolffram, S., Lovegrove, J. A., Gibbins, J. M., The role of polyphenolic compounds in the diet as inhibitors of platelet function. *Proc. Nutr. Soc.* 2003, *62*, 469 – 478.

[90] Hubbard, G. P., Wolffram, S., de Vos, R., Bovy, A., *et al.*, Ingestion of onion soup high in quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in man: a pilot study. *Br. J. Nutr.* 2006, *96*, 482 – 488.

[91] Hubbard, G. P., Wolffram, S., Lovegrove, J. A., Gibbins, J. M., Ingestion of quercetin inhibits platelet aggregation and essential components of the collagen-stimulated platelet activation pathway in humans. *J. Thromb. Haemost.* 2004, *2*, 2138 – 2145.

[92] Rechner, A. R., Kroner, C., Anthocyanins and colonic metabolites of dietary polyphenols inhibit platelet function. *Thromb. Res.* 2005, *116*, 327 – 334.

[93] Togna, G. I., Togna, A. R., Franconi, M., Marra, C., Guiso, M., Olive oil isochromans inhibit human platelet reactivity. *J. Nutr.* 2003, *133*, 2532 – 2536.

[94] Perona, J. S., Martinez-Gonzalez, J., Sanchez-Dominguez, J. M., Badimon, L., Ruiz-Gutierrez, V., The unsaponifiable fraction of virgin olive oil in chylomicrons from men improves the balance between vasoprotective and prothrombotic factors released by endothelial cells. *J. Nutr.* 2004, *134*, 3284 – 3289.

[95] Karantonis, H. C., Antonopoulou, S., Perrea, D. N., Sokolis, D. P. *et al.*, In vivo antiatherogenic properties of olive oil and its constituent lipid classes in hyperlipidemic rabbits. *Nutr. Metab. Cardiovasc. Dis.* 2006, *16*, 174 – 185.

[96] Antonopoulou, S., Fragopoulou, E., Karantonis, H. C., Mitsou, E. *et al.*, Effect of traditional Greek Mediterranean meals on platelet aggregation in normal subjects and in patients with type 2 diabetes mellitus. *J. Med. Food* 2006, *9*, 356 – 362.

[97] Bellido, C., Lopez-Miranda, J., Blanco-Colio, L. M., Perez-Martinez, P., *et al.*, Butter and walnuts, but not olive oil, elicit postprandial activation of nuclear transcription factor kappaB in peripheral blood mononuclear cells from healthy men. *Am. J. Clin. Nutr.* 2004, *80*, 1487 – 1491.

[98] Carluccio, M. A., Massaro, M., Bonfrate, C., Siculella, L., *et al.*, Oleic acid inhibits endothelial activation : A direct vascular antiatherogenic mechanism of a nutritional component in the mediterranean diet. *Arterioscler. Thromb. Vasc. Biol.* 1999, *19*, 220 – 228.

[99] Toborek, M., Lee, Y. W., Garrido, R., Kaiser, S., Hennig, B., Unsaturated fatty acids selectively induce an inflammatory environment in human endothelial cells. *Am. J. Clin. Nutr.* 2002, *75*, 119 – 125.

[100] van Oostrom, A. J., Alipour, A., Plokker, T. W., Sniderman, A. D., Cabezas, M. C., The metabolic syndrome in relation to complement component 3 and postprandial lipemia in patients from an outpatient lipid clinic and healthy volunteers. *Atherosclerosis* 2007, *190*, 167 – 173.

[101] Charo, I. F., Ransohoff, R. M., The many roles of chemokines and chemokine receptors in inflammation. *N. Engl. J. Med.* 2006, *354*, 610 – 621.

[102] Hennig, B., Toborek, M., McClain, C. J., High-energy diets, fatty acids and endothelial cell function: implications for atherosclerosis. *J. Am. Coll. Nutr.* 2001, *20*, 97 – 105.

[103] Mata, P., Varela, O., Alonso, R., Lahoz, C. *et al.*, Monounsaturated and polyunsaturated n-6 fatty acid-enriched diets modify LDL oxidation and decrease human coronary smooth muscle cell DNA synthesis. *Arterioscler. Thromb. Vasc. Biol.* 1997, *17*, 2088 – 2095.

[104] Khan-Merchant, N., Penumetcha, M., Meilhac, O., Parthasarathy, S., Oxidized fatty acids promote atherosclerosis only in the presence of dietary cholesterol in low-density lipoprotein receptor knockout mice. *J. Nutr.* 2002, *132*, 3256 – 3262.

[105] Rodriguez-Villar, C., Perez-Heras, A., Mercade, I., Casals, E., Ros, E., Comparison of a high-carbohydrate and a high-monounsaturated fat, olive oil-rich diet on the susceptibility of LDL to oxidative modification in subjects with Type 2 diabetes mellitus. *Diabet. Med.* 2004, *21*, 142 – 149.

[106] Owen, R. W., Mier, W., Giacosa, A., Hull, W. E., *et al.*, Phenolic compounds and squalene in olive oils: the concentration and antioxidant potential of total phenols, simple phenols, secoiridoids, lignansand squalene. *Food Chem. Toxicol.* 2000, *38*, 647 – 659.

[107] Avellone, G., Di Garbo, V., Abruzzese, G., Bono, M., *et al.*, Cross-over study on effects of Mediterranean diet in two randomly selected population samples. *Nutr. Res.* 2003, *23*, 1329 – 1339.

