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Authorised EU health claims for water-soluble tomato concentrate (WSTC)

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DOI: 10.1533/9780857098481.2.92

Abstract: Potent antiplatelet factors were identified in water-soluble tomato concentrate (WSTC) which significantly inhibited platelet aggregation. Hyperactive platelets, in addition to their roles in thrombosis, are also important mediators of atherogenesis. It is therefore important to find alternative safe antiplatelet inhibitors for the vulnerable population who have hyperactive platelets in order to reduce the risk of cardiovascular disease (CVD). Human volunteer studies demonstrated the potency and bioavailability of active compounds in WSTC. WSTC, also known as Fruitflow®, became the first product in Europe to obtain an approved positive health claim under Article 13.5 of the European Food Safety Authority (EFSA) regulations. Fruitflow® is now commercially available in different countries.

Key words: antiplatelet factors, cardiovascular disease (CVD), platelet aggregation, tomato extracts, water-soluble tomato concentrate (WSTC).

5.1 Introduction

Tomato (*Lycopersicon esculentum*) is popularly consumed raw, as an ingredient in many dishes and sauces, and in drinks (Agarwal and Rao, 1998; Canene-Adams *et al.*, 2005). Tomato contains substantial varieties of components including water-soluble vitamins, vitamin K, alpha-tocopherol, minerals, flavonoids, phytosterols and lycopene, as well as several other carotenoids (Blum *et al.*, 2005; Mancini *et al.*, 1995; Verhoeyen *et al.*, 2002). The carotenoid lycopene is the main phytochemical in tomatoes, whereas alpha-, beta-carotenes, lutein and zeaxanthin are also found in minor quantities in tomatoes. Table 5.1 shows the nutrient composition of tomatoes.

Consumption of tomatoes has been suggested as a dietary factor in lowering the

Table 5.1 Nutrient composition of tomatoes (per 100 g)

Nutrients	Raw tomatoes	Ketchup	Tomato juice	Tomato sauce	Tomato soup
Potassium, mg	237	382	229	331	181
α-tocopherol, mg	0.54	1.46	0.32	2.08	0.50
Vitamin A, IU	833	933	450	348	193
Vitamin C, mg	12.7	15.1	18.3	7.0	27.3
Folate, μg	15	15	20	9	7
β-carotene, μg	449	560	270	290	75
α-carotene, μg	101	0	0	0	0
Lycocpene, μg	2573	17007	9037	15152	5084
Lutein+zeaxanthin¸ μg	123	0	60	0	1
Phytoene, μg	1860	3390	1900	2950	1720
Phytofluene, μg	820	1540	830	1270	720

risk associated with several diseases including cardiovascular disease (CVD) and cancer (Jacques *et al.*, 2013; Weisburger, 2002). Those living in the Mediterranean area have been shown to have a lower risk of chronic diseases such as CVD and breast, colon and prostate cancer compared to their other European and North American counterparts (Agarwal and Rao, 2000; Rissanen *et al.*, 2000, 2002).

When determining risk levels genetic factors and age must be taken into account, but diet may also play major roles in health and disease. Lycopene and other polyphenol compounds in tomatoes are thought to play major roles in reducing risk of CVD (Rao, 2002); however, the wide variety of bioactive compounds present in tomatoes may affect many different cellular targets involving the cardiovascular system and thus can modulate the outcome of the disease risk. Recent research into the role of tomato products in health and disease risk reduction extends beyond antioxidant function to include other protective mechanisms such as antithrombotic and anti-inflammatory functions. Recently identified water-soluble antiplatelet factors in tomatoes may also contribute in reducing the risk of CVD (Dutta-Roy *et al.*, 2001; O'Kennedy *et al.*, 2006a,b; Rao, 2002; Willcox *et al.*, 2003). Several reviews are available on the overall health benefits of tomatoes (Agarwal and Rao, 2000; Canene-Adams *et al.*, 2005; Giovannucci, 1999; Weisburger, 2002; Willcox *et al.*, 2003); however, this chapter will focus only on the newly discovered antiplatelet properties of water-soluble tomato concentrate (WSTC).

5.2 Epidemiology of tomato consumption and cardiovascular disease (CVD) risk

Epidemiological studies have suggested that consumers of tomatoes have a lower risk of many types of chronic diseases such as CVD and certain forms of cancer (Giovannucci, 1999, 2002, Giovannucci *et al.*, 2002; Miller *et al.*, 2002). Lycopene has been thought to be the major component in tomatoes that offers the protection

against CVD risk (Etminan *et al.*, 2004; Ghavipour *et al.*, 2012; Giovannucci, 1999; Maruyama *et al.*, 2001; Olfer'ev *et al.*, 2004; Rao, 2002). Both epidemiological studies and animal and cell culture studies revealed that lycopene has multifaceted biological actions. The potential health benefits of lycopene range from hypocholesterolaemic, cardio-protective and osteoporotic effects to anti-mutagenic activity and anti-cancer and anti-inflammatory potential (Blum *et al.*, 2005; Palozza *et al.*, 2012; Ried and Fakler, 2011; Simone *et al.*, 2011). Lycopene probably represents a biomarker of exposure of the subjects to tomato and/or tomato product consumption, rather than a biomarker of effects. In fact, studies based on dietary intake have generally failed to detect significant independent associations between lycopene and CVD risk (Hak *et al.*, 2004; Jacques *et al.*, 2013; Kohlmeier *et al.*, 1997; Sesso *et al.*, 2005). Tomatoes have a wide variety of bioactive compounds and each bioactive compound has a unique biochemical profile that is reflected in the diversity of the molecular mechanism involved in their potential health benefit. More research is clearly needed to identify these compounds in tomatoes for their biological activities, which will potentially provide invaluable insight into the mechanisms underlying the potential for beneficial effects of tomatoes in humans, particularly in terms of reducing the risk of chronic diseases. Recently discovered water-soluble antiplatelet factors in tomatoes may also play an important role in reducing the risk of CVD by modulating platelet reactivity in non-antioxidant pathways (Dutta-Roy *et al.*, 2001; O'Kennedy *et al.*, 2006a,b; Rao, 2002; Willcox *et al.*, 2003).

5.3 Human platelets and vascular homeostasis

In the adult human body, 1×10^{12} blood platelets continuously flow over 1000 m^2 of vascular surface with normally minimal adhesion or aggregation. Upon disruption of the vessel wall or at the sites of activated or damaged endothelium (atherosclerotic plaque), rapid and complex interactions occur between platelets, vascular cells and the coagulation system (Camera *et al.*, 2012; Harker and Fuster, 1986; Pamukcu *et al.*, 2011a; Dutta-Roy *et al.*, 1986). Increased platelet aggregation is achieved by a variety of mechanisms: assembly, increasing expression of aggregating and adhesive receptors, and secretion (Kroll and Schafer, 1989). In physiological and pathological processes such as the induction of thrombosis and arteriosclerosis, platelet aggregation is essential (Dutta-Roy and Sinha, 1987; Dutta-Roy *et al.*, 1989; Hamet *et al.*, 1983; Kroll and Schafer, 1989). Blood platelets play a key role in normal haemostasis and are important for the maintenance of physiological blood flow. Normal haemostasis is initiated when platelets are exposed to the sub-endothelial matrix, where they adhere to collagen via specific cell-surface receptors. This adhesion step is followed by platelet activation that is accompanied by synthesis and release of pro-aggregatory molecules such as thromboxane (Tx) A_2 and ADP, which amplify platelet responses to collagen and recruit additional platelets to the site of injury (Coller *et al.*, 1995; Dutta-Roy *et al.*, 1986; Kroll and Schafer, 1989). The concerted action of collagen,

ADP and TxA_2 activates specific signalling pathways, generating a number of second messengers and leads to functional expression of a GPIIb-GPIIIa complex of the fibrinogen receptor on the platelets (Coller *et al.*, 1995; Dutta-Roy *et al.*, 1986; Kroll and Schafer, 1989).

Research has shown that pro-haemostatic mechanisms can be counterbalanced and regulated by physiological antihaemostatic molecules that work in a concerted and redundant way, which results in the release of prostacyclin (PGI_2), nitric oxide and the endothelium-dependent hyperpolarising factor by the endothelium, as well as the ADP hydrolysing activity that is associated with endothelial cell membrane apyrase (CD39; Dutta-Roy, 1994; Dutta-Roy *et al.*, 1991; Harker, 1986; Harker and Fuster, 1986). Mediating the aggregation of platelets is achieved through the intracellular formation of PGG_2, PGH_2 and TxA_2 from arachidonic acid, 20:4n-6, among other things (Dutta-Roy *et al.*, 1996). The most effective natural inhibitor of platelet aggregation is PGI_2, which is an arachidonic acid metabolite of endothelial cells (Dutta-Roy *et al.*, 1989). The mediation of prostaglandin-induced inhibition of platelet aggregation is achieved by an increase in cAMP synthesis due to the activation of adenylate cyclase (Dutta-Roy *et al.*, 1989). This activation is done by binding PGI_2 or PGE_1 to specific platelet surface receptors (Dutta-Roy and Sinha, 1987).

Since platelets are involved in the thrombotic event as well as in the initiation and progression of atherosclerotic plaque (Palomo *et al.*, 2012), hyperactive platelets in many conditions such as diabetes mellitus, obesity, insulin resistance, obesity and smoking may contribute to the pathogenesis of vascular complications by promoting microthrombus formation and accelerated athero-thrombotic diseases (Ferroni *et al.*, 2004a,b; Park and Harris, 2009; Pamukcu *et al.*, 2011b; Huang *et al.*, 2012; Shimodaira *et al.*, 2013). In fact, an increased prothrombotic state induced by platelet hyperactivity is a major risk factor in the development of heart attacks, strokes and venous thromboembolism (Davi and Patrono, 2007; Diener *et al.*, 2006, Ferroni *et al.*, 2007, 2008). Lowering platelet reactivity is therefore considered to be the cornerstone in reducing the risk of CVD (Davi and Patrono, 2007; Ferroni *et al.*, 2007). Dietary components have been shown to modify platelet activation and/or haemostasis pathways through a variety of mechanisms (Dutta-Roy, 2002; Dutta-Roy *et al.*, 1999). The nutritional approaches may provide a beneficial approach or an addition to current pharmacological treatments for CVD. The pathologic states such as hyperlipidaemia, hypertension, obesity, insulin resistance, smoking, diabetes and high fat diets have proven to be intensively associated with platelet hyper-reactivity (Natarajan *et al.*, 2008). It is therefore important to find alternative safe, reversible antiplatelet inhibitors for these vulnerable people who have hyperactive platelets without a clinically expressed disease. In fact, these are also the challenges facing those who use antiplatelet drugs for CVD prevention (Coccheri, 2012). With the appearance of more and more problems of current antiplatelet agents, people are beginning to question conventional antiplatelet strategies that only target the signalling pathway of platelet activation (Xiang *et al.*, 2008a,b).

Current antiplatelet treatments are mainly based on the inhibition of two

important pathways of platelet activation: TxA_2 mediated (aspirin) and (ADP)–P2Y12 receptor mediated. Data suggest that aspirin resistance and its side effects are of concern in CVD therapeutic as well as preventive treatment (Coccheri, 2012). Given the serious and unintended adverse health effects of aspirin therapy, it led to the discovery of a broad range of natural compounds, including foods and spices, with demonstrable platelet-inhibiting activity. The likely health benefits of reducing platelet activity in a general population or vulnerable population by means of a functional food are being increasingly realised. It is now acknowledged that populations whose diet results in a suppression of platelet activation (e.g. a high sea fish diet or a Mediterranean diet) obtain measurable cardiovascular health benefits.

5.4 Isolation and characterisation of water-soluble tomato extract: effects on human platelet aggregation

In order to investigate the presence of antiplatelet factors in different fruits, antiplatelet activity of the 100% fruit juice was investigated. The anti-aggregating effects of different fruit juices were extensively investigated. Due to the acidity of the different fruit extracts, the pH of the extracts was adjusted to pH 7.4, which would ensure the acidic pH would have no effect on the platelet aggregation response. The experiment used a 100% juice (w/v) extract, with the exception of the avocado, apple, nectarine, banana and mango juices. Table 5.2 shows the inhibitory effect of different fruit extracts on human platelet aggregation *in vitro* (Dutta-Roy *et al.*, 2001, Duttaroy and Jorgensen, 2004). Tomato and kiwi fruit extracts were found to have the maximum inhibitory effect (70–75%), whereas the apple and pear extracts had very little activity (2–5%), and intermediate platelet activity aggregation was noted in grapefruit, melon and strawberry juices (33–44%). Maximum inhibition of the tomato extract (72%) was obtained when 50 µl (100% juice) of tomato extract was added to 500 µl of prepared platelets, meaning tomato extract has a dose-dependent inhibition of platelet aggregation. The antiplatelet potential of the fruits tested were not related to the antioxidant potential of the fruit extract. Since the antiplatelet activity in fruits is quite different from their antioxidant properties, it is possible that these activities are due to the presence of compounds that have a different chemical structure from the antioxidants. Tomato extract inhibited collagen-, ADP- and arachidonic acid-induced platelet aggregation *in vitro*. Moreover, tomato extract also inhibited thrombin-induced platelet aggregation (Dutta-Roy *et al.*, 2001). The IC50 (minimum concentration required for 50% inhibition of platelet aggregation induced by ADP in 500 µl PRP) of tomato extract was around 20 µl (100% juice). A comparison study between extracts obtained from pulp and the fluid around the seeds in tomatoes indicated that the fluid around the seeds had the maximum antiplatelet activity.

The antiplatelet factor present in the tomato extract was further isolated by boiling the juice, followed by ultra-centrifugation. Delipidation of the tomato

Table 5.2 Effects of fruit extracts on inhibition of platelet aggregation in humans

Fruit	Scientific names	Family	% Fruit	% Inhibition of platelet aggregation
Tomato	*Lycopersicon esculentum*	Solanaceae	100	70.0 ± 12.0
Kiwifruit	*Actinidia deliciosa*	Actinidiaceae	100	72.0 ± 5.0
Grapefruit	*Citrus paradisi*	Rutaceae	100	44.2 ± 8.1
Melon (honeydew)	*Cucumis melo*	Cucurbitaceae	100	42.1 ± 12.0
Strawberry	*Fragaria virginiana*	Rosaceae	100	33.1 ± 7.0
Melon (cantelope)	*Cucumis melo cantalupensis*	Cucurbitaceae	100	27.5 ± 11.0
Banana	*Musa paradisiaca*	Musaceae	50	22.4+5.0
Mango	*Mangifera indica*	Anacardiaceae	50	22.1 ± 6.3
Pineapple	*Ananas comosus*	Bromeliaceae	100	19.8 ± 9.4
Orange (jaffa)	*Citrus sinensis* cv. Jaffa	Rutaceae	100	18.5 ± 7.6
Grape (green)	*Vitis vinifera*	Vitaceae	100	16.4 ± 8.9
Plum	*Prunus mexicana*	Rosaceae	100	15.6 ± 9.1
Grape (red)	*Vitis vinifera*	Vitaceae	100	13.8 ± 7.8
Avocado	*Persea americana*	Lanraceae	20	12.1 ± 4.5
Nectarine	*Prunus persica nucipersica*	Rosaceae	50	9.1 ± 4.2
Apple	*Malus domestica*	Rosaceae	50	5.2 ± 2.1
Pear	*Pyrus fauriei*	Rosaceae	100	2.0 ± 2.0

ultrafiltrate (100 000 g) in the tomato extract revealed that the antiplatelet factor(s) were not lipid-soluble compounds. The molecular mass of the antiplatelet compounds in the tomato juice was less than 1000 Da, meaning they were highly water-soluble and were stable after boiling, and gel filtration using a Bio gel P2 column further purified the delipidated aqueous fraction (Dutta-Roy *et al.*, 2001).

The activity was fractionated into two peaks – peak-3 and peak-4 (major peak) (Fig. 5.1). Subsequently, peak-4 was further purified by high performance liquid chromatography (HPLC) using an ion exchange column. Nuclear magnetic resonance (NMR) and mass spectroscopy studies indicated that peak F2 (obtained from peak 4) contained adenosine and cytidine. Deamination of peak F2 with adenosine deaminase almost completely abolished its antiplatelet activity, confirming the presence of adenosine in this fraction. In comparison, deamination of peak-4 resulted in only partial loss of inhibitory activity while the activity of peak-3 remained unaffected (Dutta-Roy *et al.*, 2001). These results indicate that tomatoes contain antiplatelet compounds in addition to adenosine. Unlike aspirin, the tomato-derived compounds inhibit thrombin-induced platelet aggregation. As well as adenosine, tomato extract contains other antiplatelet compounds that had not been destroyed by adenosine deaminase treatment, and that showed anti-thrombin activity. On examination of aspirin and tomato extracts, the tomato extract was the only one to display significantly inhibited thrombin-induced platelet aggregation by adenosine (Table 5.3). The tomato extract thus may be effective in the case of thrombin-induced platelet aggregation where aspirin is not capable of inhibition of platelet aggregation.

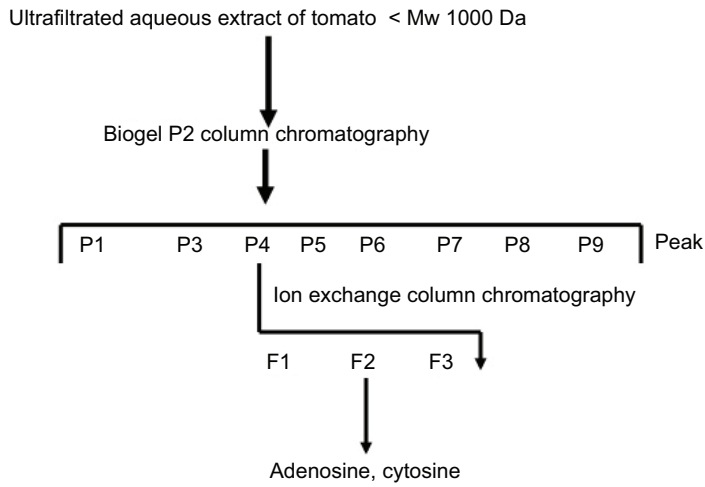

Fig. 5.1 Elution of tomato extract (ultrafiltrate) on Biogel P2 column producing several fractions. Further purification of peak 4 by high performance liquid chromatography (HPLC) ion exchange column. Typically 1.5 ml of 0.5 g/ml of freeze dried tomato ultrafiltrate were applied to Biogel P2 gel filtration and eluted with 0.01 M acetic acid buffer, pH 3.3 containing 0.15 M NaCl. The fractions were tested for their antiplatelet activity. Peak 4 as then further purified using ion exchange HPLC column. Adapted from Dutta-Roy *et al.* (2001).

Table 5.3 Effect of tomato extract platelet aggregation induced by different aggregating agents

Agonists	% Inhibition of platelet aggregation	
	Tomato extract (50μg/ml PRP)	Aspirin (10mM)
ADP	66	18
Collagen	45	25
Arachidonic acid	15	95
Thrombin	67	–

Note: Platelet aggregation induced by ADP, collagen and archidonic acid was carried out in platelet-rich plasma (PRP) whereas thrombin induced aggregation was carried out in washed platelets (Dutta-Roy *et al.*, 2001). Typically platelets were aggregated by adding aggregating agents after incubating platelets with 50 μg/ml of tomato extract for 15 min at 37 °C.

The antiplatelet activity of tomato extract was later confirmed by several different studies (Fuentes *et al.*, 2012b; Lazarus *et al.*, 2004; Yamamoto *et al.*, 2003). The recent study by Fuenetos *et al.* (2012a) confirmed my findings that the antiplatelet activity of tomatoes was partly due to the presence of adenosine. It is interesting to note in this context that fresh tomato contains about 30 times more adenosine than lycopene, which therefore may contribute significantly in reducing CVD risk by modulating platelet reactivity.

5.5 Further characterisation of water-soluble tomato extract

The water-soluble tomato extract as described above contained several compounds including soluble sugars. The soluble sugars did not show any *in vitro* antiplatelet activity (O'Kennedy *et al.*, 2006a). These inactive components were removed through the use of solid-phase extraction with styrene divinylbenzene cartridge at pH 2.5. The isolated non-sugar material, total active fraction (tAF), showed strong resistance to platelet aggregation *in vitro* and comprised 4% of the aqueous extract's dry matter. Figure 5.2 summarises the preparation of a water-

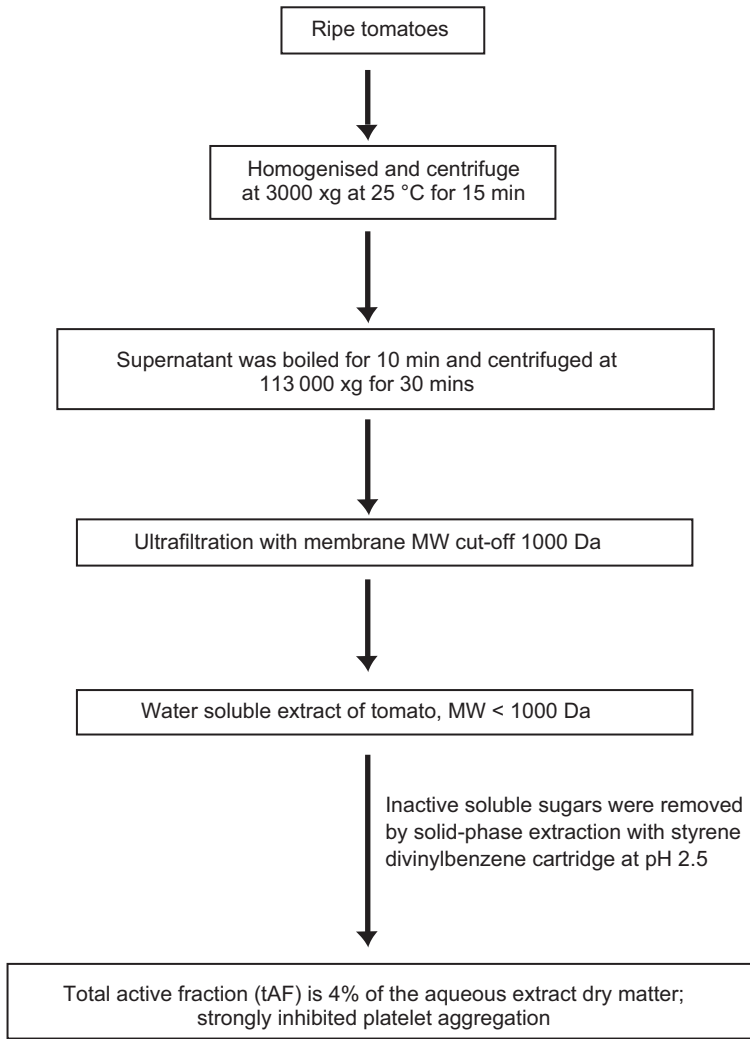

Fig. 5.2 Outline of water-soluble tomato concentrate preparation. Adapted from Dutta-Roy *et al.* (2001) and O'Kennedy *et al.* (2006a).

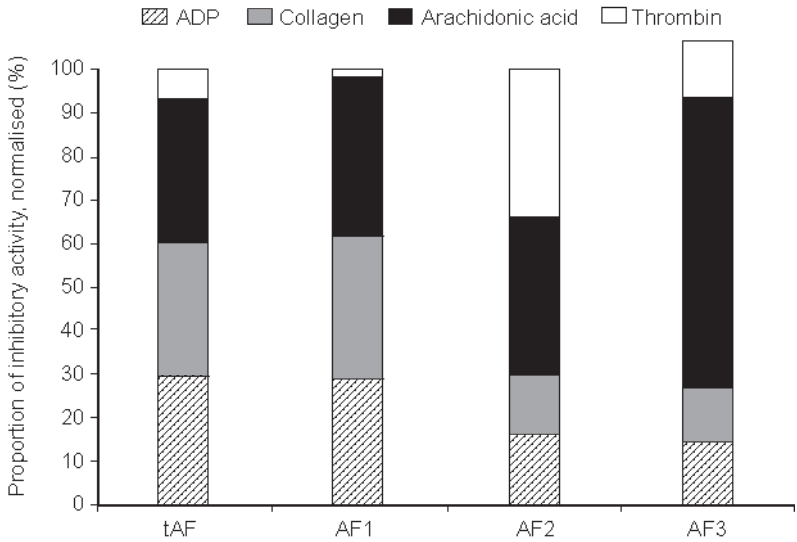

Fig. 5.3 Proportional inhibitory activity shown by the total active fraction (tAF) and its subfractions (AF1, AF2 and AF3) toward ADP- (10 µmol/L), collagen- (4 mg/L), arachidonic acid- (500 mg/L), and thrombin- (2U/L) mediated platelet aggregation. Proportional inhibitory activity shown by the total active fraction (tAF) and its subfractions (AF1, AF2 and AF3) toward ADP- (10 µmol/L), collagen- (4 mg/L), arachidonic acid- (500 mg/L), and thrombin- (2U/L) mediated platelet aggregation. A comparison ($n = 5$) was made between the antiplatelet activity of the tAF at a final concentration of 1.98 g/L and the antiplatelet activities of each subfraction at final concentrations of 0.58, 0.24 and 1.00 g/L, respectively. Source: *Am J Clin Nutr* 2006, **84**, 570–579, American Society for Nutrition.

soluble extract from tomatoes. Further fractionation of tAF into AF1, AF2 and AF3 fractions by semi-preparative HPLC resulted in a range of components with significantly different modes of antiplatelet activity (O'Kennedy *et al.*, 2006a). The fraction AF1 contributed 32% to the total dry matter of tAF, while AF2 and AF3 contributed 13% and 55% respectively (O'Kennedy *et al.*, 2006a,b). AF1's strong resistance to ADP- and collagen-mediated platelet aggregation is due to the fact that it contained a large range of nucleosides and derivatives such as adenosine, cytidine, inosine, guanosine, AMP and GMP. The remaining components in the inhibition of the platelet aggregation have yet to be identified. Sub-fraction AF2 shows significantly lower levels of inhibition of ADP- and collagen-induced aggregation, but it displays a considerably greater resistance to thrombin-induced aggregation than the other two sub-fractions. Many low-molecular-weight compounds were contained in AF2, many of which are sensory components of the tomato that are present in AF2 at very low concentrations.

Phenolic compounds are the main components of the AF3 sub-fraction and simple phenolic acids such as ferulic and caffeic acids, as well as some glycosidic derivatives, have been identified in it. In addition, flavonoids such as quercetin, kaempferol, rutin and luteolin were isolated (O'Kennedy *et al.*, 2006a,b). The AF3 sub-fraction resisted arachidonic acid-induced aggregation significantly more

than the other two subfractions, as shown in Fig. 5.3. The majority of the isolated components that display antiplatelet activity are not flavonoid derivatives, and as a result information on their characterisation and individual antiplatelet activities is as yet unreported (O'Kennedy *et al.*, 2006a,b). Some of the components present in this extract, such as adenosine, rutin and catechin, were shown to possess antiplatelet activity (Dutta-Roy *et al.*, 1999; Jasuja *et al.*, 2012). The inhibition of ADP-, collagen-, thrombin- and arachidonic acid-mediated platelet aggregation by tomato extract components appears to be linked to the inhibition of glycoprotein IIb/IIIa and platelet secretory mechanisms (O'Kennedy *et al.*, 2006a). However, further work at the molecular level is required for definitive conclusions on their mechanisms of action.

5.6 Human trials using the water-soluble tomato extract

Several human trials were performed using the water-soluble tomato extract, but so far only two human trials have been published (O'Kennedy *et al.*, 2006a; O'Kennedy *et al.*, 2006b). Two treatment supplements contained 6 g and 18 g of tomato extract syrup in 200 ml and 50 ml of orange juice respectively. The supplement containing 18 g of tomato extract syrup contained the same amount of tAF as is found in six fresh tomatoes (O'Kennedy *et al.*, 2006a). A placebo supplement drink without tomato extract was also prepared for use as a control treatment (O'Kennedy *et al.*, 2006a,b). Ninety healthy human subjects with normal platelet function were selected for a crossover study that was randomised, double-blind and placebo-controlled. Three hours after consumption of extract-enriched or control supplement the subjects' baseline haemostatic function was measured, and equivalent control drinks were prepared at each volume. Inhibition of aggregation was observed for both ADP- and collagen-mediated aggregation in a dose-dependent manner. In the randomised, double-blind placebo-controlled crossover significant reductions in platelet aggregation were observed 3 h after supplementation with the tomato extract equivalent to two and six tomatoes, while no significant effects were measured for the control group. Male subjects showed greater sensitivity to the extract, as evidenced by significantly larger reductions in platelet aggregation in response to ADP or collagen, than the female subjects. Platelet function in response to ADP or collagen was altered in 97% of trial subjects after consumption. While the average response in studies was in the range of 8–23% inhibition of baseline platelet aggregation, a subset of subjects showed a higher response (in the range 20–35% inhibition). These individuals had higher than average plasma concentrations of two emerging markers of cardiovascular risk: C-reactive protein and homocysteine, both of which have been reported to affect platelet function. It would appear, therefore, that the individuals who show the greatest response are those who could potentially derive greater benefit from a dietary antiplatelet agent. Figure 5.4 shows the different treatment effects (dose–response) observed in aggregation using suboptimal ADP concentrations in one male subject after consuming tomato extract (O'Kennedy *et al.*, 2006b). No adverse side effects of

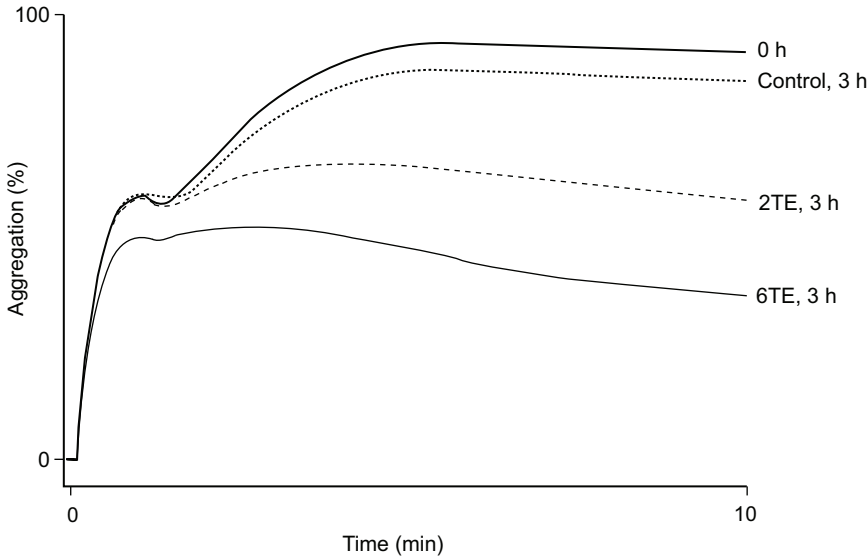

Fig. 5.4 Dose-dependent inhibition of ADP-induced platelet aggregation observed in one subject 3 h after consumption of the control drink and treatment supplements containing 2 tomato extract (TE) or 6TE. ADP-induced aggregation in one subject 3 h after consumption of the control drink and treatment supplements containing 2 or 6 tomato equivalents (2TE and 6TE, respectively). The percentage change in platelet aggregation from baseline at this agonist concentration was –4.2%, –33.7% and –47.8% with the control, 2TE, and 6TE treatments, respectively. Source: *Am J Clin Nutr* 2006, **84**, 561–569, American Society for Nutrition.

the supplementation were reported, and no effects on clotting time variables were detected after supplementation.

The other *ex vivo* cannulation study in healthy humans was performed using each treatment drink (50 ml or 200 ml) containing 18 g of tomato extract syrup. This syrup contained the equivalent tAF found in six fresh tomatoes (O'Kennedy *et al.*, 2006a). A 7 h time-course study was carried out in human subjects who had been fitted with a cannula ($n = 23$) in order to determine the *ex viv*o efficacy of a tomato extract-based supplement drink and to track the start and duration of the antiplatelet effects. Results showed that, after being supplemented with the tomato extract but not after being supplemented with the control drink, ADP-induced platelet aggregation was significantly lower than the baseline values. Results showed the differences between the tomato extract and control drinks were significant at both optimal (–1.58 ± 0.71%; vs. control group: 2.10 ± 1.15%; $P = 0.03$) and suboptimal (–15.23 ± 2.19%; vs. 1.86 ± 3.56%; $P < 0.001$) ADP concentrations. Changes in the baseline aggregation were observed at each timepoint in the tomato extract group. When the platelets were stimulated with suboptimal concentrations of ADP, significant differences at 3 and 6 h were observed in the tomato extract group than the control group. At any timepoint, neither the control nor tomato extract groups displayed any significant differences arising from the different carrier volumes.

5.7 EU approval of the health claim of the platelet inhibitory property of the water-soluble tomato extract

In 2006 the European Union (EU) adopted a regulation on the use of nutrition and health claims for foods which lays down harmonised EU-wide rules for the use of health or nutritional claims on foodstuffs. One of the key objectives of this regulation is to ensure that any claim made on a food label in the EU is clear and substantiated by scientific evidence. Provexis Limited, UK, submitted an application pursuant to Article 13(5) of Regulation (EC) No 1924/2006 via the Competent Authority of the United Kingdom, to the European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies to approve the scientific substantiation of a health claim related to WSTC I and II and reduction of platelet aggregation. The scope of the application was proposed to fall under a health claim based on newly developed scientific evidence and/or claim including a request for the protection of proprietary data. The food constituent that is the subject of the health claim is a lycopene-free and fat-free WSTC developed in two variant forms named WSTC I (completely water-soluble syrup) and its low-sugar derivative WSTC II, supplied in powder format. The WSTCs were standardised on the total quantity of 37 identified constituents and were shown to inhibit platelet aggregation *in vitro* to different degrees. Based on the submitted documents by the Provexis, the commission adopted a decision on 19 December 2009 authorising a health claim related 'water-soluble tomato concentrate (WSTC) I and II helps maintain normal platelet aggregation, which contributes to healthy blood flow'. The WSTC was later commercially termed Fruitflow®.

The claims for the effect were based on eight human studies, seven of which were claimed as proprietary and conducted with WSTC, and seven non-human studies, three of which were claimed as proprietary. The effects of WSTC on platelet aggregation *ex vivo* were investigated in carefully selected male and female subjects between 35 and 70 years of age in the seven proprietary human intervention studies. The EFSA Panel decided that the selection of the subjects and the method used to assess platelet aggregation were both appropriate for such studies.

In 2010, Provexis submitted for modification concerning an extension of the authorised health claim (as described above), additional proposed conditions of use for powdered single-serve sachets, tablets and capsules. The EFSA Panel concluded that the bioavailability of potentially active compounds in WSTCs, when administered as powder, tablets or capsules, would not be different from that observed in other food matrices for which the health claim was authorised (i.e. fruit juices, flavoured drinks or yoghurt drinks) as long as these are easily dissolved in water. In fact, the Panel reviewed one unpublished study in order to approve the modified application: a double-blind placebo-controlled study with randomised crossover design with three interventions corresponding to 3 g WSTC I (syrup), WSTC II 150 mg (powder) produced at ambient temperature and WSTC II 150 mg (powder) produced at 65 °C, and one control. All test and control materials were administered in capsules with hydroxypropyl methylcellulose as food matrix

together with 200 mL of water. The results revealed reduced platelet aggregation (ADP agonist) in the three WSTC formulations, compared to the corresponding control and baseline values, and showed no significant differences between the three formulations. In all three formulations the inhibitory responses to collagen 2 mg/L were also significant.

This study indicated that the biological activity of both forms of WSTC (I and II) administered in capsules was comparable to equivalent doses provided in fruit juice. The EFSA Panel concluded that a cause and effect relationship had been established between the consumption of a WSTC (i.e., WSTC I and II in correspondence to the specifications provided by the applicant) and the reduced platelet aggregation under the new conditions of use (i.e. consumed as powder, tablets or capsules) as proposed by the applicant. The EFSA Panel was of the opinion that there was no basis to restrict the conditions of use to this age range (35–70 years) in the adult population. The conditions of use in the EU authorised claim are for information to be given to the consumer that the beneficial effect must be obtained with a daily consumption of 3 g WSTC I or 150 mg WSTC II in up to 250 ml of either fruit juices, flavoured drinks or yoghurt drinks (unless heavily pasteurised) or with a daily consumption of 3 g WSTC I or 150 mg WSTC II in food supplements when taken with a glass of water or other liquid.

Provexis submitted several unpublished studies to the commission to consider in their application, and some of these are described below:

- In a double-blind randomised controlled trial (RCT) a platelet aggregation reduction of 8–25% was observed 3 h after the consumption of tomato extract, corresponding to 3 g and 9 g of WSTC I in 200 mL orange juice.
- Compared to placebo, a single-blinded crossover RCT demonstrated a significant reduction in platelet aggregation between 1.5 and 3 h after consumption of the 9 g WSTC I in either 50 or 250 mL of orange juice, which persisted for 12 h. By 12 h in a non-controlled crossover study platelet aggregation was inhibited by 7–8% but at 18 and 24 h it returned to baseline values after the consumption of a single dose of 3 g of WSTC I.
- Platelet aggregation was significantly reduced in a double-blind crossover RCT (compared to a tomato-free control drink) after 14 and 28 days of daily consumption of 3 g of WSTC I in 200 mL orange juice.
- A crossover RCT showed a significant reduction in platelet aggregation 3 h after consumption of a single dose of 250 mL (but not with a single dose of 1 L) of a fruit juice drink containing 12 g of WSTC I/L. The test showed a similar outcome when repeated for 5 days after subjects had consumed 1 L of the WSTC drink daily.
- A pilot, non-controlled study showed platelet aggregation was significantly reduced (compared to the baseline) 3 h after consumption of a single dose of 3 g of WSTC I in 250 mL orange juice and a single dose of 150 mg or 600 mg of WSTC II in 100 mL yoghurt drink. It also showed no significant differences between the three preparations.
- A significant decrease (26.5%) in platelet aggregation following consumption

of 250 mL filtered tomato juice (not WSTC) in diabetic subjects as compared to controls was observed in a double-blind parallel RCT.

These human studies all consistently show reduced platelet aggregation after consumption of WSTC under the conditions of use proposed by Provexis. Fruitflow® thus became the first product in Europe to obtain an approved positive health claim under Article 13.5 of the EU Regulation.

5.8 Commercially available Fruitflow® products

The proprietary functional beverage SIRCO, containing Fruitflow®, was launched in the UK in 2005. This product was initially available in 250 mL and 1 L volumes comprising the daily recommended intake. After EU approval, the Fruitflow® products were legally marketed with the claim: 'Helps maintain normal platelet aggregation, which contributes to healthy blood flow.' In June 2010, Provexis gave DSM exclusive worldwide rights to market Fruitflow®. In March 2011, DSM's heart healthy ingredient Fruitflow® was named one of the most innovative products of the year at the prestigious Food Ingredients South America awards 2012. Currently, all Fruitflow® products are in its syrup form. A powder version of Fruitflow® is now being developed. The powder version would allow the ingredient to be formulated for tablet- and capsule-form dietary supplements. Table 5.4 summarises the different Fruitflow® products now available in different countries.

5.9 Conclusion

Hyperlipidaemia, hypertension, obesity, insulin resistance, smoking, diabetes and high fat diets have been proved intensively associated with platelet hyper-reactivity. In addition to their roles in thrombosis, hyperactive platelets are also important mediators of atherogenesis itself (Fig. 5.5). It is therefore important to find alternative safe, reversible antiplatelet inhibitors for the vulnerable population

Table 5.4 Different Fruitflow® products in the market

Brand name	Company	Type of product	Country
Sirco	Sirco	Fluid	United Kingdom
Swanson Fruitflow	Swanson	Capsule	USA
SiS REGO plus Fruitflow gel	Provexis	Fluid	United Kingdom
Biocol Flow	Biocol	Capsule	Germany
Thromboflow	Dr. Wartz	Capsule	Germany
Langers Fruitflow	Langer	Fluid	USA
Tomaflow	Biogena	Fluid	Germany
Optiflow™	Brickerlabs	Liquid	USA
Coronaflow	Aenova	Capsule	Germany

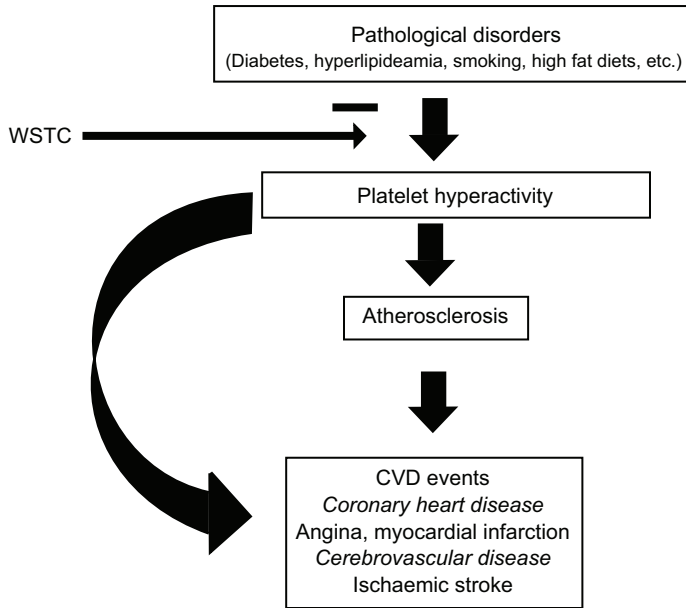

Fig. 5.5 Role of platelets in CVD. Platelets can be hyperactive in different pathological conditions (diabetes, obesity, smoking) and with high lipid diets can contribute to atherogenesis processes. In addition, active platelets can take part directly in CVD events. WSTC may be beneficial in CVD by preventing platelet hyperactivity.

who have hyperactive platelets, in order to maintain vascular homeostasis. Dietary components have been shown to modify platelet activation and/or haemostasis pathways through a variety of mechanisms. Therefore the benefits of foods providing consistent inhibition of platelet function could be beneficial in maintaining vascular homeostasis and integrity. WSTC is bioavailable, and reversible, with a broad spectrum of inhibitory activities against a number of issues, including ADP, collagen, arachidonic acid and, most importantly, thrombin-mediated platelet aggregation. WSTC has thus been shown to provide a health benefit in modulating platelet-vascular homeostasis.

5.10 Acknowledgement

I am thankful to the Throne Holst Foundation, Norway.

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