

Functional food Regulations in Europe: A case study

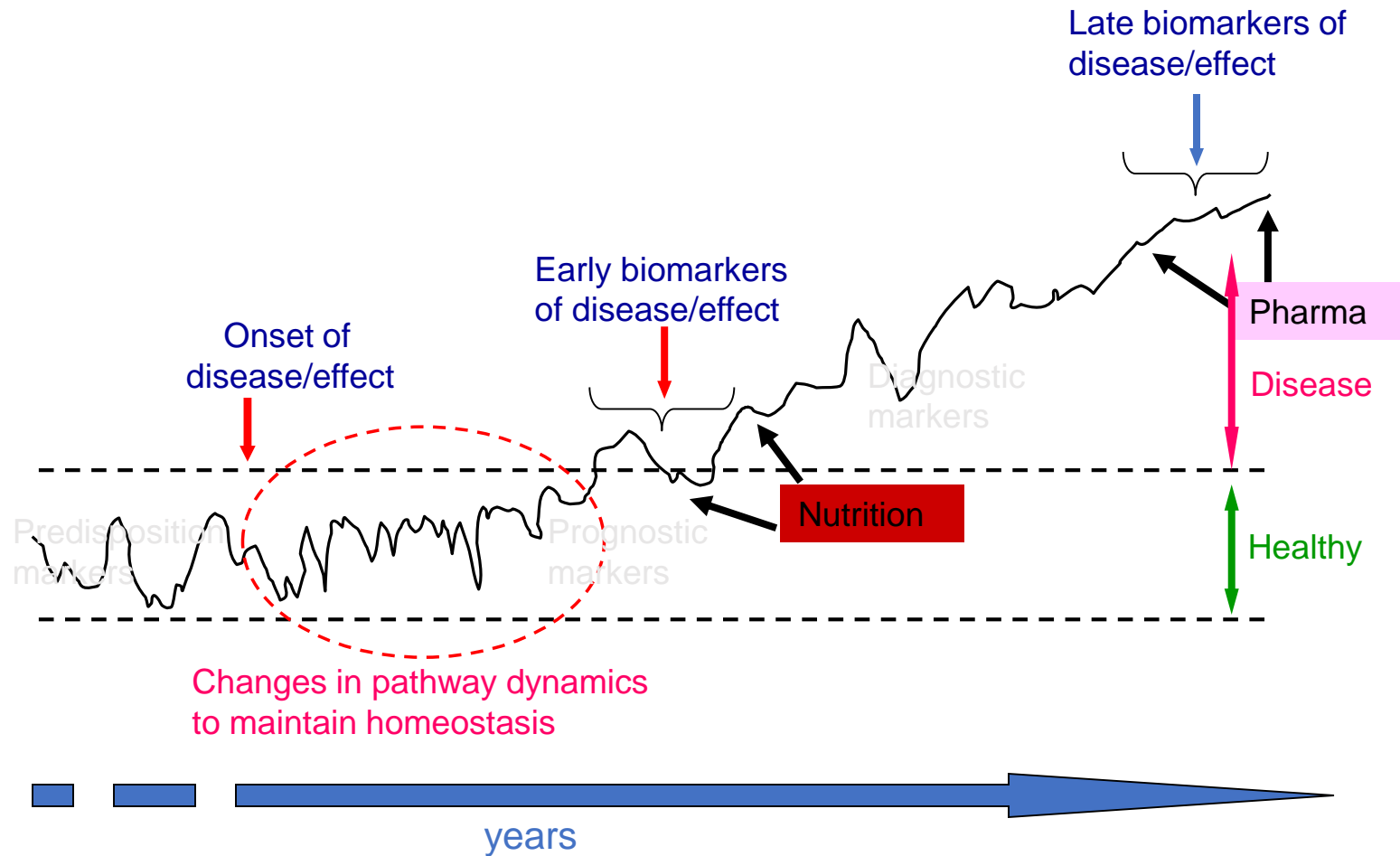


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Events in the progression of disease

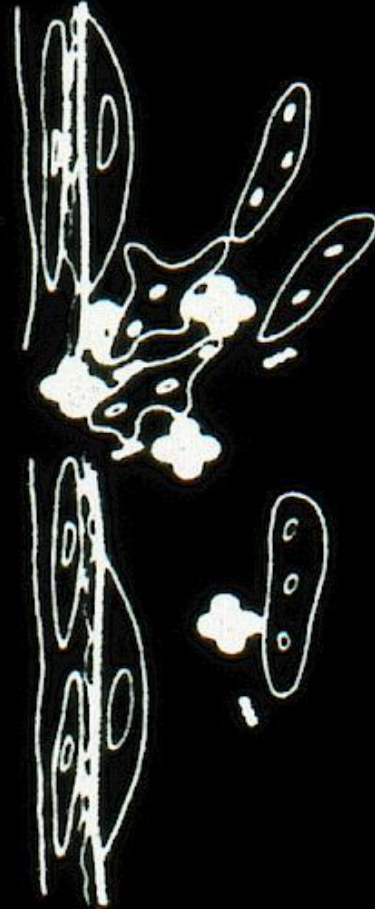


INTERACTION OF PLATELETS WITH THE VESSEL WALL

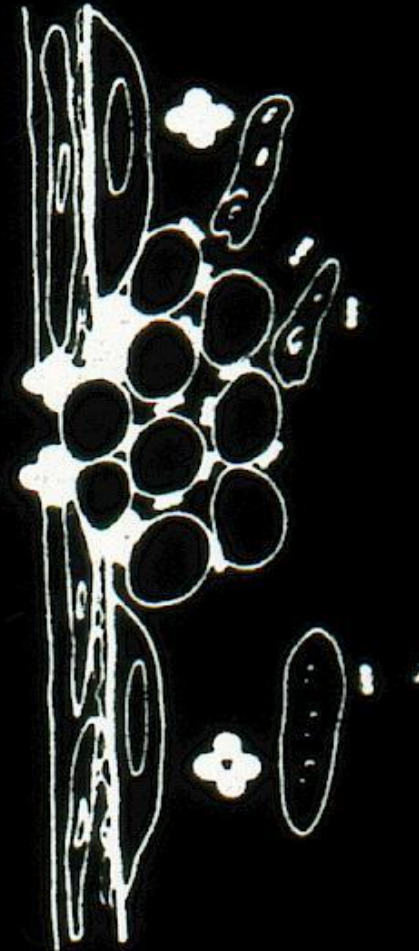
A VESSEL BEFORE INJURY



B INJURED VESSEL



C HEMOSTATIC PLUG



HYPERACTIVE PLATELETS ASSOCIATED WITH DISEASE

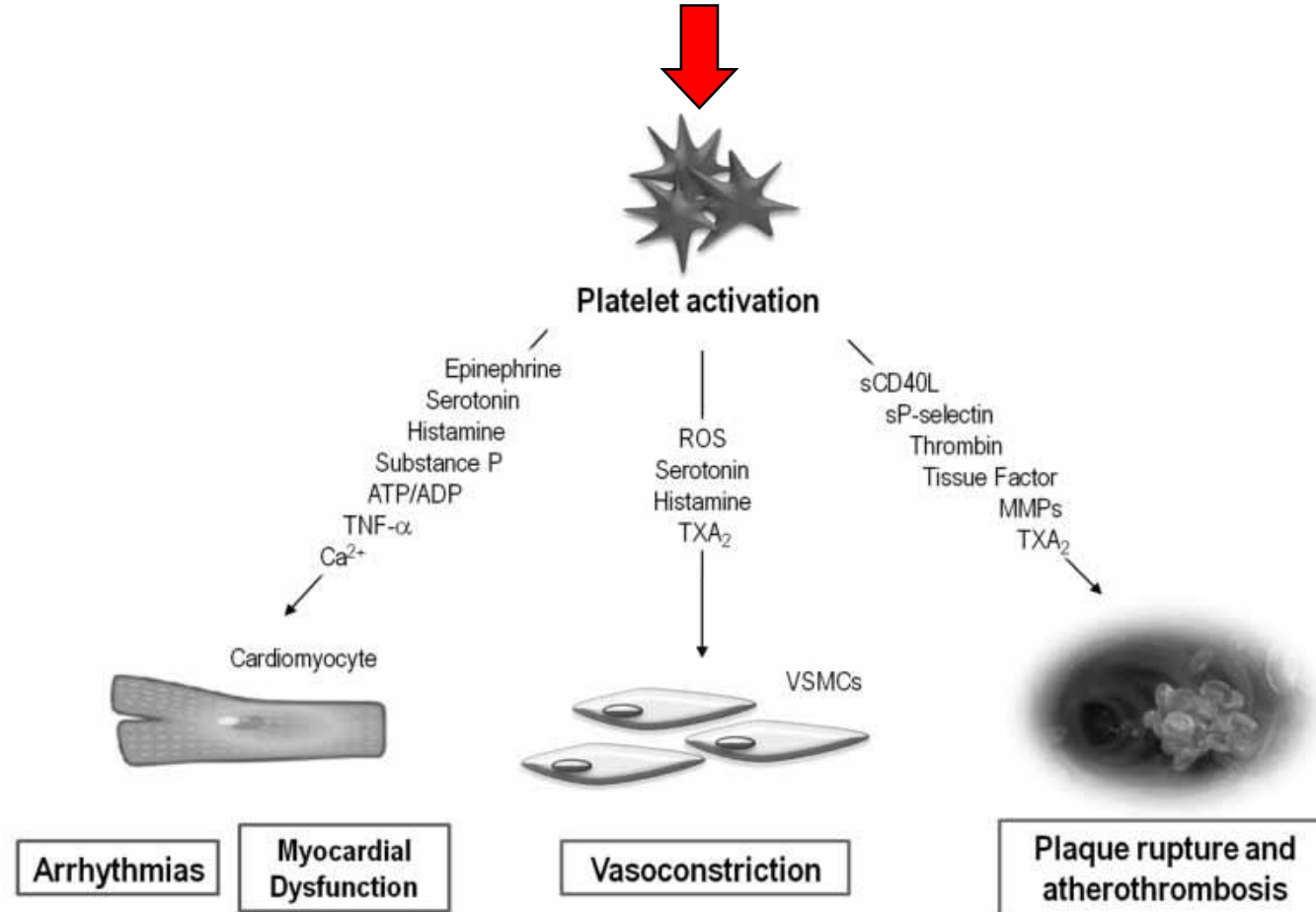
Conditions that lead to hyperactive platelets:

- Diabetes mellitus
- Insulin resistance
- Obesity
- Ageing
- Over nutrition, bad diets
- Sedentary lifestyles
- Pollution
- Oxidative stress, inflammation and hyperlipidemia
- Drugs, contraceptives
- Cancers
- Hypertension

Platelets become hyperactive or produce circulating micro-aggregates in the clinically defined conditions shown

Pathological Disorders

(Hypertension, *Diabetes, hyperlipideamia, smoking, High fat diets etc.*)



Pathological Disorders

(Diabetes, hyperlipideamia, smoking, High fat diets etc.)

↓

Platelet hyperactivity

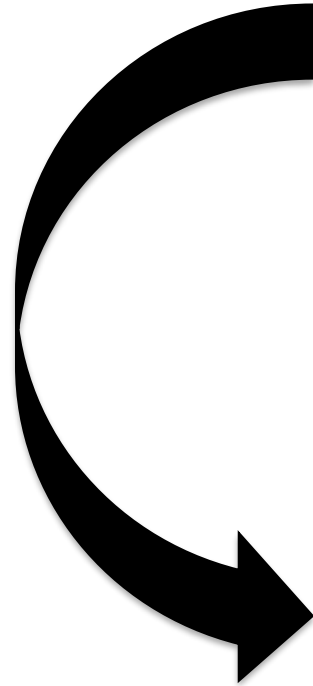
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Atherosclerosis

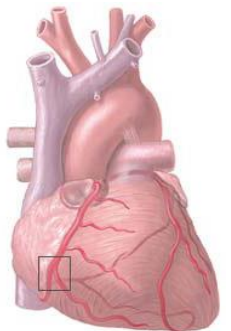
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CVD events

Coronary heart disease
Angina, Myocardial infarction
Cerebrovascular Disease
Ischaemic stroke



Blockage in right coronary artery



Importance of Anti-platelet dietary supplements

Aspirin is the most common non-prescription drug

Side effects of ASPIRIN

1. Gastric bleeding
2. Aspirin resistance
3. Ineffective against
 - i. Sheer stress/ADP/Thrombin-induced platelet activation
 - ii. Ruptured atherosclerotic plaques-induced platelet activation

New drug

GPIIb/GPIIIa antagonists

Effects of various aqueous extract of fruit extracts on platelet aggregation in vitro

Fruits	% Inhibition of ADP-induced aggregation
Tomato	76
Grapefruit	44
Melon	42
Strawberry	33
Orange	18
Grape	16
Plum	16
Cranberry	9
Pear	2
Apple	1

Duttaroy et al, Platelets 12, 218, 2001

Duttaroy & Jørgensen Platelets 15, 287, 2004

Tomato extract preparation protocol

Ripe Tomatoes



**Homogenisation and centrifugation
at 3000xg at 25C for 15 min**



Ultrafiltration with membrane MW cut-off 1000 da



Aqueous extract (Soluble sugars 85-90%)



Inactive Soluble sugars 85-90%) are removed
By Solid-phase extraction with styrene
divinylbenzene cartridge at pH 2.5))

**Total Active fraction (tAF) is
4% of the aqueous extract dry
matter**



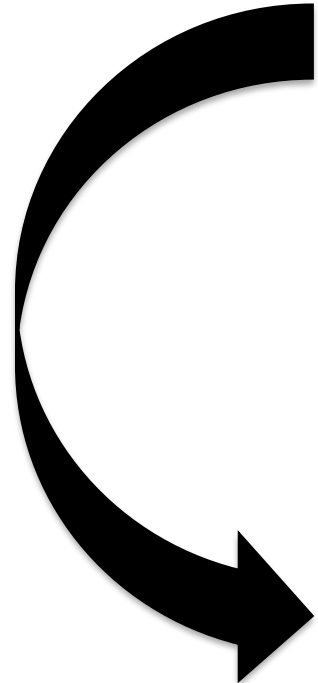
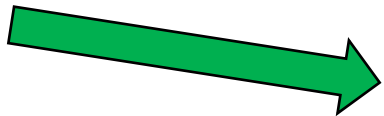
Duttaroy et al, Platelets 2001
O' Kennedy et al AJCN, 2006

Tomato Extract: Efficacy evidence from Human studies

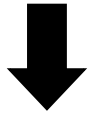
References	Design	N / Gender / Study Population	Duration / Dosage	Outcomes measured	Significant effect
1. O’Kennedy et al 2006a	R, SB, PC Cross-over	23 / M F Healthy	Single dose 18 g WSTC I (equivalent to 3 daily dose, tAF 195 mg)	Platelet Aggregation PT and TCT	+ -
2. O’Kennedy et al 2006b	R, DB, PC Cross-over	93 / M F Healthy	Single dose 18 g or 6 g WSTC I (equivalent to 3 and one daily and one dose, tAF 195 and 65 mg)	Platelet Aggregation PT and TCT	+ -
3. O’Kennedy et al (early study)	Single intervention	15 / M F Healthy	Single dose 3 g WSTC I	Platelet Aggregation PT and TCT	+ -
4. O’Kennedy et al (unpublished, see review)	R, DB, PC Cross-over	22 / M F Healthy	28 d 6 g WSTC I (equivalent to one daily dose, tAF 65 mg)	Platelet Aggregation PT and TCT	+ -
5. O’Kennedy et al (unpublished, see review)	R, DB, PC Cross-over	45 / M F Healthy	Single dose 6 g WSTC I (one daily dose, tAF 65 mg) 150 mg WSTC lia I (one daily dose, tAF 65 mg) 150 mg WSTC lib I (one daily dose, tAF 65 mg)	Platelet Aggregation PT and TCT	+ -
6. O’Kennedy et al (unpublished, see review)	R, positive C Cross-over	20 / MF Healthy	Single dose or 5 d 3 g WSTC I in 250 mL (one daily dose, tAF 65 mg) 12 g WSTC I in 1 L (four daily dose, tAF 260 mg)	Platelet Aggregation PT and TCT	+ -
7. O’Kennedy et al (unpublished, see review)	R, postiiive C Cross-over	9/ MF Healthy	Single dose 3 g WSTC I in OJ (one daily dose, tAF 65 mg) 150 mg WSTC II in Yoghurt (one daily dose, tAF 65 mg) 600 mg WSTC II in Yoghurt (4 daily dose, tAF 260 mg)	Platelet Aggregation PT and TCT	+ -
8. O’Kennedy et al (accepted for publication)	R, DB, PC Cross-over	46/MF Healthy	75 mg low-dose aspirin (8 d) 6 g WSTC I (1 d) (one daily dose, tAF 65 mg)	Platelet Aggregation PT and TCT	+ -

Review of evidence published in : O’Kennedy N, Raederstorff D, Duttaroy AK. (2016) Fruitflow®: the first European Food Safety Authority-approved natural cardio-protective functional ingredient. Eur J Nutr. 2016 Jul 7. [Epub ahead of print] Review

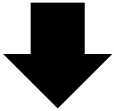
Abbreviations: R=randomized, DB=Double-blind, SB=Single-blind, PC=Placebo-control, C=Control, M=Male, F=Female, PT=Prothromin, TCT=thrombin clotting time, WSTC=water-soluble tomato extract (Fruitflow), tAF= total active fraction



Pathological Disorders
(*Diabetes, hyperlipideamia, smoking, High fat diets etc.)*)



Platelet hyperactivity



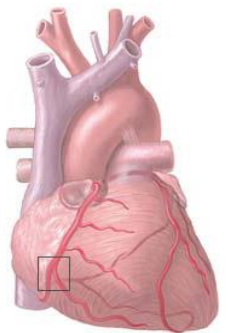
Atherosclerosis



CVD events
Coronary heart disease
Angina, Myocardial infarction
Cerebrovascular Disease
Ischaemic stroke



Blockage in right coronary artery



Publications on Fruitflow

1. Duttaroy, A.K. , and Crosbie, L., Gordon MJ (2001) Effects of tomato extract on platelet aggregation in vitro. *Platelets*, 12, 218-227.
2. O' Kennedy,N., Crosbie,L., Lieshout, M.V., Webb, D.J., Broom, J.,and Duttaroy, A.K. (2006) Antiplatelet activity of tomato extract. Effects on platelet function in vitro and ex vivo - a time course cannulation study in healthy humans. *American Journal of Clinical Nutrition* 84, 570-579.
3. O' Kennedy,N., Crosbie,L., Whelan,S, Luther,S., Horgan, G., Webb, D.J., Broom, J.,and Duttaroy, A.K. (2006) Effects of tomato extract on platelet function - a double-blinded crossover study in healthy humans. *American Journal of Clinical Nutrition* 84,561-569 .
4. Biswas D, Uddin MM, Dizdarevic LL, Jørgensen A, Duttaroy AK. Inhibition of angiotensin-converting enzyme by aqueous extract of tomato. *Eur J Nutr*. 2014 Dec;53(8):1699-706. doi: 10.1007/s00394-014-0676-1.
5. O'Kennedy N, Raederstorff D, Duttaroy AK. Fruitflow®: the first European Food Safety Authority-approved natural cardio-protective functional ingredient. *Eur J Nutr*. 2016 Jul 7. Review. PMID: 27388464
6. O'Kennedy N, Crosbie L, Song HJ, Zhang X, Horgan G, Duttaroy AK. A randomised controlled trial comparing a dietary antiplatelet, the water soluble tomato extract Fruitflow with 75mg aspirin in healthy volunteers. *Eur J Clin Nutr*. 2016 Nov 23. doi: 10.1038/ejcn.2016.222.
7. Uddin, MM, Biswas, D, Ghosh, S, O'Kennedy N, Duttaroy, AK (2017) A randomised, placebo controlled, double blind, cross-over study of Fruitflow® in moderately hypertensive males, [Int J Food Sci Nutr](#). 2017 Sep 18:1-9. doi: 10.1080/09637486.2017.1



Fruitflow[®]: the first European Food Safety Authority (EFSA)-approved natural cardio-protective functional ingredient

**Building A Successful Health Claims Dossier
for EFSA Approval**

What is Functional Food

The *European Food Safety Authority (EFSA)* defines functional foods as:

A food, which beneficially affects one or more target functions in the body, beyond adequate nutritional effects, in a way that is relevant to either an improved state of health and well-being and/or reduction of risk of disease.

A functional food can be a natural food or a food to which a component has been added or removed by technological or biotechnological means, and it must demonstrate their effects in amounts that can normally be expected to be consumed in the diet

Functional Food and its health claim in EU

Health claims are defined as pertaining to relationships between food and health either with regard to a function of the body (Article 13 claims),

or with regard to reducing a risk factor for a disease (Article 14a claims),

or with regard to children's development (Article 14b claims).

Nutrient claims are defined as pertaining to foods with particular nutritional properties with regard to either the energy, or the nutrients, they provide.

EU Regulation of Food Health Claims



REGULATION (EC) No 1924/2006 on nutrition and health claims made on foods (2006)

DIRECTIVE 2002/46/EC on the approximation of the laws of the Member States relating to food supplements (2002)

REGULATION (EC) No 178/2002 the general principles of food law, establishing the European Food Safety Authority (2002)

News:

EFSA Finds 80% of Health Claims Lack Evidence

Over **44,000 “general function” health claims** have been submitted to EFSA on behalf of the manufacturers. **4,637 claims to be considered** by the European Food Safety Authority (EFSA)

Of the approximately **900 claims the EFSA has examined** so far, **80 % have been rejected**

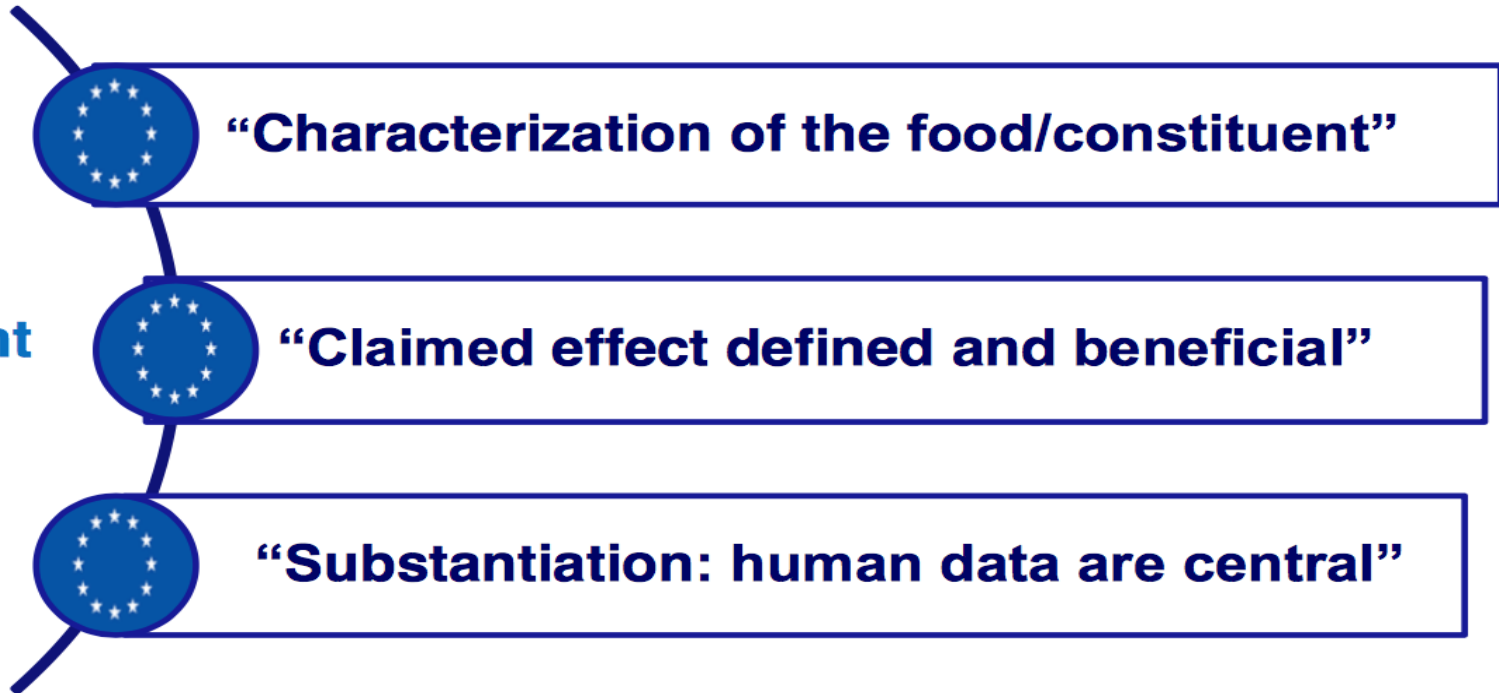


MAIN CRITERIA OF HEALTH CLAIMS REGULATION

Reg. (EC) No 1924/2006

- Authorization of health claims in the EU is based on a **scientific assessment** following the highest possible standards.
- **Consumer protection, fair competitiveness and innovation**

EFSA assessment



Scientific substantiation requires a favourable outcome in ALL



HEALTH CLAIMS CLASSIFICATION

Regulation (EC) 1924/2006

Art.13.1

**Generally
accepted
scientific
evidence**

List Claims

Art.13.5

**Newly
developed
scientific data /
proprietary data**

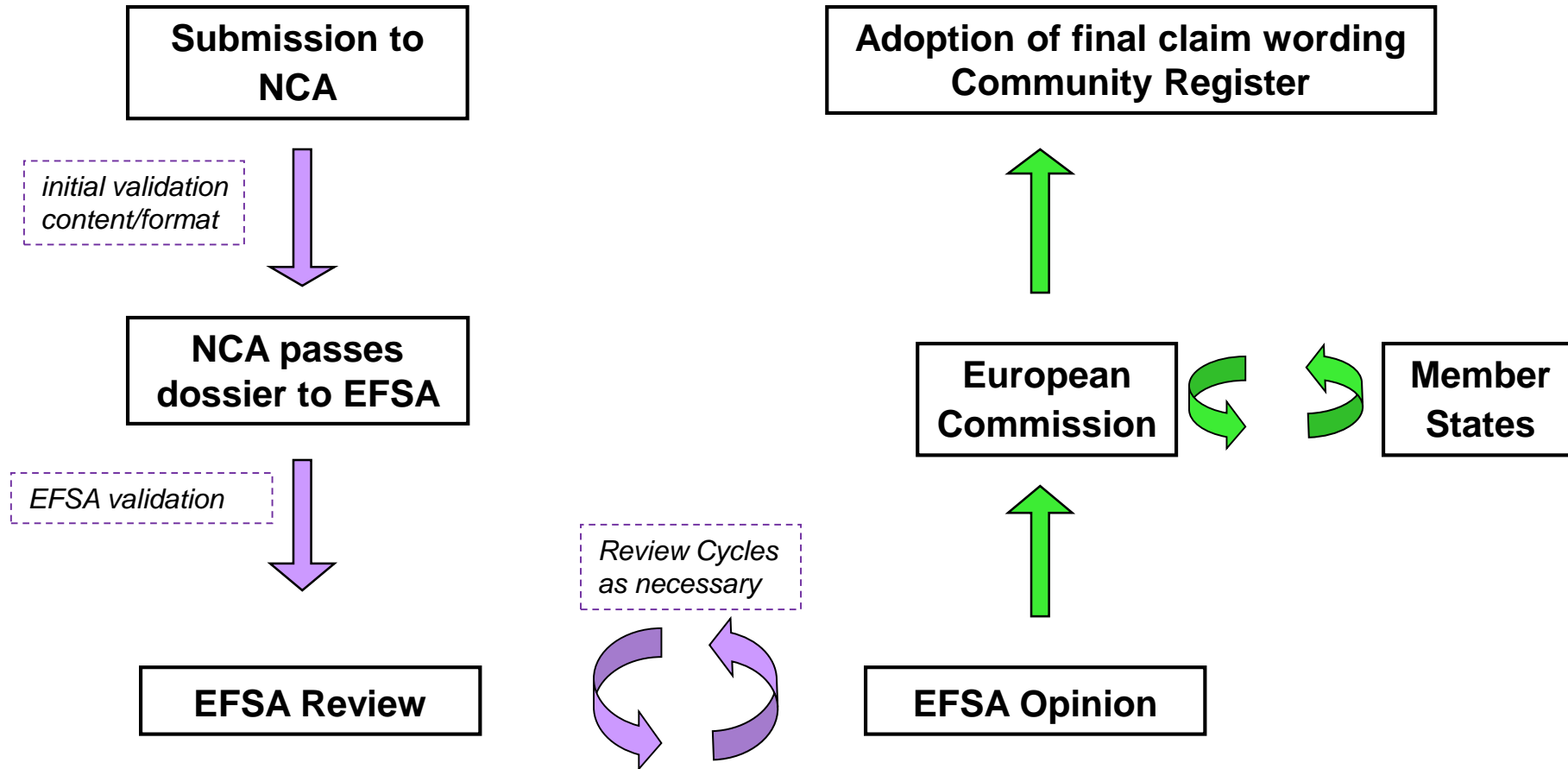
Applications

Art.14

**Reduction of
disease
Risk**

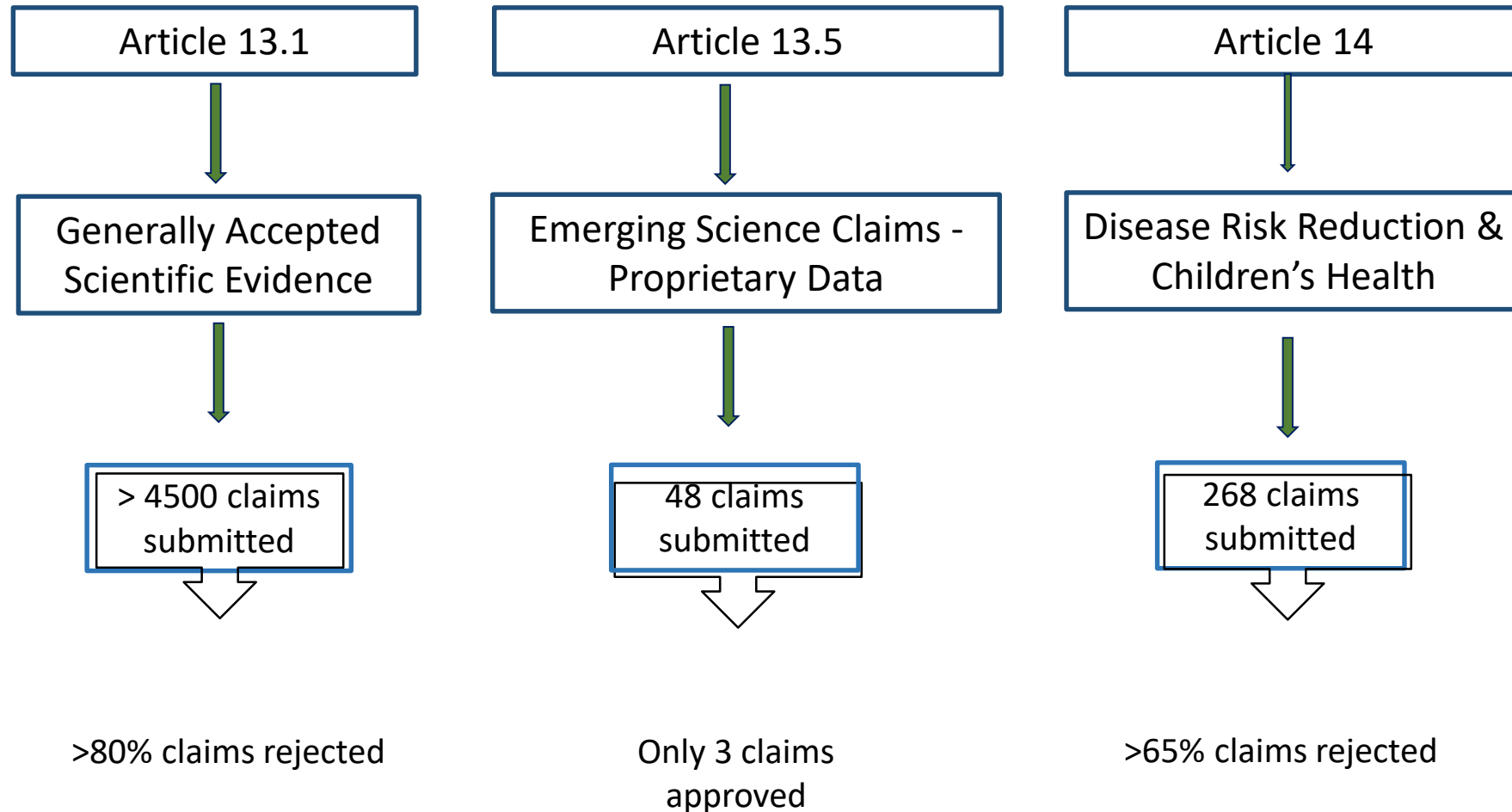
**Children's
development &
health**

EU Health Claims Process – EC Regulation 1924/2006



EC Regulation 1924/2006

Regulation covering Nutrition & Health Claims on Foods



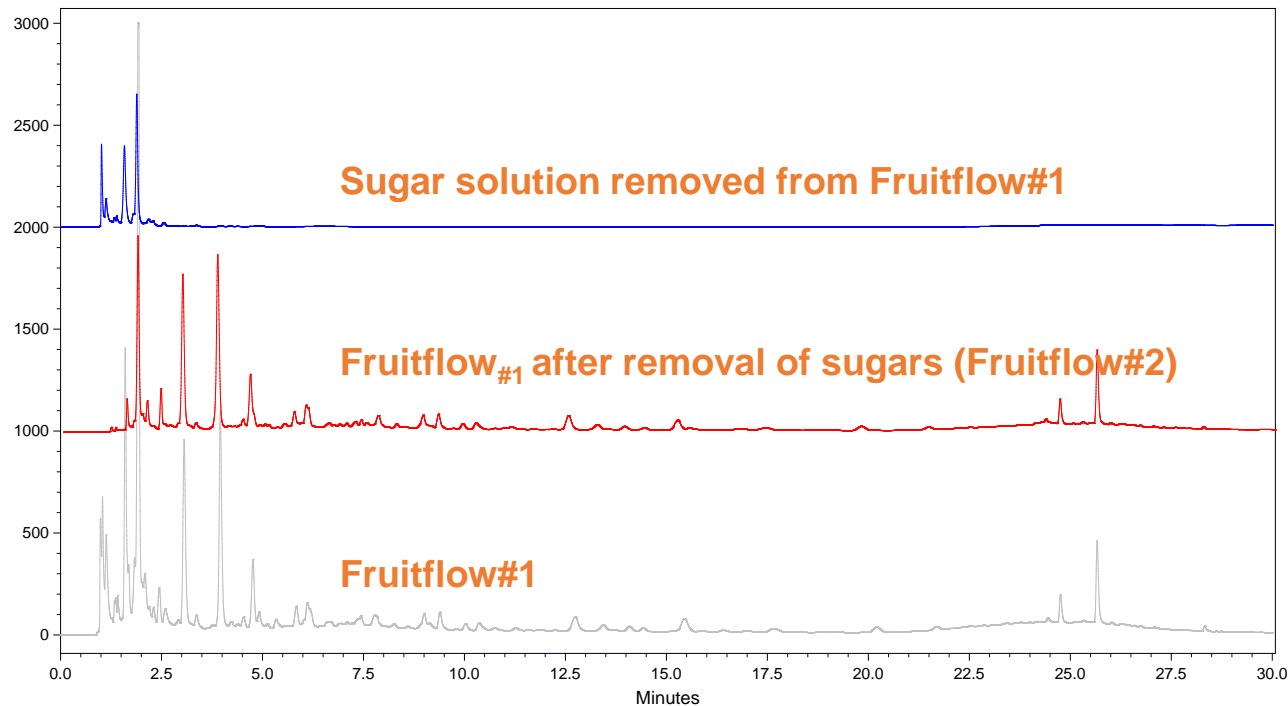
Fruitflow[®] – Water Soluble Tomato Concentrate

- First functional food ingredient to obtain a European Health Claim (EC Regulation 1924/2006) under Article 13.5
- **Fruitflow helps maintain normal platelet aggregation which contributes to healthy blood flow**
- Package of randomised & controlled human clinical trials demonstrating efficacy & safety
- Partnered with DSM Nutritional Products for commercialisation & further development



Fruitflow – establishing standardised ingredient formats

- Two different formats of the ingredient, one with and one without sugars, have been developed and fully characterised.



EFSA – Reasons for Rejection

- The words too familiar to EU health claim applicants.....
 - “.....a cause and effect relationship has not been established between the consumption of.....”
- Reasons for negative opinions
 - benefit to human health not accepted
 - insufficient characterisation of final product
 - insufficient human clinical data
 - inconsistent trial results or unreliable outcome measures
 - studies not conducted using final product form/conditions of use



Bioactive substance

The substance has not been sufficiently characterised



Beneficial physiological effect

The effect is not a beneficial physiological effect

The effect is not a health claim



Cause and effect relationship

The evidence does not research a cause and effect relationship

Incorrect methodology used

Incorrect biomarkers used

In vitro studies used

Animal studies used

The evidence does not reveal a cause and effect relationship

The evidence was not accessible

Criteria

Specifications

EFSA – Dossier for Substantiation of a Claimed Benefit

- **Important dossier sections**

- Product characteristics

- Written overall summary of pertinent human data

- Including tabulated summary of human studies

- Written overall summary of pertinent animal model / in vitro mechanistic studies

- Systematic review of all the available evidence showing the link between the selected biomarker and the health benefit

- Full details of all pertinent human study data derived from the review, and any proprietary studies not contained within this review



Biomarker - health benefit link

- **Key choices**

- Health claim

The desired health claim may not reflect exactly the health benefit which can be substantiated

- Biomarker

Possibly the most critical decision to be made

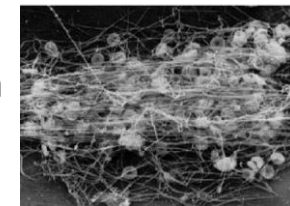
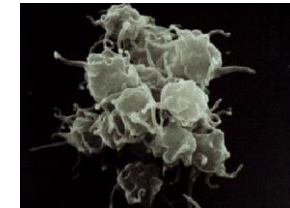
Selection of possible biomarkers



Assessment of the totality of the evidence linking biomarkers to claimed health benefit

Assessment of reduced platelet aggregability as a health benefit

- Systematic review carried out, encompassing:
 - Secondary prevention strategies
 - Platelet function is an established target in treatment and secondary prevention of CVD
 - Primary prevention strategies
 - High-risk groups only: risk-benefit analysis shows that increased risk of bleeding outweighs potential benefits in low-risk groups
 - Review of dietary antiplatelets and comparison with established medical regimes
 - Garlic, omega-3 oils, cocoa, red wine and tomato only dietary antiplatelets with significant data to support benefit, typical reduction in platelet aggregation of 12 – 20%



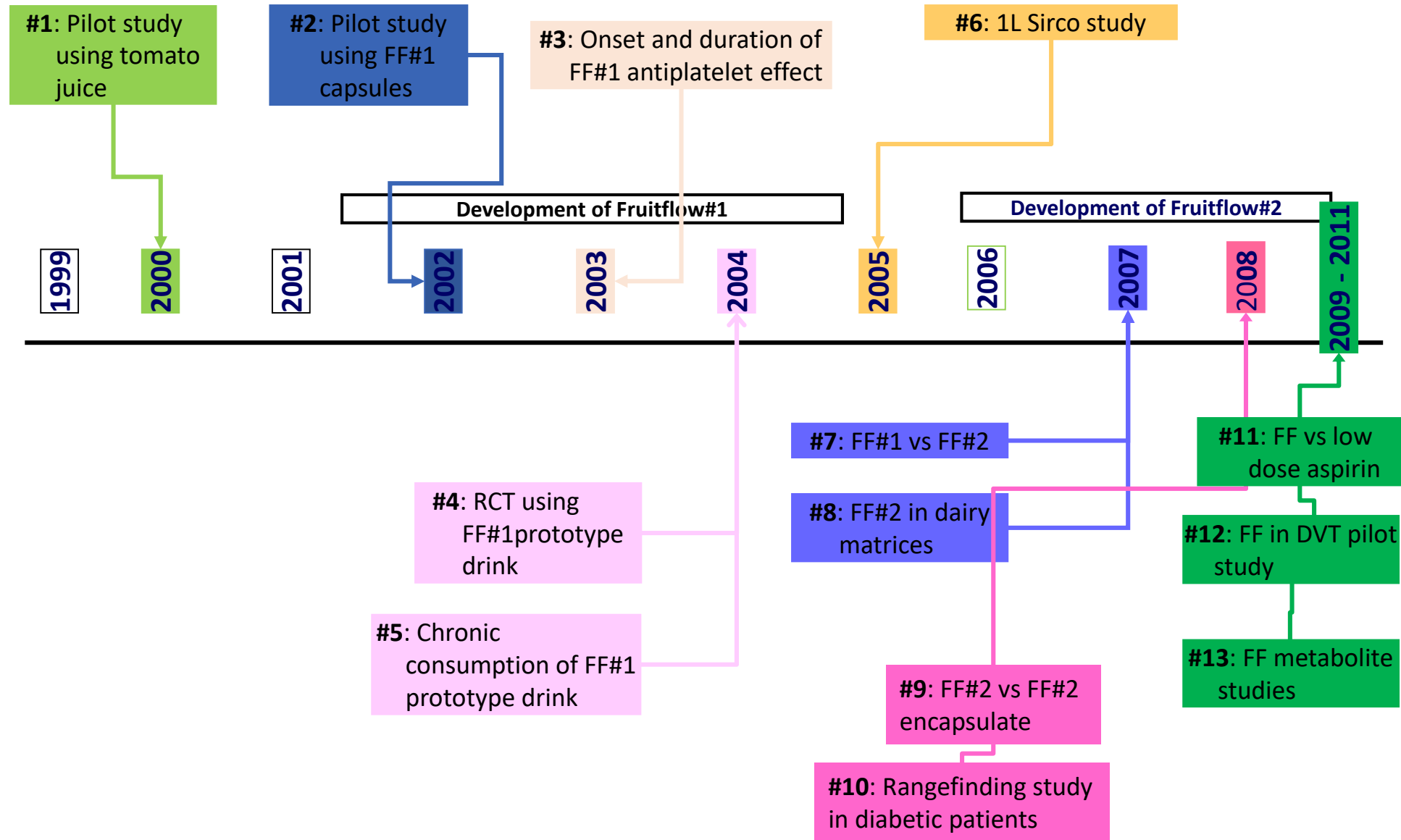
Adequacy of human trial data

- Claim substantiation dossiers are expected to contain a written summary of human trial data, based on human trial data detailed within the dossier, with particular illustrations of / comments on:
 - Study populations and conditions
 - Magnitude of effect and physiological relevance
 - Sustainability of effects over time
 - Amount of substance needed to achieve the effect, usual intakes in the general population and whether these amounts could reasonably be obtained as part of a balanced diet

Adequacy of human trial data

- **How many studies are required ?**
 - **Absolute number is not the most important factor**
 - **If the EFSA dossier sections cannot adequately be addressed** from published studies, more studies are needed
 - **If available human study data is contradictory** a very clear explanation is necessary and more studies may be required to prove the point – ambiguity in interpretation here probably results in failure
 - If available human studies do not focus on the **target population** for the claim or do not use the **specified food constituent** as the main intervention, the studies will not be considered relevant by EFSA

Adequacy of human trial data: programme of work



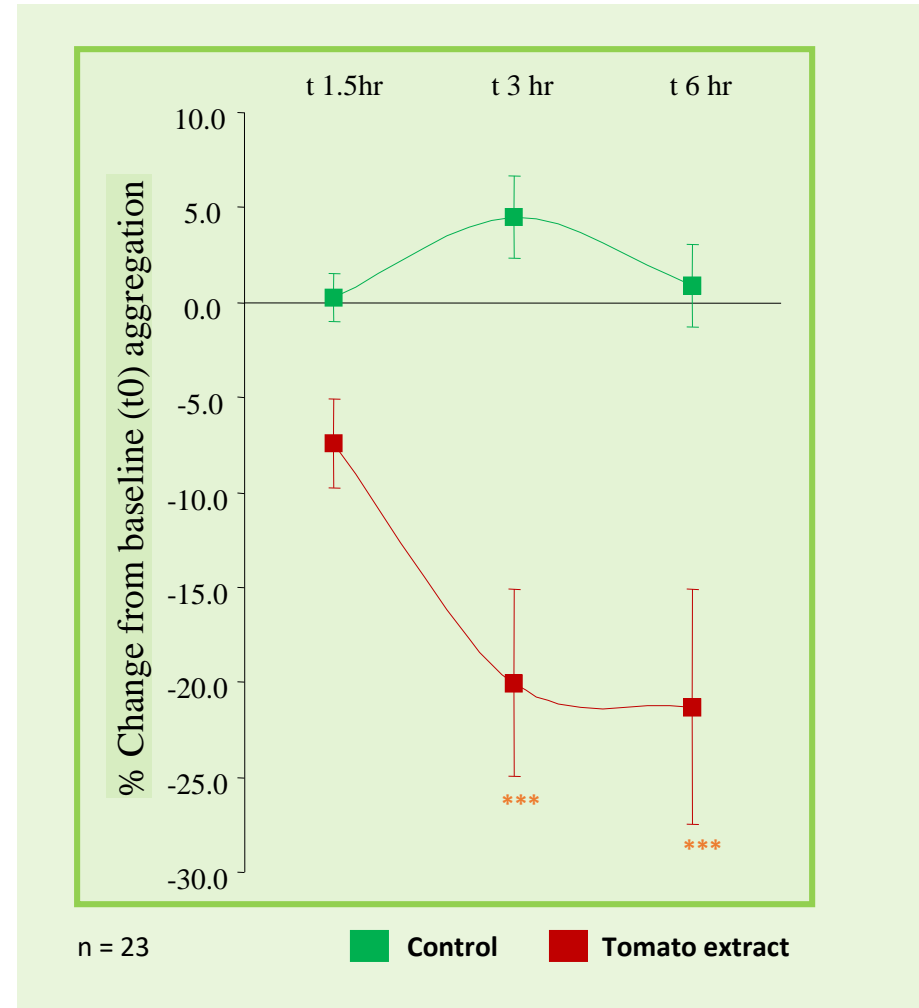
Adequacy of human trial data

- Claim substantiation dossier - 8 pertinent human studies (7 proprietary)
 - Studies should be organised according to hierarchy: randomised controlled (RC), randomised non-controlled (RN), controlled non-randomised, other studies
 - “These human studies consistently show a reduction in platelet aggregation following consumption of WSTC under the conditions of use proposed by the applicant” - EFSA

Trial	Primary Purpose	N=	Control	Target Population	Proposed conditions of use
#1	Tomato juice pilot	20	RCT	No	No
#2	Acute effect	27	RCT	Yes	Yes
#3	Acute effect	93	RCT	Yes	Yes
#4	Acute effect	15	RN	Yes	Yes
#5	Chronic effect	22	RCT	Yes	Yes
#6	Over-consumption	20	RN	Yes	Yes
#7	2 Fruitflow formats	45	RCT	Yes	Yes
#8	Matrix effects	9	RCT	Yes	Yes

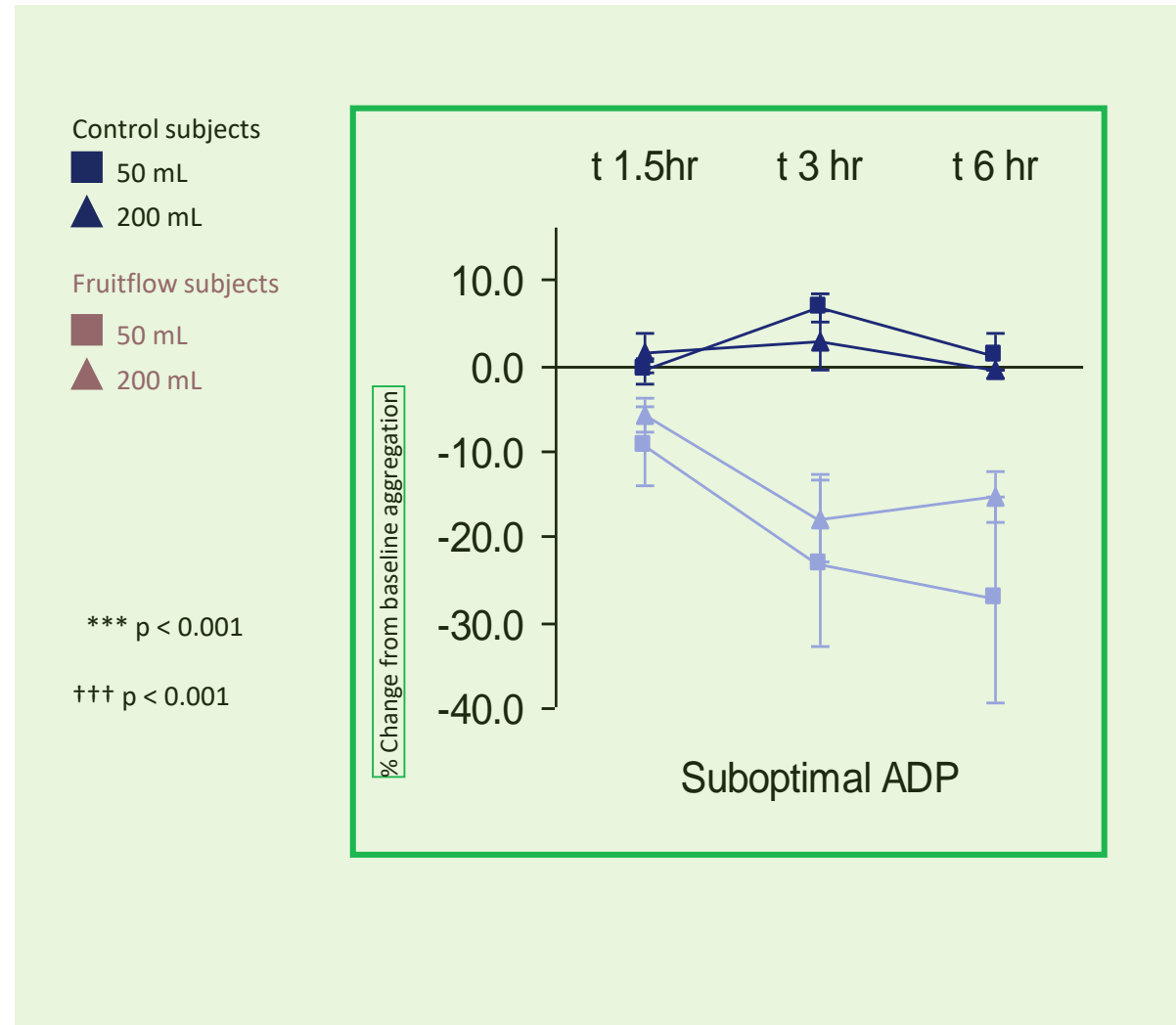
Onset and duration of *in vivo* antiplatelet effect

- Averaged results across different drink volumes
- Onset of antiplatelet effect seen within 3 hours after ingestion – measured as a significant difference from baseline platelet function
- Duration of effect variable, but after 18 hours platelet function returned to baseline in all subjects tested



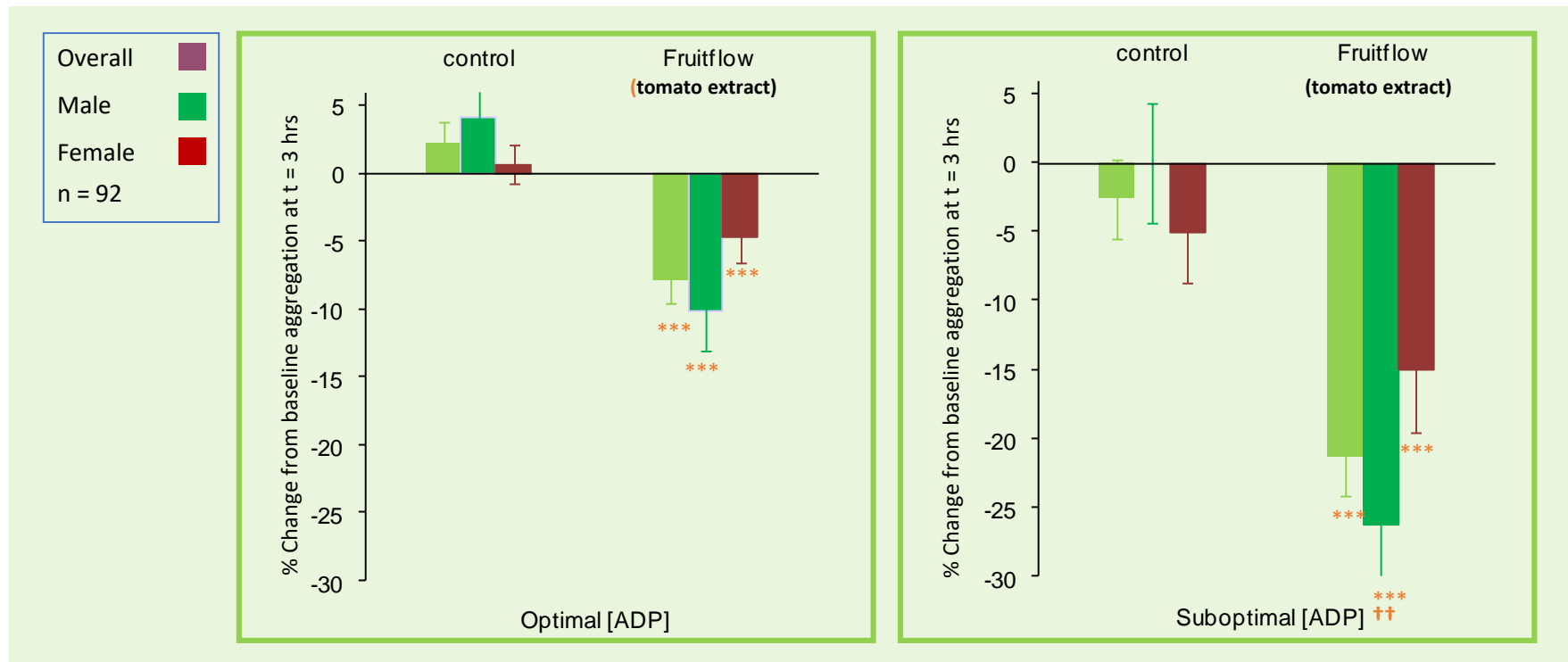
Volume of the consumed dose affects the ex vivo platelet response

- The volume of the Fruitflow drink can have an effect on the magnitude of the antiplatelet effect
- The duration of the antiplatelet effect may be lessened at larger volumes
- However the onset of the antiplatelet effect does not appear to be affected



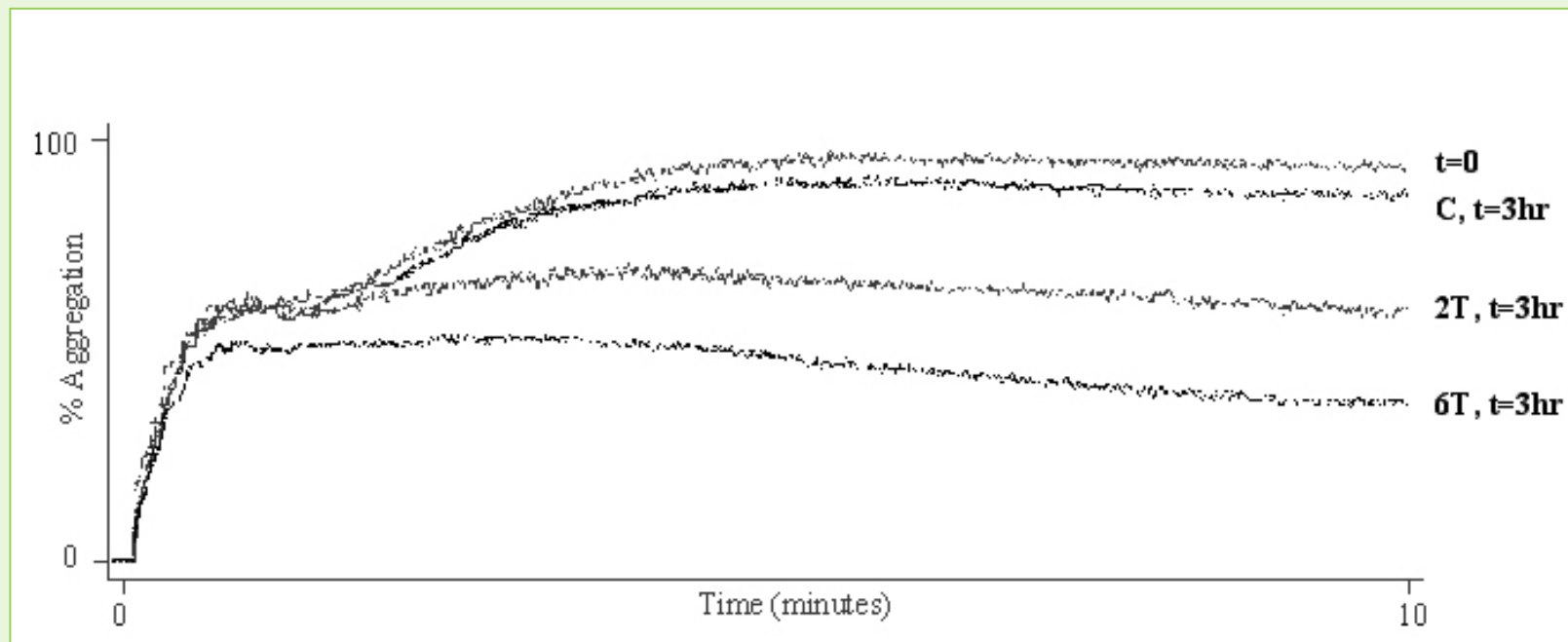
In vitro anti-platelet activity translates to *ex vivo* anti-platelet efficacy

- At suboptimal ADP levels, Fruitflow produced an average 21% decrease in platelet aggregation (men responded more than women)
- At optimal levels of ADP, the effects of Fruitflow can be overcome – for example when a physiological response to injury is needed



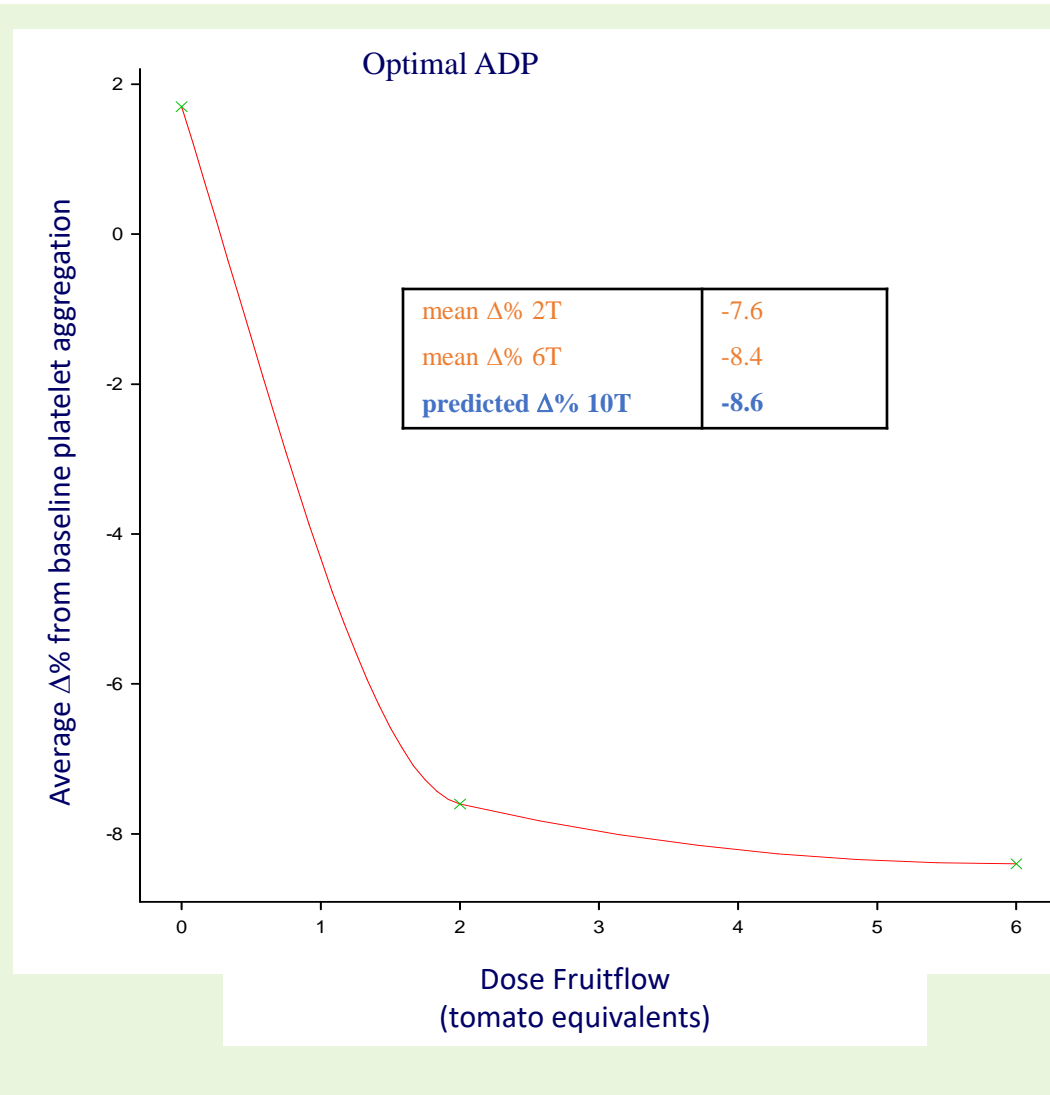
Dose-response relationship

- Consuming different amounts of Fruitflow alters the 3-hour platelet response in a dose-dependent manner



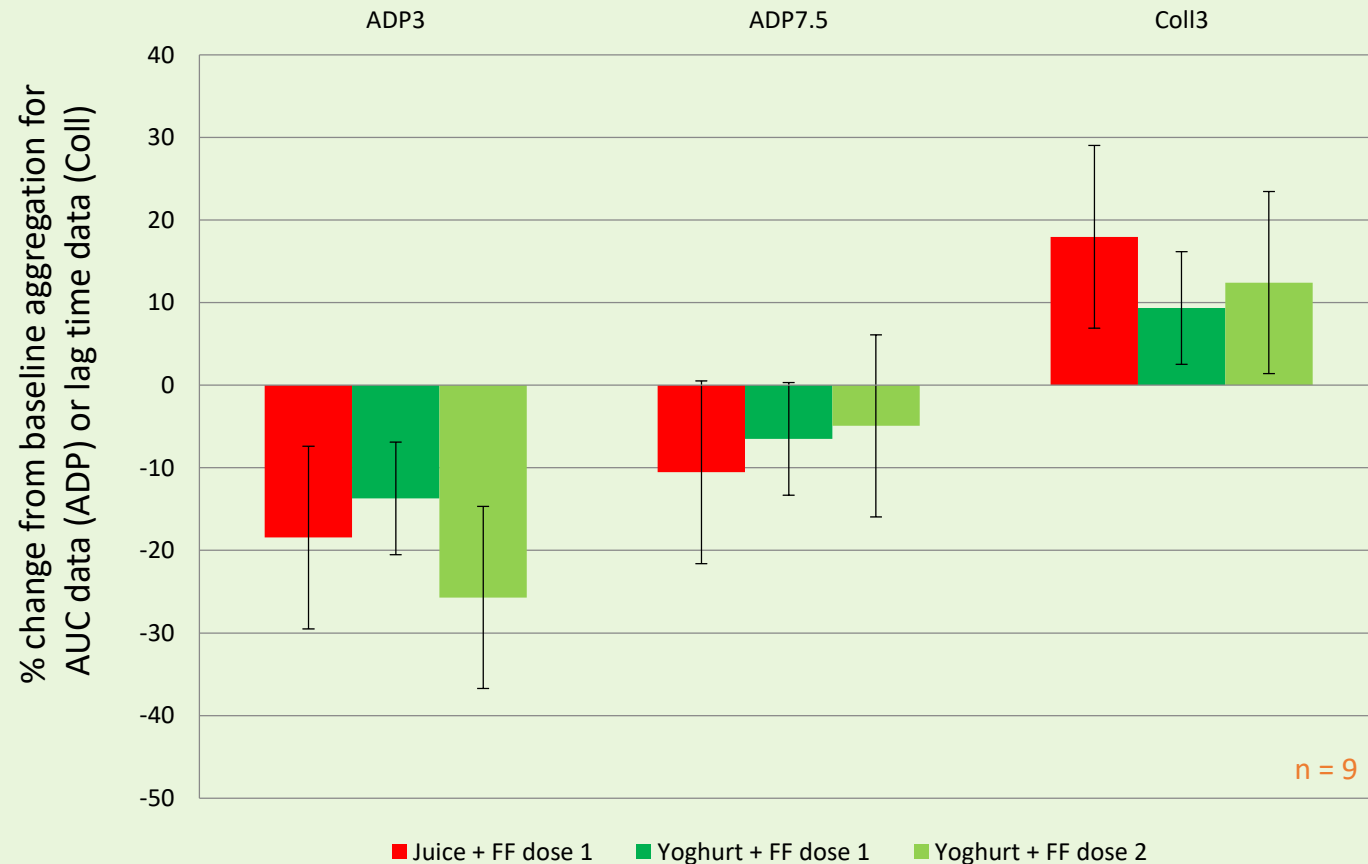
Dose-response relationship

- The effects of different doses can also be compared using dose-response curves
- At a dose-equivalence to 2 tomatoes, FF already gives an effect close to maximal (under conditions of high platelet stimulation)



Effects of food matrix on extract bioavailability

- Pilot study examining the acute antiplatelet effects of FF administered in fruit juice and yoghurt matrices: positive control equivalence study, crossover design
- Differences between matrices not significant



Establishment of a causal relationship

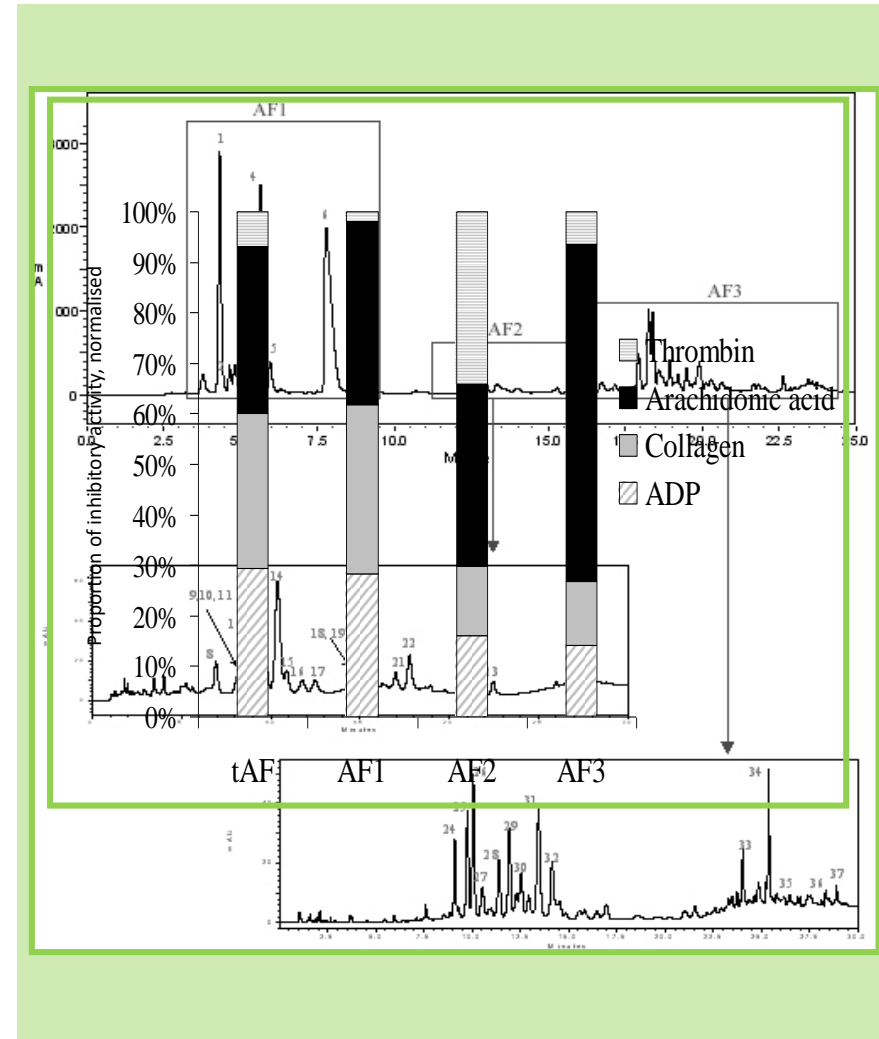
- An objective examination of the causal relationship between consumption and effect is expected, with particular attention to:
 - Biological plausibility
 - Alternate explanations
 - Specificity of the cause-effect relationship

Biological plausibility – mode of action studies

Tomato antiplatelet components (total active fraction, tAF) were isolated and characterised

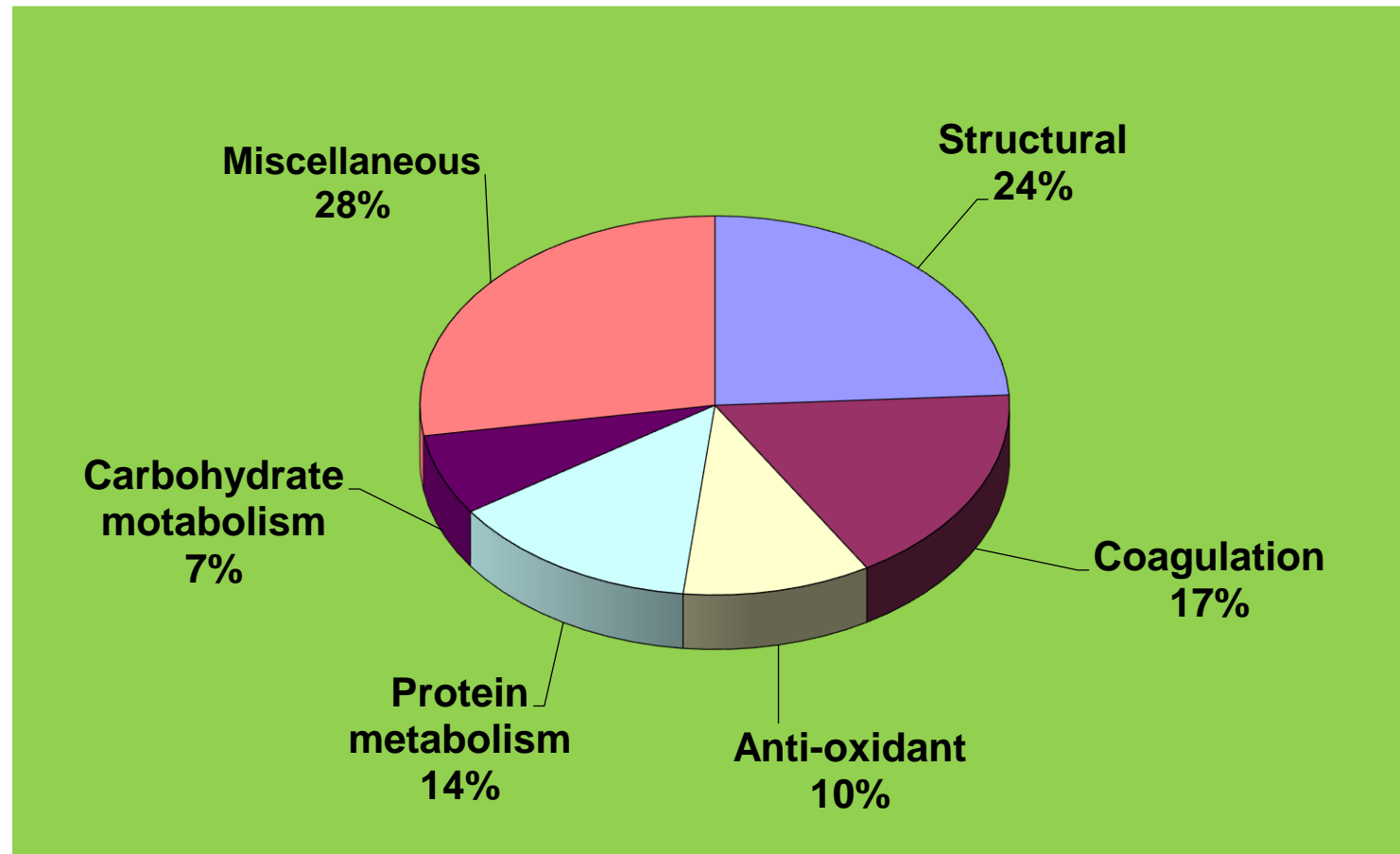
tAF components can be separated into distinct bioactive subfractions, AF1, AF2 and AF3

The three subfractions contain different types of compound and exhibit different modes of action in vitro



Mechanisms elucidated through proteomic studies

tAF components cause changes across a wide spectrum of specific platelet functional pathways



Mechanisms translated into functional effects

Functional effects of tAF have been observed on both early and later stage platelet activation markers

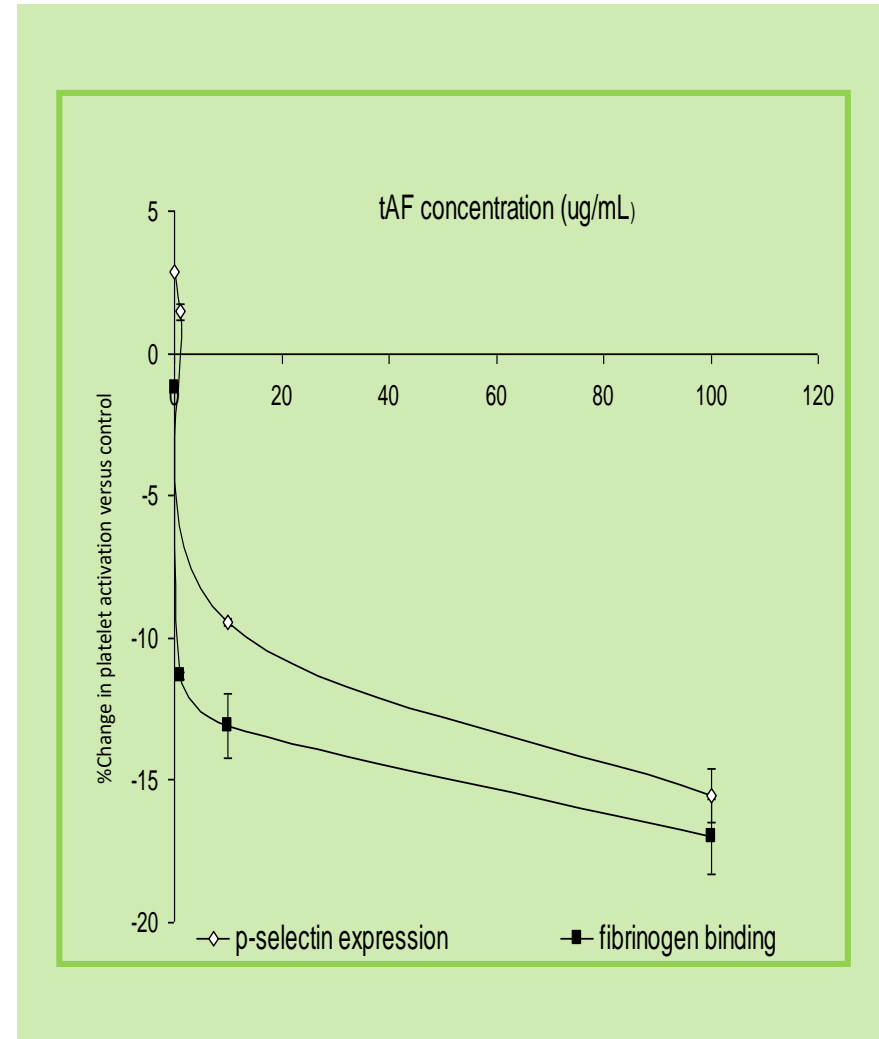
Early stage: Platelet adhesins

p-selectin

Later stage: Integrin activation

GPIIb/IIIa

GPIb-V-IX



Functional Food Health Claims - Background

- EC Regulation No: 1924/2006
 - Introduced July 2007, covering “nutrition and health claims made on foods”
 - Primary purpose is to protect consumers, to ensure claims are truthful, clear & reliable and finally to facilitate choice.
 - 3 main sub-sections
 - Article 13.1 – consolidated circa 4,500 historical “on-market” claims (pre-2010)
 - Article 13.5 - ‘newly developed scientific evidence’
 - Article 14 - ‘reduction of disease risk & children’s health’
 - 2 years after introduction (May 2009) – first product* obtains a positive EFSA opinion

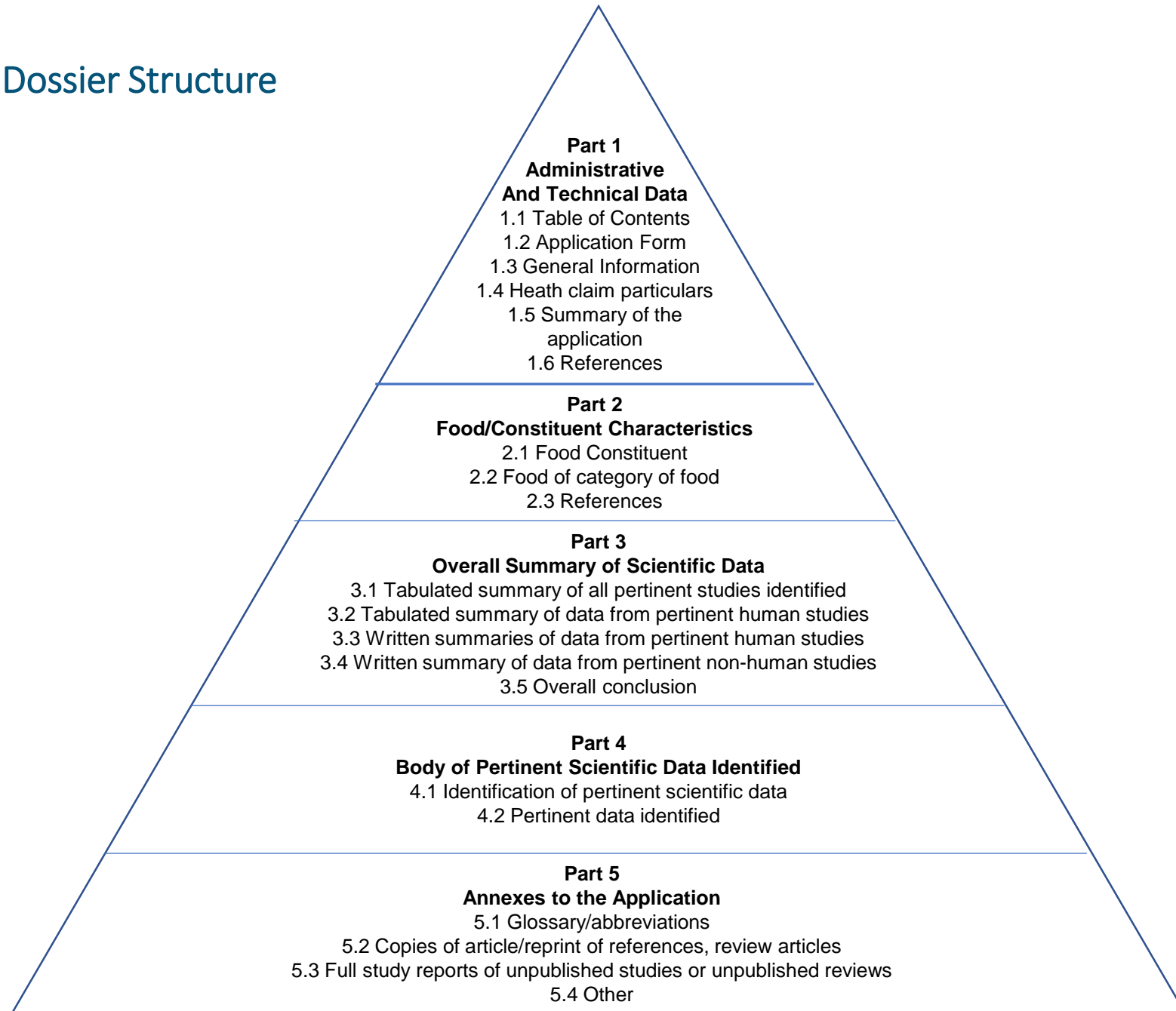
Year	Published Art 13.5 Opinions**
2009	9
2010	12
2011	16
2012	33
2013	25
2014	14
2015	11
2016	6
2017	8

* Fruitflow™ (Water Soluble Tomato Concentrate) – helps maintain healthy blood flow

** P2F Ventures Limited - Figures from monitoring all published Article 13.5 opinions from EFSA’s NDA Panel



The EFSA Dossier Structure



Positive Article13(5) Opinions

Date	Product	Company	Health Claim
2009	Fruitflow™ (tomato extract)	Provexis (UK)	contributes to healthy blood flow
2010	Ribena™ Toothkind (blackcurrant drink)	GSK (UK)	reduction tooth demineralisation
2011	Slowly Digestible Starch	Kraft Foods (Belgium)	Reduced glycaemic response (post-prandial)
2011	Sugar Beet Fibre	Nordic Sugar (Denmark)	increases faecal bulk
2012	Cocoa Flavanols	Barry Callebaut (Belgium)	contributes to healthy blood flow
2014	Chicory Inulin	BENEO-Orafti (Belgium)	maintenance of normal defecation
2015	Lactitol (disaccharide - galactose/sorbitol)	DuPont Nutrition (UK)	contributes to normal defecation
2016	Creatine	AlzChem AG (Austria)	muscle strength

Who succeeds & the challenge of valuing claims



Creapure™ – b2b ingredient



Lactitol – b2b ingredient



Aticoa™ – b2b ingredient



FibreX™ – b2b ingredient & retail product



Orafti™ – b2b ingredient



Fruitflow™ – b2b ingredient

Article 13(5) – 2016/2017 – 14 New Applications

Product	Company	Health Claim	
Germinated Brown Rice	Loc Troi Group (Netherlands*)	Maintenance long term blood glucose, blood pressure, cholesterol and reduction of weight (4 separate claims)	X
High protein moderate carbohydrate (HPMC) meals	Marks & Spencer (UK)	Helps reduction in body weight and body fat	X
Circumin	Suomen Oy (Finland)	Normal functioning of the joints (by reducing markers of inflammation)	X
Stablor™, beverage with specific proportions of amino acids – tryptophan to neutral amino acid ratio	Laboratories Nutrition et Cardiometabolisme (France)	Decrease in visceral fat whilst preserving lean mass	X
Nutrimune, a pasteurised cow's skim milk fermented with <i>Lactobacillus paracasei</i> CBA L74	HJ Heinz B.V., Netherlands	Immune defence against pathogens in GI tract and Upper Respiratory Tract (URT)	X
DHA-enriched fish oil	Pierre Fabre Medicament (France)	Helps slow age-related cognitive decline (memory and executive function)	X
Casein protein hydrolysate (bovine milk-derived)	Food for Health (Ireland)	Reduction of post-prandial blood glucose responses	X
<i>Lactobacillus plantarum</i> 299v	Probi AB (Sweden)	Increase of non-haem iron absorption	X
DHA	DSM Natural Products (UK)	Improvement of memory function	X
Polydextrose	Tate & Lyle (UK)	Maintenance of normal defecation (increasing stool bulk)	X
Creatine	AlzChem AG (Austria)	Maintenance of muscle (grip) strength	✓

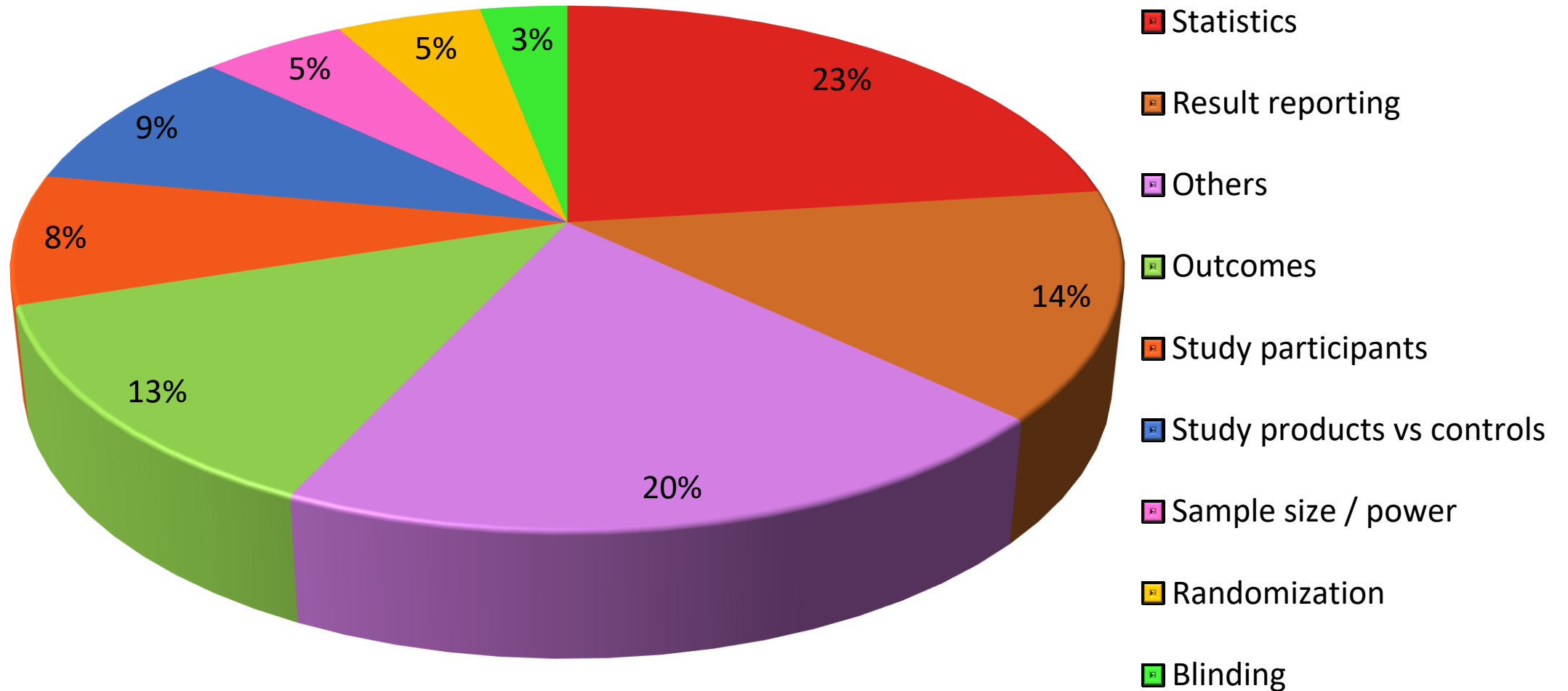
* Vietnamese group

Negative Article 13(5) Opinions – Why do they fail

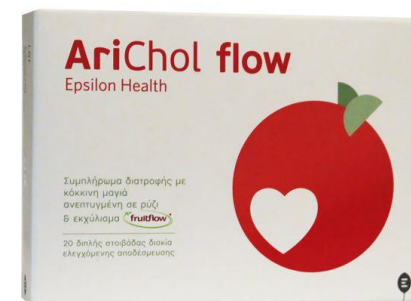
Product	Claim	NDA Panel Opinion
Germinated Brown Rice	Maintenance long term blood glucose, blood pressure, cholesterol & reduction of weight (4 separate claims)	<ul style="list-style-type: none"> • Only 1 human intervention study • Target outcome was not the primary outcome measure • Methodological limitations (open-label design, unclear randomisation procedure etc) • Un-validated outcome measures (for the specific population) • Inappropriate statistical methods
High protein moderate carbohydrate (HPMC) meals	Helps reduction in body weight and body fat	<ul style="list-style-type: none"> • 14 human intervention studies – mixed results so inconclusive !! [nearly all the short term studies (<12 weeks) were positive BUT nearly all the longer term studies (> 12 weeks) were negative]
Circumin	Normal functioning of the joints (by reducing markers of inflammation)	<ul style="list-style-type: none"> • Majority of human studies submitted evaluated “patients” with OA/RA (patients are not target population for EFSA health claims - unless justify extrapolation of findings)
Stablor™, beverage with specific proportions of amino acids – tryptophan to neutral amino acid ratio	Decrease in visceral fat whilst preserving lean mass	<ul style="list-style-type: none"> • 4 human intervention studies (but 3 studies rejected for having no control group) • Only 1 human intervention study remained • One analysis of that study (intent-to-treat) was negative whereas another (per-protocol) was positive
Nutrimune, pasteurised cow’s skim milk fermented with <i>L. paracasei</i> CBA L74	Immune defence against pathogens in GI tract and Upper Respiratory Tract (URT)	<ul style="list-style-type: none"> • 2 human intervention studies (1 quickly rejected) • Inappropriate statistical analysis of multi-centre trial (imbalanced groups/dropouts) • Inconsistent processes for determining URTI (subjective subject diary not examination)
DHA enriched fish oil	Helps slow age-related cognitive decline (memory & executive function)	<ul style="list-style-type: none"> • 5 human intervention studies (4 carried out with foods not matching product spec) • Remaining study showed no statistically significant differences
Casein protein hydrolysate (bovine milk derived)	Reduction of post-prandial blood glucose responses	<ul style="list-style-type: none"> • Effect driven by increased insulin production • EFSA guidance clearly states that lowering glucose by driving up insulin production not acceptable mechanism
Polydextrose	Maintenance of normal defecation (increasing stool bulk)	<ul style="list-style-type: none"> • 3 human intervention studies • mixed results (1 positive study and 2 negative studies) so inconclusive

What data is EFSA questioning ?

Source: EFSA Technical Meeting (Nov 2013)



THANK YOU



Naturalny produkt dla utrzymania prawidłowego przepływu krwi

