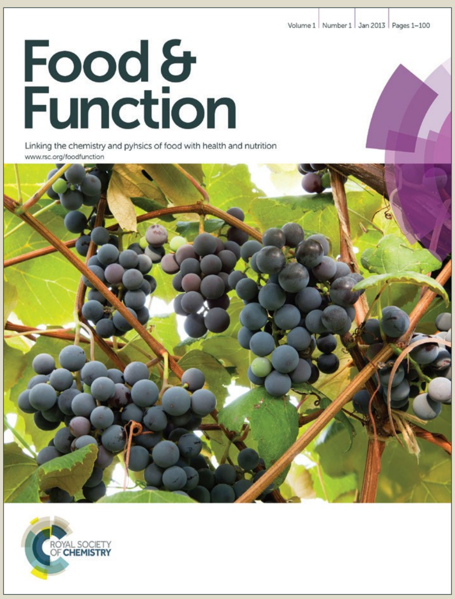

Food & Function

Strawberry as a health promoter: an evidence based review

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ABSTRACT

Since 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 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 these compounds exert a synergistic and cumulative effect in 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.

Keywords: strawberry, polyphenols, inflammation, cardiovascular diseases, cancer, neurodegenerative diseases

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 noteworthy health benefits[4,5] among fruits because of their high 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 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/100 g fresh fruit), and, albeit to a lower extent, a sufficiently good source of several other vitamins, such as thiamin, riboflavin, niacin, vitamin B6.[8]

Another significant nutritional feature is the concentration of folate (24 µg/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 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 to regulating blood sugar levels by slowing digestion, while the fiber content may control calory 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 directly and indirectly antimicrobial, anti-allergy, anti-hypertensive properties, as well as the capacity of 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 in form of pelargonidin and cyanidin derivates.[13-15] The second most abundant phytochemicals in strawberry 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 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.

Strawberry and human health

In 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 (CVD), obesity, metabolic syndrome, certain types of cancers and even neurological diseases.

Strawberry 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 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., 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 excess of body fat and poor diet. The central orchestrator of the inflammatory response is the nuclear factor kappa B (NF-κB), a redox-sensitive transcription factor that, once activated, 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 relation 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 been also tested on 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 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) 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.[18]

Moreover, the protective effects of strawberry diet have been demonstrated in rats exposed to 1.5Gy 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 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 signal, such as COX-2 and NF-kB.[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. The postprandial test was conducted on 26 overweight adultswho 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 that involved the same group of 26 overweight adults, randomized to a 6-weeks strawberry or placebo beverage followed 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 population;[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 TNFα 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 able to inhibit carrageenan-induced paw inflammation in Jcl-ICR mice, probably due to the enhancement of MAP kinase activation by this flavanol.[25]

Strawberry and cardiovascular diseases

Currently, CVD 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 CVD,[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, specially 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 of 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]

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 isoprostanes 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 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]

A 2-week strawberry supplementation also increases 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 significant increase in the erythrocyte resistance to spontaneous and AAPH- induced hemolysis that persist 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 study of 87.242 hypertensive women,[38] anthocyanin consumption (mainly from strawberries and blueberries) reduces 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 the B-ring hydroxylation and methyoxylation 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/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.

Strawberry 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 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 caught significant attention for the management of the 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 metabolic syndrome features.[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 strawberries (50 g/day ~ 500 g fresh strawberries) reduced total and LDL-cholesterol, serum malondialdehyde, small LDL particles and adhesion molecules, improving the features of metabolic syndrome and associated lipid oxidation and inflammation in obese adults.[39]

Otherwise, in 40 healthy individuals the 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, postprandial hyperglycemia was higher compared to 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 has 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 presents a lower postprandial insulin requirement, may help in the prevention of type II diabetes and metabolic syndrome and may be probably 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 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 detoxification of carcinogens, scavenging of reactive oxygen species, decrease of oxidative DNA damage,[49,50] reduction of cancer cell proliferation through apoptosis[51] and cell-cycle arrest,[48] downregulation of 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 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-kB–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 of p16 and p13Arf and an increase of Trp53 and Bcl2 expression were revealed by the treatment.[54]

In humans, in a cohort study with 490.802 participants, higher consumption of Rosaceae botanical subgroup, including strawberries, was associated with a protective effect against human esophageal squamous cell carcinoma[55] and head and neck cancer[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 premalignant lesion, demonstrating that dietary intake of strawberries (60 g/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 of reduced expression of phosphorylation of phosphatidylinositol 3-kinase, Akt, and NF-kB.[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 tumor have been performed in mice.[15,60,61] Administration of strawberry aqueous extracts such as drinking water inhibited 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 on 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 lung.[15] In addition, the strawberry treatment was able to block the proliferation of tumor cells in mice bearing breast adenocarcinoma through induction of intrinsic pathway of apoptosis.[61]

Several polyphenolic compounds such as anthocyanins, kaempferol, quercetin, fisetin, ellagitannins, ellagic acid have been reported in strawberries.[13-15] They show anticancer properties in *in vitro* and *in vivo* studies as well as in human intervention trials and are known to augment 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 level and "rejuvenated" antioxidant enzymes (catalase, super oxide dismutase and glutathione peroxidase), especially at the dose of 200 mg/kg body weight, demonstrating that it could be safely used as a chemopreventive agent in 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 cancer.[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 the micro vessel density.[69] Similarly, fisetin increased cisplatin cytotoxicity in human embryonal carcinoma NT2/D1 mouse xenograft model, stimulating FasL expression, activating caspases and proapototic protein 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 the 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 that 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.

Strawberry 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 associations 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 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 slopes of cognitive decline by long-term berry and flavonoid intakes were estimated. The results revealed that high intakes of blueberries and strawberries were associated with slower rates of cognitive decline, indicating that flavonoids intake appears to reduce 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, 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 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 product, 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-kB and subsequent pro-inflammatory cytokines, and on anticarcinogenic and antiproliferative activity, 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, highlighting 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 to traditional therapies for the best achievement of therapeutic goals. However, further research are strongly encouraged to underline some critical aspects that are still not adequately debated in the present literature, as the bioavailability of strawberry bioactive compounds and metabolites in subjects with one or more risk factors for chronic diseases, the optimal dose of strawberry that could improve biomarkers of inflammation and oxidative stress, the synergic/antagonist effects of strawberry consumptions with the commonly used drugs in the treatment of chronic diseases as CVD and cancers, the assessment of temporal relationship between strawberry consumption and diseases incidence through long-term and wide prospective and interventional studies.

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Table 1. Anti-inflammatory effects of strawberries in animals and humans.

Model	Dosage and duration	Key Effects	Reference
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) 30 minutes before laser injury thrombus formation in 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 diet, 2%w/w), or a control diet, 8 weeks prior to irradiation of ^{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-kB	19,20
26 overweight adults (10 male and 16 female) consumed a HCFM	The HCFM was accompanied by strawberries (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 strawberries (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 (80g) 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/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 TNFα production.	24
Jcl-ICR mice	Fisetin (50 mg/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

Abbreviations. RANTES: regulated on activation, normal T cell expressed and secreted; IL-1β: interleukin 1β; COX-2: ciclooxygenase 2; NF-kB: 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.

Table 2. Cardioprotective activity of strawberry consumption in humans.

Model	Dosage and duration	Key Effects	Reference
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 isoprostanes 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.Improvment 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 female	Anthocyanins, catechins, apigenin (mainly from strawberries) for 14 years	1. Improvement of vasodilatation. 2. Reduction of hypertension risk.	38

Table 3. Effects of strawberry consumption on metabolic syndrome.

Model	Dosage and duration	Key Effects	Reference
27 individuals (2 males and 25 females) with metabolic syndrome	Freeze-dried strawberries (50 g/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 female	Berry puree, including strawberry (150 g)	1. Reduction of postprandial insulin response. 2. Improvement of glycemic profile of the breads.	43

Abbreviations. GLP-1: glucagon-like peptide-1.

Table 4. Anti-cancer effects of strawberries in animals and humans.

Types of cancer	Model	Dosage and duration	Key Effects	Reference
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/d or 60 g/d for 6 months	1. Reduction of the histologic grade of dysplastic premalignant lesions. 2. Downregulation of COX-2, iNOS, NFkB.	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 NFκB phosphorylation.	58
	1.558.147 partecipants	100 and 200 g/day	Nonlinear association between fruit intake and colorectal cancer risk	59
	Male wistar rats (1,2-dimethyl hydrazine induced cancer)	200 mg/kg 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	60

			adenomas.	
	Swiss albino mice (benzo(a)pyrene-induced cancer)	25 mg/kg of fisetin for 8 to16 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 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 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 of kaempferol	Apoptosis and suppression of tumor cell proliferation.	67
Human embryonal carcinoma	NT2/D1 xenograft mouse	20 mmol/L 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 of fisetin for 2 times in a weeks	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

Abbrevations. HCP: hamster cheek pouch; ESCC: esophageal squamous cell carcinoma; EAC: esophageal adenocarcinoma; COX-2: cyclooxygenase 2; iNOS: inducible nitric oxide synthase, NFkB: 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.

Table 5. Neuroprotective effects of strawberries in animals and humans.

Model	Dosage and duration	Key Effects	Reference
Older women in the Nurses' Health Study	Long-term diet (1995-2001) containing 1 or 2 serving 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, 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 ofHD	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

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