Strawberry as a health promoter: An evidence based review

9 authors, including:

Francesca Giampieri
Università Politecnica delle Marche
126 PUBLICATIONS   **3,819** CITATIONS

Tamara Forbes
Università Politecnica delle Marche
66 PUBLICATIONS   **964** CITATIONS

Massimiliano Gasparrini
Università Politecnica delle Marche
67 PUBLICATIONS   **890** CITATIONS

José Miguel Alvarez-Suarez
Universidad de Las Américas
64 PUBLICATIONS   **2,415** CITATIONS

Some of the authors of this publication are also working on these related projects:

COST Action CA15203 Mitochondrial fitness mapping View project

http://www.goodberry-eu.eu/ View project

REVIEW

CrossMark
click for updates

Strawberry as a health promoter: an evidence based review

Francesca Giampieri,[a] Tamara Y. Forbes-Hernandez,[b,c] Massimiliano Gasparrini,[b] José M. Alvarez-Suarez,[d] Sadia Afrin,[b] Stefano Bompadre,[e] Josè L. Quiles,[f] Bruno Mezzetti[a] and Maurizio Battino*[b,g]

Since a high intake of fruits and vegetables is inversely related to the incidence of several degenerative diseases, the importance of a balanced diet in relation to human health has received increased consumer attention worldwide. Strawberries (*Fragaria X ananassa*, Duch.) are a rich source of a wide variety of nutritive compounds such as sugars, vitamins, and minerals, as well as non-nutritive, bioactive compounds such as flavonoids, anthocyanins and phenolic acids. All of these compounds exert a synergistic and cumulative effect on human health promotion and in disease prevention. Strawberry phenolics are indeed able (i) to detoxify free radicals blocking their production, (ii) to modulate the expression of genes involved in metabolism, cell survival and proliferation and antioxidant defense, and (iii) to protect and repair DNA damage. The overall objective of the present review is to update and discuss the key findings, from recent *in vivo* studies, on the effects of strawberries on human health. Particular attention will be paid to the molecular mechanisms proposed to explain the health effects of polyphenols against the most common diseases related to oxidative stress driven pathologies, such as cancer, cardiovascular diseases, type II diabetes, obesity and neurodegenerative diseases, and inflammation.

Introduction

Dietary guidelines around the world recommend the increased consumption of fruits and vegetables, as good sources of dietary fiber, essential nutrients, and beneficial phytochemicals, to improve global health and reduce chronic disease risk.[1] A diet rich in fruits and vegetables is indeed associated with a lower incidence of several degenerative pathologies, including obesity, cardiovascular and neurological diseases, and cancer;[2,3] therefore, increasing the consumption of fruit may be a practical strategy for prevention. Berries provide note-

worthy health benefits[4,5] among fruits because of their highly nutritive compounds, including minerals, vitamins, fatty acids, and dietary fiber, as well as a wide range of polyphenolic phytochemicals (flavonoids, phenolic acids, lignans, and tannins).[6] Among berries, strawberries are popularly consumed not only in fresh and frozen forms but also as processed and derived products, including yogurts, beverages, jams, and jellies. Recently, strawberry extracts have also been used as ingredients in functional foods and dietary supplements, combined with other colorful fruits, vegetables, and herbal extracts.[7]

Regarding their nutritional and phytochemical composition, strawberries contain fat-soluble vitamins, including carotenoids, vitamin A, vitamin E and vitamin K, but one of the aspects of major nutritional relevance is their high content of vitamin C (about 60 mg per 100 g fresh fruit), and, albeit to a lower extent, a sufficiently good source of several other vitamins, such as thiamin, riboflavin, niacin, and vitamin B6.[8]

Another significant nutritional feature is the concentration of folate (24 μg per 100 g fresh fruit):[8] among fruit, strawberries are one of the richest natural sources of this indispensable micronutrient, which represents an essential factor in health promotion and disease prevention.[9,10] Strawberries are also a notable source of manganese, and a good source of iodine, magnesium, copper, iron and phosphorus. Moreover, both their dietary fiber and fructose contents may contribute

[a]*Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università Politecnica delle Marche, Via Ranieri 65, 60131 Ancona, Italy*
[b]*Dipartimento di Scienze Cliniche Specialistiche ed Odontostomatologiche (DISCO)-Sez. Biochimica, Facoltà di Medicina, Università Politecnica delle Marche, Via Ranieri 65, 60131 Ancona, Italy. E-mail: m.a.battino@univpm.it; Fax: +39 071 2204123; Tel: +39 071 2204646*
[c]*Area de Nutrición y Salud, Universidad Internacional Iberoamericana (UNINI), Campeche, C.P.24040, Mexico*
[d]*Facultad de Ciencias de la Salud, Universidad Nacional de Chimborazo, Riobamba, Ecuador*
[e]*Dipartimento Scienze Biomediche e Sanità Pubblica, Facoltà di Medicina, Università Politecnica delle Marche, Via Ranieri 65, 60131 Ancona, Italy*
[f]*Department of Physiology, Institute of Nutrition and Food Technology "José Mataix", Biomedical Research Centre, University of Granada, Spain*
[g]*Centre for Nutrition & Health, Universidad Europea del Atlantico (UEA), Santander, 39011, Spain*

to regulating blood sugar levels by slowing digestion, while the fiber content may control calorie intake by its satiating effect.

In addition to traditional nutrients, strawberries are among the richest dietary sources of phytochemicals, mainly represented by phenolic compounds, a large and heterogeneous group of biologically active non-nutrients, showing many non-essential functions in plants and huge biological potentialities in humans.[11] Indeed, strawberry phenolics are best known for their antioxidant and anti-inflammatory action, and possess direct and indirect antimicrobial, anti-allergy, and anti-hypertensive properties, as well as the capacity for inhibiting the activities of some physiological enzymes and receptors, preventing oxidative stress-related diseases.[12] The major class of strawberry polyphenols are flavonoids, mainly anthocyanins, the most quantitatively important phenolic compounds present in strawberries are in the form of pelargonidin and cyanidin derivatives.[13–15] The second most abundant class of phytochemicals in strawberries are ellagitannins (i.e., sanguiin-H-6), followed by flavonols (i.e., quercetin and kaempferol-3-malonylglucoside), flavanols (i.e., catechins and procyanidins), and phenolic acids (i.e., caffeic and hydroxybenzoic derivates).[13–15]

In the past few years, the antioxidant power of fruit has been considered as an indicator of the beneficial bioactive compounds present in foodstuffs and, therefore, of their healthfulness. This parameter is strictly correlated to the presence of efficient oxygen radical scavengers whose activity, however, has been proven mostly in vitro. Moreover, considering the low bioavailability of polyphenols in vivo,[13–15] it seems that their real contribution to the overall cellular antioxidant capacity appears to be negligible. For these reasons, more complex mechanisms have begun to be investigated, beyond the mere antioxidant capacity.[15] This review focuses mainly on recent data, related to in vivo studies that have been conducted with strawberries, emphasizing the role of phytochemicals;

recent and important advances have been achieved in understanding the molecular mechanisms of polyphenols present in strawberries involved in their health effects against chronic and degenerative diseases, which will also be discussed herein.

Strawberries and human health

In the last decade, berries have been studied for their biological and functional properties, mainly using in vitro and animal models, but currently human epidemiological and interventional studies with strawberries are growing. The protective effects of strawberry consumption comprise a wide range of biological activities in the prevention of inflammation, cardiovascular diseases (CVDs), obesity, metabolic syndrome, certain types of cancers and even neurological diseases.

Strawberries and inflammation

Inflammation is the normal, protective and temporary response of the innate immune system to pathogens and injury. However, with recurrent stimuli or inefficient regulation, chronic inflammation ensues and sustains a pro-inflammatory state that is the major contributing factor in the development, progression and complication of most commonly known diseases such as cardiovascular disease, Alzheimer's, and type II diabetes. Quantifiable inflammatory responses can be triggered by different stimuli such as endotoxin (i.e., a lipopolysaccharide from bacteria), viruses, and changes in levels of reactive oxygen species (ROS), cellular redox status, fatty acids, growth factors, and carcinogens; in addition to these stimuli, inflammatory stress can also result from an excess of body fat and a poor diet. The central orchestrator of the inflammatory response is the nuclear factor kappa B (NF-κB), a redox-sensitive transcription factor that, once acti-

Francesca Giampieri

Francesca Giampieri, PhD, works as a Post-Doctoral Research Fellow at the Department of Clinical Science, at the Polytechnic University of Marche (Ancona, Italy). She graduated in the Biological Sciences (2007) and pursued a Specialization in Food Science at the Polytechnic University of Marche (2014). She has extensive experience in the field of chemistry, in the assessment of the nutritional and phytochemical composition of different foodstuffs, and in the field of biochemistry, in the evaluation of the role of dietary bioactive compounds on human health, and in particular on the biological mechanisms related to oxidative stress.

Tamara Y. Forbes-Hernandez

Tamara Y. Forbes-Hernandez is a PhD student in Biological Sciences and Clinical Specialties, at the Polytechnic University of Marche, Italy. She graduated in Food Science at the University of Havana, Cuba, (2007) and pursued a Master in Food Science and Technology at the University of Havana, Cuba, (2010). She was a Professor of Food Toxicology and Dietetics at the Institute of Pharmacy and Food, University of Havana, Cuba (2007–2012). Her research focuses on the nutritional characterization of strawberries and the evaluation of their functional properties, especially, the in vitro evaluation of their lipid-lowering effects and the involved metabolic pathways.

vated, stimulates the expression of a number of genes, including those responsible for the production of cytokines (*i.e.*, tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-1β), that act as signals between immune cells to coordinate the inflammatory reaction.[16]

In recent years the relationship between strawberry consumption and inflammation has been evaluated in some animal models (Table 1).

In a mouse model (C57BL/6 mice) of diet-induced obesity (low-fat and high-fat diets), the anti-inflammatory and blood glucose-regulating capacity of strawberries has been evaluated. The estimated intake of strawberries, 2.6% of the diet in the form of freeze-dried powder for 24 weeks per mouse, was equivalent to at least one human serving of strawberries per day. The results demonstrated that regular consumption of strawberries may contribute to the maintenance of blood glucose in obesity, and may be beneficial in regulating many aspects of systemic inflammation and inflammatory-mediated dysfunction in non-obese mice.[17]

The protective effect of strawberries has also been tested on the platelet inflammatory mediators of atherosclerosis. In C57BL/6 mice, in fact, the effects of the strawberry extract on laser-injured thrombus formation were evaluated in the mesenteric artery: in untreated mice, the mesenteric artery was totally blocked by a stable bulky thrombus at 20 minutes, while in strawberry extract-treated mice, the time necessary to form the artery thrombosis was drastically prolonged.[18] Thus, one intraperitoneally bolus injection of strawberry extract (200 mg kg^{-1}) 30 minutes before laser injury prevented thrombus formation for over 60 minutes after laser-induced damage. The negative effects of the strawberry extract on atherosclerosis occurrence seem to be related to the inhibition of two important platelet mediators of inflammation (RANTES and IL-1β), demonstrating that the amount of strawberry extract necessary in humans for proven antiplatelet effects is about 70 mg kg^{-1}.[18]

Moreover, the protective effects of a strawberry diet have been demonstrated in rats exposed to 1.5 Gy irradiation of ^{56}Fe particles that cause significant neurochemical changes in critical regions of the brain, through increasing inflammation and oxidative stress.[19,20] Rats fed for 8 weeks, prior to irradiation, with a diet containing 2% of strawberry extract showed a significant reduction in radiation-induced neurotoxicity and dysfunction. This protection is mediated by improving protective signalling and reducing inflammation and pro-oxidant load in critical regions of the brain[19] and by antagonizing the effects of oxidative and inflammatory signals, such as COX-2 and NF-κB.[20]

Several human studies investigating the effects of berries have been published,[16] but very little literature data takes into account the involvement of strawberries in inflammation and in its related diseases (Table 1).

The effect of strawberry antioxidants in a milk-based beverage form (10 g of freeze-dried strawberry powder that correspond to 94.7 mg of total polyphenols) on meal-induced postprandial inflammatory and insulin responses has been evaluated in a human subject cross-over design model. The postprandial test was conducted on 26 overweight adults who consumed a high-carbohydrate, moderate-fat meal (HCFM) to induce acute oxidative and inflammatory stress, accompanied by either a single serving of strawberry or a placebo beverage; in these subjects blood samples were collected at baseline and at multiple time points (up to 6 h) after the meal challenge. The results showed that acute strawberry consumption considerably attenuated the postprandial inflammatory response, as indicated by C-reactive protein and IL-6 levels decrease and postprandial insulin response reduction. Collectively, these data provide evidence for favourable effects of strawberry antioxidants on postprandial inflammation and insulin sensitivity.[21]

A similar study was conducted in a crossover design model that involved the same group of 26 overweight adults, randomized to a 6-week strawberry or placebo beverage plan followed

Massimiliano Gasparrini

Massimiliano Gasparrini is a PhD student at the Department of Clinical Science, Faculty of Medicine, at the Polytechnic University of Marche (Ancona, Italy). During his PhD course he is acquiring experience in the fields of chemistry and biochemistry, with particular attention to the assessment of the nutritional profile and biological and functional properties of strawberries. He focuses his research on the protective role of strawberry bioactive compounds against oxidative stress and inflammation, determining their possible beneficial effects on human health.

Josè L. Quiles

Prof. Josè L. Quiles is a full Professor of Physiology and a member of the Institute of Nutrition and Food Technology "José Mataix" at the University of Granada, Spain. He graduated in Biology at the University of Granada, Spain and pursued a Master's degree in Nutrition (2002) at the University of Granada. He got his PhD in 1995 and he worked as a postdoctoral student at the Rowett Research Institute in Aberdeen, Scotland for two years. He was also a visiting professor at the Polytechnic University of Marche Ancona, Italy. His research focuses on oxidative stress and dietary interactions.

by an HCFM. The daily consumption of a strawberry beverage, which added about 95 mg of strawberry phenols to diets per day, significantly attenuated HCFM-induced postprandial increases in plasminogen activator inhibitor (PAI)-1 and IL-1 β blood concentrations with moderate suppression of IL-6. Therefore, the effect of chronic strawberry consumption could provide protection from HCFM-induced increases of inflammatory factors in at-risk populations.[22] These studies highlight that an anti-inflammatory effect may be found with strawberries after an acute or protracted consumption.

A chronic feeding study with strawberries was also conducted in obese subjects.[23] In this work, a total of 20 healthy obese subjects completed a 7-week double-blind, randomised, cross-over trial. After the first week, they were subjected to the strawberry freeze-dried powder or control intervention for 3 weeks. For the remaining period, subjects underwent the opposite treatment. Blood was collected at baseline and at the end of weeks 3, 4, 6 and 7. The results demonstrated that a 3-week dietary intervention with strawberry powder may not have been long enough to observe differences in inflammatory markers between the two dietary groups; on the contrary, a reduction in plasma concentrations of cholesterol and small HDL-cholesterol particles, and an increase of LDL particle size was observed, suggesting a possible role of strawberries as a dietary tool to decrease obesity-related disease.[23]

Finally, in recent years particular attention has been focused on fisetin, a flavanol present in many fruits and vegetables, including strawberries. It possesses multiple biological effects, as well as anti-inflammatory and neuroprotective properties.[24] In a mouse model of stroke, the effects of fisetin on the inflammatory response and infarct size have been analysed.[24] It has been demonstrated that fisetin not only protects brain tissue against ischemic reperfusion injury when given before ischemia but also when applied 3 hours after ischemia.

It also prominently inhibited the infiltration of macrophages and dendritic cells into the ischemic hemisphere and suppressed the intracerebral immune cell activation as measured by intracellular TNF-α production. This suggests that the fisetin-mediated inhibition of the inflammatory response is part of the mechanism through which fisetin exerts neuroprotective effects in cerebral ischemia.[24] On the contrary, fisetin has not been demonstrated as being able to inhibit carrageenan-induced paw inflammation in Jcl-ICR mice, probably due to the enhancement of MAP kinase activation by this flavanol.[25]

Strawberries and cardiovascular diseases

Currently, CVDs still represent the leading cause of morbidity and death worldwide.[26,27] From a dietary approach to this problem, growing evidence supports the beneficial effects of fruit- and vegetable-rich diets in the prevention of important risk factors for CVDs,[28,29] including obesity, hypertension[26,30,31] and type II diabetes mellitus.[29] In addition, other studies have also demonstrated an inverse association between these dietary patterns and the development of CVD incidents such as coronary heart disease (CHD) and stroke.[29]

The mechanisms through which fruits and vegetables may reduce CVD risk are not completely clear and they seem to be multiple.[29] Some of their constituents like fiber, magnesium, potassium, folate and polyphenols, especially flavonoids, could be mainly responsible for some of the protective associations that link vegetable foods to CVD prevention.[26] Specifically, the main mechanisms proposed for flavonoids include an improvement in the lipid profile of plasma, an increase in its antioxidant activity, as well as an enhancement of the endothelial function,[32] by exerting anti-inflammatory effects, reducing low density lipoprotein (LDL) oxidation, inhibiting endothelial NADPH oxidase, modulating nitric oxide synthase activity/expression and increasing nitric oxide status.[33]

Prof. Bruno Mezzetti, PhD in Biotechnology from the University of Bologna, is a full Professor in Fruit Crop Breeding and Biotechnology (AGR03) and Director of the Department of Agricultural, Food and Environmental Sciences (D3A), Marche Polytechnic University (http://www.d3a.univpm.it/), Ancona IT. He is responsible for the coordination of several breeding and biotech projects on berries, including the EU COST836 and COST863 Actions and the running FP7 Euberry project. He has been involved in many international scientific publications related to different aspects of the breeding of strawberries and other berries, biotechnology, cultivation and fruit quality and health benefits for the consumer.

Bruno Mezzetti

Prof. Maurizio Battino, PhD, DSc, MS, MD (Hon), Associate Professor of Biochemistry, Dept of Clinical Sciences, Polytechnic University of Marche (Ancona, Italy) is the Director of Nutrition & Health projects and Master courses at the Center for Nutrition & Health, Universidad Europea del Atlantico (Santander, Spain). His research group, the Bioenergetics Group, investigates ways of mitigating disease processes through the correct use of specific foods (mainly berries and dietary fats) and of their bioactive compounds. The targeted diseases are those related directly to mitochondrial impairment (e.g., fibromyalgia) and/or inflammation processes and oxidative stress including metabolic syndrome, cancer, atherosclerosis and periodontal diseases.

Maurizio Battino

Table 1 Anti-inflammatory effects of strawberries in animals and humans[a]

Model	Dosage and duration	Key effects	Ref.
C57BL/6 mice fed with a low or a high-fat diet	Mice were supplemented with 2.6% of strawberry freeze-dried powder for 24 weeks.	1. Maintenance of blood glucose in obese mice. 2. Regulation of systemic inflammation and inflammatory-mediated dysfunction in non-obese mice.	17
C57BL/6 mice subjected to thrombus formation	Mice were treated with one bolus intraperitoneal injection of strawberry extract (200 mg kg^{-1}) 30 minutes before laser injury thrombus formation in the mesenteric artery.	1. Significant delay in the time taken to form the artery thrombosis (inhibition of platelet aggregation): 20 min in control, over 60 minutes in strawberry-treated mice. 2. Inhibition of two important platelet mediators of inflammation (RANTES and IL-1β).	18
Rats irradiated with ^{56}Fe particles	Rats were fed diets containing 2% strawberry extract (lyophilized and added to rodent chow, 20 g kg^{-1} diet, 2% w/w), or a control diet, 8 weeks prior to irradiation with ^{56}Fe particles.	1. Significant reduction in radiation-induced neurotoxicity and dysfunction. 2. Improvement of protective signaling and reduction in inflammation and pro-oxidant load in critical regions of the brain. 3. Alteration in cell signaling and counteraction of the effects of oxidative and inflammatory signal such as COX-2 and NF-κB.	19,20
26 overweight adults (10 male and 16 female) consumed a HCFM	The HCFM was accompanied by a strawberry (10 g of freeze-dried powder) milk-based beverage or a strawberry-flavoured beverage that served as a placebo.	1. Significant attenuation of the postprandial inflammatory response, as indicated by C-reactive protein and IL-6 decrease. 2. Reduction in postprandial insulin response.	21
26 overweight adults (10 male and 16 female) consumed a HCFM	The HCFM was consumed after 6 weeks of dietary intervention with a strawberry (10 g of freeze-dried powder) milk-based beverage or a strawberry-flavoured beverage that served as a placebo.	1. Significant attenuation of the PAI-1 concentration and IL-1β response. 2. No significant reduction in IL-6 level. 3. No significant differences for platelet aggregation, TNF-α, insulin or glucose levels.	22
20 healthy obese human subjects (7 male and 13 female)	Subjects received strawberry freeze-dried powder (80 g) or control intervention for 3 weeks. For a further 3 weeks, subjects crossed over to the opposite intervention.	1. No differences in inflammatory markers were observed between the two dietary groups. 2. Reduction in plasma concentrations of cholesterol and small HDL-cholesterol particles. 3. Increase of LDL particle size.	23
C57BL/6 mice with middle cerebral artery occlusion	Animals were injected 20 minutes before or 180 minutes after the onset of ischemia (performed for 60 minutes) with fisetin (50 mg per kg bw).	1. Protection of brain tissue against ischemic reperfusion injury when given before ischemia but also when applied 3 hours after ischemia. 2. Inhibition of the infiltration of macrophages and dendritic cells into the ischemic hemisphere. 3. Suppression of the intracerebral immune cell activation as measured by intracellular TNF-α production.	24
Jcl-ICR mice	Fisetin (50 mg per kg bw) or control was injected subcutaneously into the right and left plantar hind paw 30 minutes before carrageenan-induced inflammation.	Inflammation was not reduced.	25

[a] Abbreviations: RANTES: regulated on activation, normal T cell expressed and secreted; IL-1β: interleukin 1β; COX-2: cyclooxygenase 2; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; HCFM: high-carbohydrate, moderate-fat meal; PAI-1: plasminogen activator inhibitor 1; IL-6: interleukin 6; TNF-α: tumor necrosis factor α.

In the particular case of strawberries, data from *in vitro* experiments suggest a protective interaction of flavonoids with lipid bilayers against oxidative damage, as a result of their localization in lipoprotein domains and cell membranes, thus explaining the possible *in vivo* role of strawberries in protecting LDL from oxidation. Moreover, it has been hypothesized that bioactive compounds present in strawberries, once absorbed and metabolized, may be accumulated inside the cell membrane modifying the membrane composition, fluidity and functionality.[34,35]

However, there is only little recent research evidence from *in vivo* extended strawberry consumption studies on humans (Table 2).

In 23 healthy volunteers, Alvarez-Suarez *et al.*[32] demonstrated that one month of strawberry supplementation not only reduces total cholesterol, LDL and triglyceride levels in plasma compared with baseline, but also decreases serum malondialdehyde, urinary 8-hydroxy-2′-deoxyguanosine and isoprostane concentrations. In addition, strawberry consumption improves anti-hemolytic defenses and platelet function, decreasing central clustered platelets and making them less receptive to activation stimuli.[32] It should be remembered that this is a very critical point since the activation of platelets and their consequent binding to the endothelium is a key process in the development and progression of CVD.[36]

In another 16-day pilot study, where 12 healthy subjects ingested 500 g of strawberries every day, an improvement in the plasma antioxidant status, characterized by an increase in plasma total antioxidant capacity and in serum vitamin C concentration, was observed.[34]

Table 2 Cardioprotective activity of strawberry consumption in humans

Model	Dosage and duration	Key effects	Ref.
23 healthy adults (11 male and 12 female)	500 g of fresh strawberries for 1 month	1. Reduction of plasma total cholesterol, LDL and triglyceride levels. 2. Decrease in serum malondialdehyde, urinary 8-hydroxy-2′-deoxyguanosine and isoprostane concentrations. 3. Improvement of anti-hemolytic defenses and platelet function.	32
93 600 healthy women	Anthocyanins for 18 years	Reduction of myocardial infarction.	33
12 healthy adults (5 male and 7 female)	500 g of fresh strawberries for 16 days	Increase in plasma total antioxidant capacity and serum vitamin C concentration.	34
18 healthy adults (8 male and 10 female)	500 g of fresh strawberries for 14 days	1. Increase in fasting plasma antioxidant capacity and vitamin C. 2. Increase in the lag phase preceding plasma lipid oxidation. 3. Improvement of resistance to oxidative hemolysis in red blood cells. 4. Attenuation of mononuclear cell mortality after *ex vivo* exposure to a single acute oxidative challenge.	37
87 242 hypertensive females	Anthocyanins, catechins and apigenin (mainly from strawberries) for 14 years	1. Improvement of vasodilatation. 2. Reduction of hypertension risk.	38

A 2-week strawberry supplementation also increased the lag phase duration prior to the copper-induced formation of plasma lipid oxidation products in 18 healthy subjects. Strawberry consumption changes the plasma water-soluble and/or the lipoprotein environment improving membrane lipid susceptibility to *ex vivo* induced oxidation.[37] Other beneficial effects of strawberry consumption in 12 healthy subjects include a significant increase in the erythrocyte resistance to spontaneous and AAPH-induced hemolysis that persists for more than 1 month after the end of the strawberry consumption period,[34] and attenuation of mononuclear cell mortality after *ex vivo* exposure to a single acute oxidative challenge.[37]

In a prospective cohort study of 87 242 hypertensive women,[38] anthocyanin consumption (mainly from strawberries and blueberries) reduced the relative risk of hypertension in adults. Analyses for individual compounds suggested a risk reduction for participants in the highest quintile of apigenin and flavan-3-ol catechin intake, compared with the risk for participants in the lowest quintile. These vasodilatory properties may result from specific flavonoid structural characteristics such as B-ring hydroxylation and methoxylation pattern.

Furthermore, in a prospective cohort study of 93 600 young and middle-aged women with more than 10 years of follow-up and repeated measures of dietary intake, the anthocyanin consumption (almost 60% of the total anthocyanin intake derived from strawberries and blueberries) was associated with a reduction of CHD risk, independently of established dietary and non-dietary CVD risk factors. The consumption of strawberries in combination with blueberries, at least 3 servings per week, significantly decreased CHD risk compared to a lower consumption of these fruits.[33]

In conclusion, the main positive effects of strawberries in the development or prevention of CVD can be summarized as three: antioxidant, antihypertensive and anti-atherosclerotic effects.

Strawberries and metabolic syndrome

Like obesity and CVD, metabolic syndrome is one of the chronic diseases whose incidence continues to rise worldwide. This illness, also known as insulin resistance syndrome or syndrome X, is characterized by the simultaneous occurrence of at least three of the following medical conditions: central or visceral obesity, insulin resistance, hypertension, high serum triglycerides and an altered low to high-density cholesterol levels ratio. It is also associated with elevated biomarkers of inflammation and lipid oxidation.[39]

Dietary patterns are recognized as one of the most determinant environmental factors in the emergence and development of the disease, encouraging food and pharmaceutical industries in the identification and commercialization of medicinal foods/beverages to address these public health challenges. In that sense, fruits, particularly berries, have attracted significant attention for the management of metabolic syndrome.

Different studies conducted in cellular and animal models of obesity and diabetes have proved that strawberry supplementation in particular, or purified anthocyanin treatment, can normalize blood glucose levels and inhibit glucose uptake and transport.[39] It has also been demonstrated that strawberry extract may constrain the activity of carbohydrate and lipid digestive enzymes such as α-glucosidase and α-amylase, as well as pancreatic lipase activity and angiotensin I-converting enzyme, which may be related to the therapeutic management of hypertension and hyperglycemia, the main features of metabolic syndrome.[39]

Also in human interventional studies, the effects of strawberries in postprandial hyperglycemia, lipid oxidation and inflammatory responses have been documented (Table 3).

In 27 selected subjects with at least three features of metabolic syndrome, supplementation with freeze-dried strawber-

Table 3 Effects of strawberry consumption on metabolic syndrome[a]

Model	Dosage and duration	Key effects	Ref.
27 individuals (2 males and 25 females) with metabolic syndrome	Freeze-dried strawberries (50 g per day ~500 g fresh strawberries) for 8 weeks	1. Reduction of total and LDL-cholesterol, serum malondialdehyde, small LDL particles and adhesion molecules. 2. Improvement of lipid oxidation and inflammation.	39
30 healthy adults (10 men and 20 women)	Strawberry jam at different concentrations	Attenuation of postprandial hyperglycemia.	40
12 healthy adults (2 men and 20 women)	Berry puree, including strawberry (150 g)	1. Reduction of capillary and venous plasma glucose and serum insulin concentrations. 2. Modest effect on the GLP-1 response. 3. Improvement of glycemic profile.	42
20 healthy females	Berry puree, including strawberry (150 g)	1. Reduction of postprandial insulin response. 2. Improvement of the glycemic profile of bread.	43

[a] Abbreviations: GLP-1: glucagon-like peptide-1.

ries (50 g per day ~500 g fresh strawberries) reduced total and LDL-cholesterol, serum malondialdehyde, small LDL particles and adhesion molecules, improved the features of metabolic syndrome and associated lipid oxidation and inflammation in obese adults.[39]

Otherwise, in 40 healthy individuals supplementation with strawberry jam attenuated postprandial hyperglycemia when compared to a matched glucose load,[40] while in the presence of visceral obesity, impaired glucose metabolism and dyslipidemia, and postprandial hyperglycemia was higher compared to in healthy subjects.[41] Consequently the effects of strawberries in improving postprandial metabolism might have significant implications in the control of metabolic syndrome.

Torronen et al.,[42] investigated in 12 healthy subjects the postprandial glucose, insulin and glucagon-like peptide 1 (GLP-1) responses to sucrose consumed with and without a berry puree containing strawberries. Compared to the control meal, ingestion of the berry puree resulted in lower capillary and venous plasma glycaemia and serum insulin concentrations as well as in a modest effect on the GLP-1 response. It also reduced the maximum increases of capillary and venous glycaemia and insulin concentrations and improved the glycaemic profile.

In humans the effects of strawberries on postprandial metabolic responses to starch have been also evidenced. Strawberry consumption attenuates postprandial insulin response to bread with no effect on the glucose response in 20 healthy women.[43] These results seem to be a consequence of the interaction among strawberry constituents, like anthocyanins and not to ellagitannins, because other berries with higher contents of ellagitannins had no clear effect on the insulin response.[43]

Therefore, regular consumption of strawberries, which present a lower postprandial insulin requirement, may help in the prevention of type II diabetes and metabolic syndrome and may probably be recommended for individuals at high risk.

Strawberries and cancer

There is consolidated evidence to classify strawberries as a functional food with several preventive and therapeutic health benefits.[44] Strawberries possess anticarcinogenic, antioxidative and genoprotective properties against multiple human and mouse cancer cell types in in vitro[45,46] and in vivo animal models,[47,48] but human studies are still rare and new investigations particularly focused on patients with precancerous conditions are strongly advisable.

Anticarcinogenic effects of strawberries are mediated mainly through the detoxification of carcinogens, scavenging of reactive oxygen species, the decrease of oxidative DNA damage,[49,50] the reduction of cancer cell proliferation through apoptosis[51] and cell-cycle arrest,[48] downregulation of the activator protein-1 and NF-κB, inhibition of Wnt signaling, TNF-α[46] and angiogenesis.[52,53]

In this section, our main focus is to discuss the role of the anticarcinogenic effect of strawberries in modulating the development and progression of tumors in vivo (Table 4).

Regarding oral cancer, freeze-dried or lyophilized strawberries have been recorded for the inhibition of chemically induced oral cancer treated rodents via the inhibition of N-nitrosomethylbenzylamine metabolism and DNA adduct formation, the reduced frequency of preneoplastic lesions, and the downregulation of both inflammatory (iNOS, COX-2, phospho-NF-κB-p65 and phospho-S6) and proliferation markers (Ki-67).[54] In addition, lyophilized strawberries were evaluated also for their potentiality to inhibit 7,12-dimethylbenz(a)anthracene-induced tumorigenesis in an established hamster cheek pouch model of oral cancer and for their ability to modify the expression of several genes relevant to oral cancer development.[54] An important reduction of histological lesions as well as a decrease in p16 and p13Arf and an increase in Trp53 and Bcl2 expression were revealed by the treatment.[54]

In humans, in a cohort study with 490 802 participants, higher consumption of the Rosaceae botanical subgroup, including strawberries, was associated with a protective effect against human esophageal squamous cell carcinoma[55] and head and neck cancers[56] compared to lower intakes and other botanical groups. Moreover, freeze-dried strawberry powder has shown a preventive effect in a Phase II clinical investigation for 75 subjects diagnosed with esophageal dysplastic

Table 4 Anti-cancer effects of strawberries in animals and humans[a]

Types of cancer	Model	Dosage and duration	Key effects	Ref.
Oral cancer	HCP model	5% or 10% lyophilized strawberries for 12 weeks	1. Decrease in number of tumors. 2. Changes in histological lesion. 3. Modulation of gene expression.	54
	490 802 participants (292 898 male and 197 904 female)	Rosaceae botanical subgroup (including strawberries) 1/2 cup fruit, or 6 oz juice for 12 months	1. Significant decrease in esophageal squamous cell carcinoma. 2. Significant decrease in head and neck cancer.	55,56
	75 patients with esophageal premalignant lesions	Freeze-dried strawberry powder at either 30 g per day or 60 g per day for 6 months	1. Reduction of the histologic grade of dysplastic premalignant lesions. 2. Downregulation of COX-2, iNOS, NF-κB.	57
Colon cancer	CD-1 Mice (AOM or DSS induced cancer)	2.5%, 5.0% or 10.0% of lyophilized strawberries for 20 weeks	1. Inhibition of tumor development and reduction of nitrotyrosine production. 2. Down-regulation of proinflammatory mediator expression. 3. Decrease of PI3K, Akt, ERK and NF-κB phosphorylation.	58
	1 558 147 participants	100 and 200 g per day	Nonlinear association between fruit intake and colorectal cancer risk.	59
	Male Wistar rats (1,2-dimethyl hydrazine induced cancer)	200 mg kg^{-1} of kaempferol for 16 weeks	1. Reduction of erythrocyte lysate and liver thiobarbituric acid reactive substances. 2. Decrease of colonic superoxide dismutase and catalase activities.	66
Lung cancer	Swiss ICR mice (NMBA-induced cancer)	35% strawberries for 7 months	Inhibition of body weight loss, cytogenetical damage, liver degeneration, pulmonary emphysema and lung adenomas.	60
	Swiss albino mice (benzo(a) pyrene-induced cancer)	25 mg kg^{-1} of fisetin for 8 to 16 weeks	1. Anticarcinogenic activity. 2. Reduces histological lesions. 3. Restores the levels LPO, enzymic and nonenzymic anti-oxidants.	68
	Lewis lung carcinoma-bearing mice	223 mg kg^{-1} of fisetin for 2 weeks on different days	1. Improvement of the antitumor effect of CPA. 2. Reduction of micro vessel density.	69
Breast cancer	Transgenic mice expressing the HER-2/neu oncogene	15% MESB for 4 months	Reduction of the number and size of metastases.	15
	Swiss albino mice	2 g kg^{-1} MESB for after 12 days of tumor development to 45 days	1. Reduction of tumor volume in a time-dependent manner. 2. Antiproliferative activity by apoptosis.	61
Osteosarcoma	BALB/cnu/nu mice	25 or 50 mg kg^{-1} of kaempferol	Apoptosis and suppression of tumor cell proliferation.	67
Human embryonal carcinoma	NT2/D1 xenograft mouse	20 mmol L^{-1} of fisetin for 10 days	Activation of both the mitochondrial and the cell death receptor pathway.	63
Prostate cancer	Athymic nude mice (AR-positive CWR22RU1 human PCa cells)	10–60 µmol L^{-1} of fisetin 2 times in a week	1. Inhibition of AR transactivation function. 2. Reduction of tumor growth and serum PSA levels.	70
Melanoma	451Lu xenografted nude mice	40 to 80 µM of fisetin for 45 days	1. Decrease of tumor development and Mitf expression. 2. Reduction of cell viability and disruption of Wnt/β-catenin signaling pathway.	62

[a] Abbreviations: HCP: hamster cheek pouch; ESCC: esophageal squamous cell carcinoma; EAC: esophageal adenocarcinoma; COX-2: cyclooxygenase 2; iNOS: inducible nitric oxide synthase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; PI3K: phosphatidylinositol-3-kinase; Akt: protein kinase B; ERK: extracellular signal-regulated kinase; LPO: lipid peroxidation; CPA: cyclophosphamide; AR: androgen receptor; Mitf: Microphthalmia-associated transcription factor.

premalignant lesions, demonstrating that the dietary intake of strawberries (60 g per day for 6 months) is able to inhibit the progression of precancerous lesions in a dose dependent way, *via* the suppression of NF-κB activation and the down-regulation of COX-2 and iNOS.[57]

For colon cancer, literature data are still controversial. In a recent study, Crj: CD-1 mice treated with different doses of freeze-dried strawberries presented a reduction in proinflammatory mediators expression, a suppression of nitrosative stress and a decrease in the reduced expression of the phosphorylation of phosphatidylinositol 3-kinase, Akt, and NF-κB.[58] On the contrary, a large-scale human intervention study, including 1 558 147 participants, showed only a weak association between the intake of strawberries and the reduced risk of colon cancer.[59]

Aqueous or methanolic extracts of strawberry treatment for lung and breast tumors have been performed in mice.[15,60,61] Administration of strawberry aqueous extracts, such as in

drinking water. inhibited the tobacco-induced formation of lung tumors as well as pulmonary emphysema, liver degeneration, loss of body weight and systemic cytogenetical damage.[60] Similarly, supplementation of strawberry methanolic extracts was able to inhibit breast carcinogenesis in transgenic mice expressing the HER-2/neu oncogene (line FVB/N 233 neu-NT) by reducing the number and size of metastases, as well as their propagation in the lungs.[15] In addition, strawberry treatment was able to block the proliferation of tumor cells in mice bearing breast adenocarcinoma through induction of the intrinsic pathway of apoptosis.[61]

Several polyphenolic compounds such as anthocyanins, kaempferol, quercetin, fisetin, ellagitannins and ellagic acid have been reported in strawberries.[13–15] They show anti-cancer properties in *in vitro* and *in vivo* studies as well as in human intervention trials and are known to augment the effects of chemotherapeutic agents.[53,62,63] Recently, kaempferol has been shown to modulate multiple molecular targets including p53 and STAT3, through the activation of caspases and ROS generation in tumor-bearing mice,[64] preserving normal cell viability.[65] Similarly, the anti-cancer effects of kaempferol were evaluated in colorectal cancer in rats. The results showed that kaempferol supplementation lowered 1,2-dimethyl hydrazine induced erythrocyte lysate and liver thiobarbituric acid reactive substance levels and "rejuvenated" antioxidant enzymes (catalase, super oxide dismutase and glutathione peroxidase), especially at a dose of 200 mg per kg body weight, demonstrating that it could be safely used as a chemopreventive agent for this type of cancer.[66] Moreover, the anti-cancer properties of kaempferol were evaluated in BALB/cnu/nu mice inoculated with human osteosarcoma U-2 OS cells, and the results showed the inhibition of tumor growth through apoptosis induction *via* endoplasmic reticulum stress activation.[67]

Fisetin possesses anti-oxidant, anti-inflammatory and anti-proliferative effects in a wide variety of cancers.[68] Most of the studies have been performed *in vivo*, in particular in lung cancer,[68,69] prostate cancer,[70] teratocarcinoma[63] and skin cancer.[62] For example, in lung cancer fisetin significantly decreased benzo(a)pyrene [B(a)P] induced carcinogenesis in Swiss albino mice, reducing the degree of histological lesions, restoring the levels of lipid peroxidation and enzymic and non-enzymic anti-oxidants and improving anti-proliferative efficacy.[68] In addition, in LLC-bearing mice treated with fisetin a marked decrease in tumor volume was found, probably due to the antiangiogenic effect of fisetin, as treated tumors presented a significant reduction in micro vessel density.[69] Similarly, fisetin increased cisplatin cytotoxicity in a human embryonal carcinoma NT2/D1 mouse xenograft model, stimulating FasL expression, activating caspases and the proapoptotic proteins Bak and Bid and decreasing cyclin B1, leading to cell death.[63] In prostate cancer, in athymic nude mice implanted with AR-positive CWR22RU1 human PCa cells, treatment with fisetin resulted in the inhibition of tumor growth and reduction in serum PSA levels.[70] Finally, the inhibitory effect of fisetin was evident also in melanoma tumor xenografted nude mice at different doses, with a slow progression of 451Lu tumor development and decrease in microphthalmia-associated transcription factor, a downstream protein of the Wnt/β-catenin pathway, considered an important prognostic marker of melanoma.[62]

In recent years, most studies have been developed with cell lines and rodents, and unfortunately limited attention has been paid to humans, so further human cancer prevention trials are strongly encouraged for the future development of specific phytochemicals or metabolites as chemopreventive agents using the principles of pharmacognosy.

Strawberries and neurological diseases

Other possible health benefits related to strawberry consumption have been investigated in the last few years (Table 5).

Devore *et al.*[71] have published results about the association of a long-term dietary intake of berries and flavonoids with cognitive decline in a large, prospective cohort of older women in the Nurses' Health Study. From 1980, a semi-quantitative food frequency questionnaire has been administered every four years to the Nurses' Health Study participants. In 1995–2001, cognitive function was measured in 16 010 participants, aged ≥70 years; follow-up assessments were conducted twice, at two-year intervals. Using multivariable-adjusted, mixed linear regression, mean differences in the slopes of cognitive decline by long-term berry and flavonoid intakes were estimated. The results revealed that high intakes of blueberries

Table 5 Neuroprotective effects of strawberries in animals and humans[a]

Model	Dosage and duration	Key effects	Ref.
Older women in the Nurses' Health Study	Long-term diet (1995–2001) containing 1 or 2 servings of strawberries per week.	1. Reduction in rates of cognitive decline	71
ICR mice model of despair tests	Behavioral and neurochemical tests were conducted 60 min after fisetin treatment (5, 10 and 20 mg kg^{-1}, *via* gavage, p.o.).	1. Inhibition of the immobility time in both behavioral tests in a dose dependent way: the doses that affected the immobile response did not affect locomotor activity. 2. Increase in serotonin and noradrenaline levels in the frontal cortex and hippocampus.	72
R6/2 mouse model of HD	The mice were fed with control chow or chow containing 0.05% fisetin	1. Reduction of the impact of mutant huntingtin in HD. 2. Activation of ERK pathway.	76

[a] Abbreviations: HD: Huntington's disease; ERK: extracellular signal-regulated kinase.

and strawberries were associated with slower rates of cognitive decline, indicating that flavonoid intake appears to reduce the rates of this phenomenon in older adults.[71]

Depression is a highly prevalent psychiatric disease affecting nearly 21% of the world population and its prevalence has significantly increased by 6% during the past 15 years. According to the World Health Organization, depression will become the second leading cause of disease-related disability by the year 2020. The antidepressant potential of fisetin has been investigated by Zhen *et al.*[72] in two classical mouse models of despair tasks, tail suspension and forced swimming tests. The results suggest that fisetin (applied at 10 and 20 mg kg⁻¹, p.o.) inhibited the immobility time in both behavioral tests in a dose dependent way, while the doses that affected the immobile response did not affect locomotor activity. In addition, neurochemical assays showed that fisetin produced an increase in serotonin and noradrenaline levels in the frontal cortex and hippocampus. These findings indicate that fisetin could serve as a novel natural antidepressant agent and that this positive effect could involve the regulation of the central serotonin and noradrenaline levels.[72]

Finally, Huntington's disease (HD) is a neurodegenerative disorder that is characterized by cognitive, psychiatric and motor symptoms for which there is, to date, no cure. It is determined by the expansion of a trinucleotide repetition that encodes an abnormally long polyglutamine tract in the huntingtin protein. Mitogen-activated protein kinase signalling and particularly the Ras-extracellular signal-regulated kinase (ERK) cascade are the most common pathways implicated in HD. Studies in both cell and animal models suggest that ERK activation might provide a novel therapeutic target for the treatment of HD but compounds that specifically activate ERK are few.[73–75] Only one study, conducted on an R6/2 mouse model of HD revealed that fisetin (0.05% of the diet) can reduce the impact of mutant huntingtin in HD disease and can activate the ERK pathway, thus suggesting that this strawberry polyphenol could be useful for its managment.[76]

Conclusion

Strawberries are one of the most popular berries consumed worldwide and, since they are available throughout the year as fresh or frozen products, represent a relevant dietary source of vitamins, minerals and phytochemicals, which contribute to its health effects. Studies involving animals and humans provide evidence on the anti-inflammatory role of strawberries, mainly *via* downregulation of NF-κB and subsequent pro-inflammatory cytokines, and on anticarcinogenic and antiproliferative activities, through the modulation of oncogenic signalling pathways. Moreover, other *in vivo* studies demonstrate the protective effects of strawberries in postprandial hyperglycemia and metabolic syndrome, through the regulation of carbohydrate and lipid digestive enzymes and angiotensin I-converting enzyme. Epidemiological and clinical studies further reinforce the health effects of strawberries, highlight-

ing their antioxidant, anti-inflammatory and antihypertensive capacities. Therefore, strawberries represent a promising powerful disease-fighting food, for the prevention of chronic degenerative pathologies or in support of traditional therapies for the best achievement of therapeutic goals. However, further research is strongly encouraged to underline some critical aspects that are still not adequately debated in the present literature, such as the bioavailability of strawberry bioactive compounds and metabolites in subjects with one or more risk factors for chronic diseases, the optimal dose of strawberries that could improve biomarkers of inflammation and oxidative stress, the synergic/antagonist effects of strawberry consumption with commonly used drugs in the treatment of chronic diseases such as CVD and cancer, and the assessment of the temporal relationship between strawberry consumption and disease incidence through long-term and wide prospective and interventional studies.

Acknowledgements

References

1 L. A. Bazzano, Dietary intake of fruit and vegetables and risk of diabetes mellitus and cardiovascular diseases [electronic resource], in *Background Paper of the Joint FAO/WHO Workshop on Fruit and Vegetables for Health*, World Health Organization, Kobe, Japan, 2005, pp. 1–65.

2 Y. Chu, J. Sun, X. Wu and R. H. Liu, Antioxidant and antiproliferative activities of common vegetables, *J. Agric. Food Chem.*, 2002, **50**, 6910–6916.

3 A. Bach-Faig, E. M. Berry, D. Lairon, J. Reguant, A. Trichopoulou, S. Dernini, F. X. Medina, M. Battino, R. Belahsen, G. Miranda and L. Serra-Majem, Mediterranean Diet Foundation Expert Group. Mediterranean diet pyramid today. Science and cultural updates, *Public Health Nutr.*, 2011, **14**, 2274–2284.

4 S. Romandini, L. Mazzoni, F. Giampieri, S. Tulipani, M. Gasparrini, T. Y. Forbes-Hernandez, N. Locorotondo, M. D'Alessandro, B. Mezzetti, S. Bompadre and J. M. Alvarez-Suarez, Effects of an acute strawberry (Fragaria × ananassa) consumption on the plasma antioxidant status of healthy subjects, *J. Berry Res.*, 2013, **3**, 169–179.

5 K. Banaszewski, E. Park, I. Edirisinghe, J. C. Capozzo and B. M. Burton-Freeman, A pilot study to investigate bioavailability of strawberry anthocyanins and characterize postprandial plasma polyphenols absorption patterns by Q-TOF LC/MS in humans, *J. Berry Res.*, 2013, **3**, 113–126.

6 B. Halvorsen, M. H. Carlsen, K. M. Phillips, S. K. Bohn, K. Holte, D. R. Jacobs Jr. and R. Blomhoff, Content of redox-active compounds (i.e., antioxidants) in food con-

sumed in the United States, *Am. J. Clin. Nutr.*, 2006, **84**, 95–135.

7 S. H. Nile and S. W. Park, Edible berries: bioactive components and their effect on human health, *Nutrition*, 2014, **30**, 134–144.

8 US Department of Agriculture, Agriculture Research Service. USDA national nutrient for standard references, release 23. Fruits and fruit juices; pp. 785–7. Available at: http://www.ars.usda.gov/Services/docs.htm?docid=8964. Accessed on January 10, 2015.

9 S. Tulipani, B. Mezzetti and M. Battino, Impact of strawberries on human health: insight into marginally discussed bioactive compounds for the Mediterranean diet, *Public Health Nutr.*, 2009, **12**(9A), 1656–1662.

10 S. Tulipani, S. Romandini, J. M. Alvarez Suarez, F. Capocasa, B. Mezzetti, F. Busco, F. Bamonti, N. Novembrino and M. Battino, Folate content in different strawberry genotypes and folate status in healthy subjects after strawberry consumption, *BioFactors*, 2008, **34**, 47–55.

11 S. H. Hakkinen and A. R. Torronen, Content of flavonols and selected phenolic acids in strawberries and Vaccinium species: influence of cultivar, cultivation site and technique, *Food Res. Int.*, 2000, **33**, 517–524.

12 H. Wang, G. Cao and R. L. Prior, Total antioxidant capacity of fruits, *J. Agric. Food Chem.*, 1996, **44**, 701–705.

13 F. Giampieri, S. Tulipani, J. M. Alvarez-Suarez, J. L. Quiles, B. Mezzetti and M. Battino, The strawberry: composition, nutritional quality, and impact on human health, *Nutrition*, 2012, **28**, 9–19.

14 F. Giampieri, J. M. Alvarez-Suarez, L. Mazzoni, S. Romandini, S. Bompadre, J. Diamanti, F. Capocasa, B. Mezzetti, J. L. Quiles, M. S. Ferreiro, S. Tulipani and M. Battino, The potential impact of strawberry on human health, *Nat. Prod. Res.*, 2013, **27**, 448–455.

15 F. Giampieri, J. M. Alvarez-Suarez and M. Battino, Strawberry and human health: effects beyond antioxidant activity, *J. Agric. Food Chem.*, 2014, **62**, 3867–3876.

16 S. V. Joseph, I. Edirisinghe and B. M. Burton-Freeman, Berries: Anti-inflammatory Effects in Humans, *J. Agric. Food Chem.*, 2014, **62**, 3886–3903.

17 M. A. Parelman, D. H. Storms, C. P. Kirschke, L. Huang and S. J. Zunino, Dietary strawberry powder reduces blood glucose concentrations in obese and lean C57BL/6 mice, and selectively lowers plasma C-reactive protein in lean mice, *Br. J. Nutr.*, 2012, **108**, 1789–1799.

18 M. Alarcón, E. Fuentes, N. Olate, S. Navarrete, G. Carrasco and I. Palomo, Strawberry extract presents antiplatelet activity by inhibition of inflammatory mediator of atherosclerosis (sP-selectin, sCD40L, RANTES, and IL-1β) and thrombus formation, *Platelets*, 2014, 1–6.

19 S. M. Poulose, D. F. Bielinski, K. L. Carrihill-Knoll, B. M. Rabin and B. Shukitt-Hale, Protective effects of blueberry- and strawberry diets on neuronal stress following exposure to (56)Fe particles, *Brain Res.*, 2014, **1593**, 9–18.

20 B. Shukitt-Hale, F. C. Lau, V. Cheng, K. Luskin, A. N. Carey, K. Carrihill-Knoll, B. M. Rabin and J. A. Joseph, Changes in gene expression in the rat hippocampus following exposure to 56Fe particles and protection by berry diets, *Cent. Nerv. Syst. Agents Med. Chem.*, 2013, **13**, 36–42.

21 I. Edirisinghe, K. Banaszewski, J. Cappozzo, K. Sandhya, C. L. Ellis, R. Tadapaneni, C. T. Kappagoda and B. M. Burton-Freeman, Strawberry anthocyanin and its association with postprandial inflammation and insulin, *Br. J. Nutr.*, 2011, **106**, 913–922.

22 C. L. Ellis, I. Edirisinghe, T. Kappagoda and B. Burton-Freeman, Attenuation of meal-induced inflammatory and thrombotic responses in overweight men and women after 6-week daily strawberry (Fragaria) intake. A randomized placebo-controlled trial, *J. Atheroscler. Thromb.*, 2011, **18**, 318–327.

23 S. J. Zunino, M. A. Parelman, T. L. Freytag, C. B. Stephensen, D. S. Kelley, B. E. Mackey, L. R. Woodhouse and E. L. Bonnel, Effects of dietary strawberry powder on blood lipids and inflammatory markers in obese human subjects, *Br. J. Nutr.*, 2012, **108**, 900–999.

24 M. Gelderblom, F. Leypoldt, J. Lewerenz, G. Birkenmayer, D. Orozco, P. Ludewig, J. Thundyil, T. V. Arumugam, C. Gerloff, E. Tolosa, P. Maher and T. Magnus, The flavonoid fisetin attenuates postischemic immune cell infiltration, activation and infarct size after transient cerebral middle artery occlusion in mice, *J. Cereb. Blood Flow Metab.*, 2012, **32**, 835–843.

25 M. Funakoshi-Tago, K. Nakamura, K. Tago, T. Mashino and T. Kasahara, Anti-inflammatory activity of structurally related flavonoids, Apigenin, Luteolin and Fisetin, *Int. Immunopharmacol.*, 2011, **11**, 1150–1159.

26 M. L. McCullough, J. J. Peterson, R. Patel, P. F. Jacques, R. Shah and J. T. Dwyer, Flavonoid intake and cardiovascular disease mortality in a prospective cohort of US adults, *Am. J. Clin. Nutr.*, 2012, **95**, 454–464.

27 W. Rosamond, K. Flegal, K. Furie, A. Go, K. Greenlund, N. Haase, S. M. Hailpern, M. Ho, V. Howard, B. Kissela, S. Kittner, D. Lloyd-Jones, M. McDermott, J. Meigs, C. Moy, G. Nichol, C. O'Donnell, V. Roger, P. Sorlie, J. Steinberger, T. Thom, M. Wilson and Y. Hong, American Heart Association Statistics Committee and Stroke Statistics Subcommittee. Heart disease and stroke statistics—2008 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee, *Circulation*, 2008, **117**, e25–146.

28 L. Dauchet, P. Amouyel and J. Dallongeville, Fruits, vegetables and coronary heart disease, *Nat. Rev. Cardiol.*, 2009, **6**, 599–608.

29 L. A. Bazzano, M. K. Serdula and S. Liu, Dietary intake of fruits and vegetables and risk of cardiovascular disease, *Curr. Atheroscler. Rep.*, 2003, **5**, 492–499.

30 L. J. Appel, T. J. Moore, E. Obarzanek, W. M. Vollmer, L. P. Svetkey, F. M. Sacks, G. A. Bray, T. M. Vogt, J. A. Cutler, M. M. Windhauser, P. H. Lin and N. Karanja, A clinical trial of the effects of dietary patterns on blood pressure.

DASH Collaborative Research Group, *N. Engl. J. Med.*, 1997, **336**, 1117–1124.

31 L. Djoussé, T. Rudich and J. M. Gaziano, Nut consumption and risk of hypertension in US male physicians, *Clin. Nutr.*, 2009, **28**, 10–14.

32 J. M. Alvarez-Suarez, F. Giampieri, S. Tulipani, T. Casoli, G. Di Stefano, A. M. González-Paramás, C. Santos-Buelga, F. Busco, J. L. Quiles, M. D. Cordero, S. Bompadre, B. Mezzetti and M. Battino, One-month strawberry-rich anthocyanin supplementation ameliorates cardiovascular risk, oxidative stress markers and platelet activation in humans, *J. Nutr. Biochem.*, 2014, **25**, 289–294.

33 A. Cassidy, K. J. Mukamal, L. Liu, M. Franz, A. H. Eliassen and E. B. Rimm, High anthocyanin intake is associated with a reduced risk of myocardial infarction in young and middle-aged women, *Circulation*, 2013, **127**, 188–196.

34 S. Tulipani, J. M. Alvarez-Suarez, F. Busco, S. Bompadre, J. L. Quiles, B. Mezzetti and M. Battino, Strawberry consumption improves plasma antioxidant status and erythrocyte resistance to oxidative haemolysis in humans, *Food Chem.*, 2011, **128**, 180–186.

35 S. Chaudhuri, A. Banerjee, K. Basu, B. Sengupta and P. K. Sengupta, Interaction of flavonoids with red blood cell membrane lipids and proteins: antioxidant and antihemolytic effects, *Int. J. Biol. Macromol.*, 2007, **41**, 42–48.

36 J. M. van Gils, J. J. Zwaginga and P. L. Hordijk, Molecular and functional interactions among monocytes, platelets, and endothelial cells and their relevance for cardiovascular diseases, *J. Leukocyte Biol.*, 2009, **85**, 195–204.

37 S. Tulipani, T. Armeni, F. Giampieri, J. M. Alvarez-Suarez, A. M. Gonzalez-Paramás, C. Santos-Buelga, F. Busco, G. Principato, S. Bompadre, J. L. Quiles, B. Mezzetti and M. Battino, Strawberry intake increases blood fluid, erythrocyte and mononuclear cell defenses against oxidative challenge, *Food Chem.*, 2014, **156**, 87–93.

38 A. Cassidy, E. J. O'Reilly, C. Kay, L. Sampson, M. Franz, J. P. Forman, G. Curhan and E. B. Rimm, Habitual intake of flavonoid subclasses and incident hypertension in adults, *Am. J. Clin. Nutr.*, 2011, **93**, 338–347.

39 A. Basu and T. J. Lyons, Strawberries, blueberries, and cranberries in the metabolic syndrome: clinical perspectives, *J. Agric. Food Chem.*, 2012, **60**, 5687–5692.

40 T. Kurotobi, K. Fukuhara, H. Inage and S. Kimura, Glycemic index and postprandial blood glucose response to Japanese strawberry jam in normal adults, *J. Nutr. Sci. Vitaminol.*, 2010, **56**, 198–202.

41 M. E. Tushuizen, P. J. Pouwels, S. Bontemps, C. Rustemeijer, N. Matikainen, R. J. Heine, M. R. Taskinen and M. Diamant, Postprandial lipid and apolipoprotein responses following three consecutive meals associate with liver fat content in type 2 diabetes and the metabolic syndrome, *Atherosclerosis*, 2010, **211**, 308–314.

42 R. Törrönen, E. Sarkkinen, T. Niskanen, N. Tapola, K. Kilpi and L. Niskanen, Postprandial glucose, insulin and glucagon-like peptide 1 responses to sucrose ingested with berries in healthy subjects, *Br. J. Nutr.*, 2012, **107**, 1445–1451.

43 R. Törrönen, M. Kolehmainen, E. Sarkkinen, K. Poutanen, H. Mykkänen and L. Niskanen, Berries reduce postprandial insulin responses to wheat and rye breads in healthy women, *J. Nutr.*, 2013, **143**, 430–436.

44 A. Basu, A. Nguyen, N. M. Betts and T. J. Lyons, Strawberry as a functional food: an evidence-based review, *Crit. Rev. Food Sci. Nutr.*, 2014, **54**, 790–806.

45 S. Y. Wang, R. Feng, Y. Lu, L. Bowman and M. Ding, Inhibitory effect on activator protein-1, nuclear factor-kappaB, and cell transformation by extracts of strawberries (Fragaria x ananassa Duch.), *J. Agric. Food Chem.*, 2005, **53**, 4187–4193.

46 Y. Zhang, N. P. Seeram, R. Lee, L. Feng and D. Heber, Isolation and identification of strawberry phenolics with antioxidant and human cancer cell antiproliferative properties, *J. Agric. Food Chem.*, 2008, **56**, 670–675.

47 P. S. Carlton, L. A. Kresty, J. C. Siglin, M. A. Morse, J. Lu, C. Morgan and G. D. Stoner, Inhibition of N-nitrosomethylbenzylamine-induced tumorigenesis in the rat esophagus by dietary freeze-dried strawberries, *Carcinogenesis*, 2001, **22**, 441–446.

48 G. D. Stoner, L. S. Wang, N. Zikri, T. Chen, S. S. Hecht, C. Huang, C. Sardo and J. F. Lechner, Cancer prevention with freeze-dried berries and berry components, *Semin. Cancer Biol.*, 2007, **17**, 403–410.

49 H. Xue, R. M. Aziz, N. Sun, J. M. Cassady, L. M. Kamendulis, Y. Xu, G. D. Stoner and J. E. Klaunig, Inhibition of cellular transformation by berry extracts, *Carcinogenesis*, 2001, **22**, 351–356.

50 G. D. Stoner, L. S. Wang and B. C. Casto, Laboratory and clinical studies of cancer chemoprevention by antioxidants in berries, *Carcinogenesis*, 2008, **29**, 1665–1674.

51 N. P. Seeram, L. S. Adams, Y. Zhang, R. Lee, D. Sand, H. S. Scheuller and D. Heber, Blackberry, black raspberry, blueberry, cranberry, red raspberry, and strawberry extracts inhibit growth and stimulate apoptosis of human cancer cells in vitro, *J. Agric. Food Chem.*, 2006, **54**, 9329–9339.

52 M. Atalay, G. Gordillo, S. Roy, B. Rovin, D. Bagchi, M. Bagchi and C. K. Sen, Anti-angiogenic property of edible berry in a model of hemangioma, *FEBS Lett.*, 2003, **544**, 252–257.

53 S. J. Duthie, Berry phytochemicals, genomic stability and cancer: evidence for chemoprotection at several stages in the carcinogenic process, *Mol. Nutr. Food Res.*, 2007, **51**, 665–674.

54 B. C. Casto, T. J. Knobloch, R. L. Galioto, Z. Yu, B. T. Accurso and B. M. Warner, Chemoprevention of oral cancer by lyophilized strawberries, *Anticancer Res.*, 2013, **33**, 4757–4766.

55 N. D. Freedman, Y. Park, A. F. Subar, A. R. Hollenbeck, M. F. Leitzmann, A. Schatzkin and C. C. Abnet, Fruit and vegetable intake and esophageal cancer in a large prospective cohort study, *Int. J. Cancer*, 2007, **121**, 2753–2760.

56 N. D. Freedman, Y. Park, A. F. Subar, A. R. Hollenbeck, M. F. Leitzmann, A. Schatzkin and C. C. Abnet, Fruit and vegetable intake and head and neck cancer risk in a large United States prospective cohort study, *Int. J. Cancer*, 2008, **122**, 2330–2336.

57 T. Chen, F. Yan, J. Qian, M. Guo, H. Zhang, X. Tang, F. Chen, G. D. Stoner and X. Wang, Randomized phase II trial of lyophilized strawberries in patients with dysplastic precancerous lesions of the esophagus, *Cancer Prev. Res.*, 2012, **5**, 41–50.

58 N. Shi, S. K. Clinton, Z. Liu, Y. Wang, K. M. Riedl, S. J. Schwartz, X. Zhang, Z. Pan and T. Chen, Strawberry Phytochemicals Inhibit Azoxymethane/Dextran Sodium Sulfate-Induced Colorectal Carcinogenesis In Crj: CD-1 Mice, *Nutrients*, 2015, **7**, 1696–1715.

59 D. Aune, R. Lau, D. S. Chan, R. Vieira, D. C. Greenwood, E. Kampman and T. Norat, Nonlinear reduction in risk for colorectal cancer by fruit and vegetable intake based on meta-analysis of prospective studies, *Gastroenterology*, 2011, **141**, 106–118.

60 R. Balansky, G. Ganchev, M. Iltcheva, M. Kratchanova, P. Denev, C. Kratchanov, K. Polasa, F. D'Agostini, V. E. Steele and S. De Flora, Inhibition of lung tumor development by berry extracts in mice exposed to cigarette smoke, *Int. J. Cancer*, 2012, **131**, 1991–1997.

61 R. R. Somasagara, M. Hegde, K. K. Chiruvella, A. Musini, B. Choudhary and S. C. Raghavan, Extracts of strawberry fruits induce intrinsic pathway of apoptosis in breast cancer cells and inhibits tumor progression in mice, *PLoS One*, 2012, **7**, e47021.

62 D. N. Syed, F. Afaq, N. Maddodi, J. J. Johnson, S. Sarfaraz, A. Ahmad, V. Setaluri and H. Mukhtar, Inhibition of human melanoma cell growth by the dietary flavonoid fisetin is associated with disruption of Wnt/β-catenin signaling and decreased Mitf levels, *J. Invest. Dermatol.*, 2011, **131**, 1291–1299.

63 R. Tripathi, T. Samadder, S. Gupta, A. Surolia and C. Shaha, Anticancer activity of a combination of cisplatin and fisetin in embryonal carcinoma cells and xenograft tumors, *Mol. Cancer Ther.*, 2011, **10**, 255–268.

64 P. Rajendran, T. Rengarajan, N. Nandakumar, R. Palaniswami, Y. Nishigaki and I. Nishigaki, Kaempferol, a potential cytostatic and cure for inflammatory disorders, *Eur. J. Med. Chem.*, 2014, **86**, 103–112.

65 A. Y. Chen and Y. C. Chen, A review of the dietary flavonoid, kaempferol on human health and cancer chemoprevention, *Food Chem.*, 2013, **138**, 2099–20107.

66 P. Nirmala and M. Ramanathan, Effect of kaempferol on lipid peroxidation and antioxidant status in 1,2-dimethyl hydrazine induced colorectal carcinoma in rats, *Eur. J. Pharmacol.*, 2011, **654**, 75–79.

67 W. W. Huang, Y. J. Chiu, M. J. Fan, H. F. Lu, H. F. Yeh, K. H. Li, P. Y. Chen, J. G. Chung and J. S. Yang, Kaempferol induced apoptosis via endoplasmic reticulum stress and mitochondria-dependent pathway in human osteosarcoma U-2 OS cells, *Mol. Nutr. Food Res.*, 2010, **54**, 1585–1595.

68 N. Ravichandran, G. Suresh, B. Ramesh and G. V. Siva, Fisetin, a novel flavonol attenuates benzo(a)pyrene-induced lung carcinogenesis in Swiss albino mice, *Food Chem. Toxicol.*, 2011, **49**, 1141–1147.

69 Y. S. Touil, J. Seguin, D. Scherman and G. G. Chabot, Improved antiangiogenic and anti-tumour activity of the combination of the natural flavonoid fisetin and cyclophosphamide in Lewis lung carcinoma-bearing mice, *Cancer Chemother. Pharmacol.*, 2011, **68**, 445–455.

70 N. Khan, M. Asim, F. Afaq, M. Abu Zaid and H. Mukhtar, A novel dietary flavonoid fisetin inhibits androgen receptor signaling and tumor growth in athymic nude mice, *Cancer Res.*, 2008, **68**, 8555–8563.

71 E. E. Devore, J. H. Kang, M. M. Breteler and F. Grodstein, Dietary intakes of berries and flavonoids in relation to cognitive decline, *Ann. Neurol.*, 2012, **72**, 135–143.

72 L. Zhen, J. Zhu, X. Zhao, W. Huang, Y. An, S. Li, X. Du, M. Lin, Q. Wang, Y. Xu and J. Pan, The antidepressant-like effect of fisetin involves the serotonergic and noradrenergic system, *Behav. Brain Res.*, 2012, **228**, 359–366.

73 K. Gharami, Y. Xie, J. J. An, S. Tonegawa and B. Xu, Brain-derived neurotrophic factor over-expression in the forebrain ameliorates Huntington's disease phenotypes in mice, *J. Neurochem.*, 2008, **105**, 369–379.

74 S. Gines, M. Bosch, S. Marco, N. Gavalda, M. Diaz-Hernandez, J. J. Lucas, J. M. Canals and J. Alberch, Reduced expression of the TrkB receptor in Huntington's disease mouse models and in human brain, *Eur. J. Neurosci.*, 2006, **23**, 649–658.

75 B. L. Apostol, K. Illes, J. Pallos, L. Bodai, J. Wu, A. Strand, E. S. Schweitzer, J. M. Olson, A. Kazantsev, J. L. Marsh and L. M. Thompson, Mutant huntingtin alters MAPK signaling pathways in PC12 and striatal cells: ERK1/2 protects against mutant huntingtin-associated toxicity, *Hum. Mol. Genet.*, 2006, **15**, 273–285.

76 P. Maher, R. Dargusch, L. Bodai, P. E. Gerard, J. M. Purcell and J. L. Marsh, ERK activation by the polyphenols fisetin and resveratrol provides neuroprotection in multiple models of Huntington's disease, *Hum. Mol. Genet.*, 2011, **20**, 261–270.