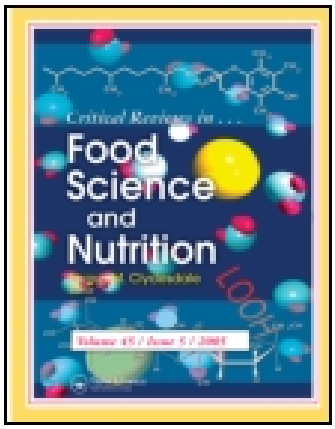

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Flavonoids and Immune Function in Human: A Systematic Review

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Flavonoids and Immune Function in Human: A Systematic Review

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Flavonoids, through a modulation of immune function, have been suggested to be involved in the role played by plant foods in disease prevention. We performed a systematic search in the MEDLINE database to review the effect of flavonoid-rich foods and flavonoids supplements on immune function. A total of 58 studies, were identified as suitable: 41 addressed in vivo proinflammatory cytokines and 15 measured ex vivo markers of immune function. According to our findings and on the basis of single food items, the number of studies in humans is limited and, for galenic supplements, only quercetin has been investigated. More evidences are needed to clarify the role of flavonoids as modulator of immune function in humans.

Keywords Immune function, flavonoid, food, human

INTRODUCTION

A large body of epidemiological evidences has provided a solid foundation for the health benefits of diets based on foods of vegetable origin (Bruckdorfer, 2008; Sofi, 2008; Mente et al., 2009; Trichopoulou et al., 2009). Large prospective studies have consistently shown that adherence to a diet low in saturated fats and rich in plant foods, such as the Mediterranean diet, has been associated to reduction in overall mortality (Sofi, 2008; Trichopoulou et al., 2009), mortality from cardiovascular diseases (CVD) (Bruckdorfer, 2008; Sofi, 2008; Mente et al., 2009) and incidence of cancer, Parkinson's and Alzheimer's diseases (Sofi, 2008). An alteration of immune response seems to be associated with many disease states, e.g., CVD, cancer, atopic disease, and metabolic syndrome, as well as with prepathological conditions such as obesity (Sanderson et al., 2010). Under physiological conditions, the immune system works to keep people healthy, defending them against pathogens; however, under conditions associated with an increased production of inflammatory cytokines, such as interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), chronic low-grade systemic inflammation can occur increasing the risk for development of chronic diseases (Cevenini et al., 2010; Kolb and Mandrup-Poulsen, 2010; Vasto et al., 2009).

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Although there is encouraging indication that fruit and vegetable consumption may improve immune system (Lampe, 1999), evidences in humans are limited and it is still unclear which food components are involved in this largely unknown mechanism (Liu, 2003). In recent years, great attention has been focused on the biological role of polyphenols, in particular flavonoids, a wide group of almost 5000 secondary plant metabolites, sharing a common carbon skeleton of two benzene rings (ring A and B), joined by a 3-carbon bridge (C6–C3–C6) (Aherne and O'Brien, 2002). Flavonoids comprise diverse subclasses, among which flavonols (quercetin and kaempferol), flavones (luteolin and apigenin), flavanols (catechins and proanthocyanidins), anthocyanidins, flavanones (naringenin and hespertine), and isoflavones (genistein and daidzein) are compounds present in great amounts in fruit, vegetable, cocoa, wine, tea, and soy (Aherne and O'Brien, 2002; Neveu et al., 2010; Pérez-Jiménez et al., 2010).

Extensive studies have provided a wealth of information on the different modality of action of flavonoids, including antimicrobial, antioxidant, and anti-inflammatory activities (Crozier et al., 2009). Recently, a wide number of in vitro studies have suggested a role of flavonoids in the modulation of immune response, through the inhibition of Th1-type and the promotion of Th2-type immunities (Murr et al., 2005). Modulation of cytokines, in particular the reduction of the Th1 cytokine IL-2 (Miles et al., 2005), may deeply affect both antigen-specific (Verbeek et al., 2004) and polyclonal (Watson et al., 2005; López-Posadas et al., 2008) proliferative responses and increase

the activation-induced cell death (Xu et al., 2008), both fundamental physiological responses involved in clonal expansion after antigen challenge. In vitro, the inhibition of TNF-α (Kim et al., 2004; Nair et al., 2006), IL-2 (Watson et al., 2005; López-Posadas et al., 2008), IFN-γ (Verbeek et al., 2004), IL-12 (Lee et al., 2009), and IL-6 (Lee et al., 2009) production by flavonoids has been documented. The inhibition of TNF-α and IL-12 may affect phagocytes (Dewas et al., 2003) and NK cells (Xu et al., 2010) activities, respectively. Moreover fermented grape marc polyphenols has been shown to suppress both the induction and the effector phases of type-I allergic response in murine asthma models (Tominaga et al., 2010).

In addition, flavonoids have been reported to exert cytoprotective effects, restoring lymphocyte proliferation, and preventing apoptosis (Carrero et al., 1998), through their ability to limit damage induced by Reactive Oxygen Species (Greenrod and Fenech, 2003), as well as inhibiting oxidative burst (Sanbongi et al., 1997). The molecular mechanisms that have been recognized to be at the base of the immune-modulating action of flavonoids in vitro, include the regulation of transcription factors such as nuclear transcription factor kB (NF-kB), activation protein 1 (AP-1), mitogen activate protein kinase, and lipid raft (Rahman et al., 2006; Serafini et al., 2010). In the past decade, clinical trials have been conducted in order to verify the in vivo efficacy of food-containing flavonoids to modulate immune system providing contrasting results (Serafini et al., 2010). Aim of this work is to systematically review the evidence on the effect of supplementation with flavonoids-rich foods and flavonoids supplements on markers of immunity in humans.

SEARCH CRITERIA

We performed a systematic search in the MEDLINE database (PubMed database; National Library of Medicine, Bethesda, MD) to review the effect of flavonoid-rich foods and flavonoids supplements on immune function. All relevant English-language articles published between 1980 and 2011 in the MEDLINE database were searched using the MeSH search: (((((immune*) OR cytokines) OR proliferation) OR lymphocyte) OR PBMC) AND ((((((((((flavonoids) OR tea) OR soy) OR wine) OR cocoa) OR chocolate) OR fruit) OR vegetable) OR galenic) OR capsule) OR extract) AND (subject* OR patient*)) NOT review [Publication Type]; Limits Activated: Humans, English. The literature search yielded 6125 citations that were screened for eligibility. After exclusion of irrelevant references, the search was further limited to human chronic intervention studies. Literature that measured both cytokines inflammatory markers in human plasma and ex vivo markers of immune function, such as induced cytokines production, lymphocyte proliferation, phagocytic, and NK activities ex vivo was included. Furthermore, the PubMed options "Related Articles" were also retrieved to find other studies on the same topic. A total of 58 studies including 77 different interventions, were identified as suitable; 41 studies (49 interventions) addressed in vivo proinflammatory cytokines (de Maat et al., 2000; Jenkins et al., 2002; Estruch et al., 2004; Sánchez-Moreno et al., 2004; Hermansen et al., 2005; Hilpert et al., 2005; Huang et al., 2005; Zern et al., 2005; Marfella et al., 2006; Ryan-Borchers et al., 2006; Ryu et al., 2006; Sánchez-Moreno et al., 2006; Azadbakht et al., 2007; Djurovic et al., 2007; Hsu et al., 2007; Vázquez-Agell et al., 2007; Marotta et al., 2007; Mukamal et al., 2007; Nieman et al., 2007a, 2007b; Egert et al., 2008; Inoue et al., 2008; Nasca et al., 2008; Bae et al., 2009; Beavers et al., 2009; Charles et al., 2009; Dalgård et al., 2009; Egert et al., 2009; Maskarinec et al., 2009; Monagas et al., 2009; Udani et al., 2009; Kawai et al., 2009; Nieman et al., 2009; Egert et al., 2010; Eichenberger et al., 2010; Karlsen et al., 2010; Zemel et al., 2010; Basu et al., 2011; Ellis et al., 2011; Knab et al., 2011; Llaneza et al., 2011), 15 studies (25 interventions) measured ex vivo markers of immune function (Watzl et al., 2000; Mathur et al., 2002; Bub et al., 2003; Watzl et al., 2003, 2004; Winkler et al., 2004; Watzl et al., 2005; Fanti et al., 2006; Nantz et al., 2006; Nieman et al., 2007; Castilla et al., 2008; Ellinger et al., 2008; Henson et al., 2008; Imhof et al., 2008; Rowe et al., 2011), and 2 studies (3 interventions) tested both markers (Boots et al., 2008; Heinz et al., 2010) after ingestion of flavonoids-rich foods, beverages, galenic supplements, and food extracts.

EFFECT OF FLAVONOIDS ON CIRCULATING CYTOKINE LEVELS

The studies investigating the effect of flavonoids-rich foods on circulating cytokine levels in humans were focused on a limited number of foods of plant origin, including tea, wine, cocoa, soy products, small variety of fruit- and/or vegetable-based products, and quercetin only as pure molecule, as described in Tables 1–4. The most addressed cytokines resulted to be TNF-α and IL-6, which were measured in more than the 70% of the studies. The amount of flavonoids ingested by volunteers in each intervention varied notably among studies, ranging from 50 (Egert et al., 2008) mg/d to 1400 (Nieman et al., 2009) mg/d of quercetin, from 318 mg/d (black tea) to 928 mg/d (green tea) of catechins, from 70 mg/d (Jenkins et al., 2002) to 112,1 mg/d (Huang et al., 2005) of soy isoflavones and from 94,66 (Marotta et al., 2007) mg/d (strawberry juice) to 2490 mg/d (green tea extract) of total flavonoids (de Maat et al., 2000). Only a limited number of studies (13) assessed flavonoids absorption in biological fluids: plasma levels ranged from 8.7 nM to 0.35 μM, when data were expressed as molar and from 24 to 1000 when data were expressed as μg/l, while urinary levels were comprised between 5 and 24 μM (Tables 1–4).

Table 1 summarizes studies conducted with tea, wine, and cocoa. Tea or tea extract consumption resulted to have scarce influence on cytokine production in vivo, with only one study out of six reporting decreased TNF-α levels, after a 7 month daily supplementation with a green tea extract containing 455 mg of catechins in hemodialized patients (Hsu et al., 2007). All the

Table 1 Overview of the reviewed intervention studies in humans providing tea, wine, and cocoa: characteristics and results for circulating citokines and flavonoids

Treatment (daily dose)	Days	Subjects	Study design	n	Effect on cytokines	Effect on flavonoids	References
Black tea (318 mg catechins)	180	Diabetics	Placebo-controlled parallel	14 × 2	TNF-α, IL-6 ↔	Urinary 4-O-methylgallic acid ↑ (13 μM)	Mukamal et al., 2007
Black tea (900 ml), Green tea (900 ml), Green tea extract (2.49 g flavonoids)	28	Healthy	Placebo-controlled parallel	16 black tea, 15 green tea, 13 green tea extract, 15 control	TNF- α, IL-1β, IL-6 ↔		de Maat et al., 2000
Green tea extract (159 mg catechins)	21	Healthy	Placebo-controlled crossover	9	IL-6 ↔		Eichenberger et al., 2010
Green tea extracts (Catechins 455 mg)	215	Hemodialysis patients	Placebo-controlled parallel	14 catechins, 30 control	TNF-α ↓		Hsu et al., 2007
Green tea (900 ml)	28	Diabetics	Placebo-controlled crossover	55	IL-6 ↔		Ryu et al., 2006
Green tea (928 mg catechins) Green tea extracts (870 mg catechins).	56	Obese with metabolic syndrome	Placebo-controlled parallel	13, 10, 12 control	IL-1β, IL-6 ↔		Basu et al., 2011
Grape extract (209 mg total phenols)	28	Postmenopausal	Placebo-controlled crossover	44	TNF-α ↓, IL-6 ↔		Zern et al., 2005
Red wine (150 mL)	21	Healthy	Placebo-controlled crossover	87	TNF-α, IL-6, TGF-β ↔		Djurovic et al., 2007
Red wine (320 mL) or gin (100 mL)	28	Healthy	Placebo-controlled crossover	80	↓ IL-1α (both red wine and gin) TNF-α ↔	Plasma epicatechin gallate ↑ (0.024 mg/L with red wine)	Estruch et al., 2004
Red wine (118 mL)	365	Diabetics	Placebo-controlled parallel	57 red wine, 58 control	TNF-α, IL-6 and IL-18↓		Marfella et al., 2006
Sparkling wine (Cava)	28	Healthy	Placebo-controlled crossover	20	IL-6 ↓		Vázquez-Agell et al., 2007
Cocoa powder (495.2 mg total polyphenols,)	28	High risk of cardiovascular disease	Placebo-controlled crossover	42	IL-6 ↔	Total polyphenols ↔	Monagas et al., 2009

↔ No change; ↑ increase; ↓ decrease.

Table 2 Overview of the reviewed intervention studies in humans providing soy: characteristics and results for circulating cytokines and flavonoids

Treatment (daily dose)	Days	Subjects	Study design	n	Effect on cytokines	Effect on flavonoids	References
Soy (70 mg isoflavones)	28	Hypercholesterolemic	Placebo-controlled crossover	23 men, 18 women	IL-6 ↑ (in women) TNF-α ↔		Jenkins et al., 2002
Soy (100 mg isoflavones)	168	Hypercholesterolemic	Placebo-controlled parallel	43, 46 control	TNF-α ↔		Hermansen et al., 2005
Soy (100 mg isoflavones)	730	Healthy	Placebo-controlled parallel	90, 93 control	IL-6 ↔		Maskarinec et al., 2009
Soy (101 mg isoflavones)	56	Postmenopausal	Placebo-controlled crossover	60	IL-6 ↔		Nasca et al., 2008
Soy (112.1 mg isoflavones)	112	Postmenopausal	Uncontrolled	20	IL-6 ↑, TNF-α, and IL-1α ↓		Huang et al., 2005
Soy (160 mg isoflavones)	84	Postmenopausal	Placebo-controlled parallel	32, 43 control	TNF-α and IL-6 ↔		Charles et al., 2009
Soy (71.6 mg isoflavones), Soy supplement (70 mg isoflavones)	112	Postmenopausal	Placebo-controlled parallel	18 soy, 15 supplement, 19 control	IFN-γ, IL-2, TNF-α ↔	Soy: Plasma: daidzein ↔; genistein ↑ (0.15 μM); equol ↑ (0.1 μM); Urine: daidzein ↑ (13 μM); genistein ↑ (5 μM); equol ↑ (18 μM); Supplement: Plasma: daidzein ↔; genistein ↑ (0.35 μM); equol ↑ (0.03 μM); Urine: daidzein ↑ (24 μM); genistein ↑ (18 μM); equol ↑ (10 μM)	Ryan-Borchers et al., 2006
Soy (90 mg isoflavones)	42	Healthy	Placebo-controlled crossover	64	IL-6 ↔		Hilpert et al., 2005
Soy (protein 10 g)	28	Overweight/obese	Placebo-controlled crossover	20	TNF-α ↑ (overweight and obese) IL-6 ↑ (obese)		Zemel et al., 2010
Soy (protein or nuts 30 g)	56	Postmenopausal with metabolic syndrome	Placebo-controlled crossover	42	TNF-α ↓ (nuts) IL-18↓(protein) IL-6, IL-2 ↔	Plasma phytoestrogen ↑ (soy nut 64%; soy protein 48%)	Azadbakht et al., 2007
Soy (soymilk, 3 cups)	28	Healthy	Placebo-controlled parallel	16 soy, 15 control	TNF-α, IL-1β, and IL-6 ↔		Beavers et al., 2009
Soy isoflavones extract (80 mg isoflavones)	180	Obese postmenopausal women	Placebo-controlled parallel	43, 44 control	TNF-α ↔		Llaneza et al., 2011

↔ No change; ↑ increase; ↓ decrease.

Table 3 Overview of the reviewed intervention studies in humans providing fruit juices and vegetables: characteristics and results for circulating cytokines and flavonoids

Treatment (daily dose)	Days	Subjects	Study design	*n*	Effect on cytokines	Effect on flavonoids	References
Bilberry juice (330 mL)	28	Healthy	Placebo-controlled parallel	31 × 2	IL-6 and IL-15 ↓, TNF-α ↑, IFN-γ, IL-17, IL-13, IL-12, IL-8, IL-7, IL-5, IL-4, IL-2,IL-1 ↔	Plasma quercetin ↑ (12 nM) and p-coumaric acid ↑ (8.7 nM)	Karlsen et al., 2010
Blueberry–apple juice (15 mg quercetin)	28	Healthy	Uncontrolled	7	TNF-α ↔	Plasma quercetin ↑ (50 nM)	Boots et al., 2008
Camu juice (70 mL)	7	Smokers	Uncontrolled	10	IL-6 ↓, IL-8 ↓		Inoue et al., 2008
Fermented papaya preparation (9 g)	182	HCV-related cirrhosis or healthy	Placebo-controlled parallel	25 papaya, 25 control (cirrhosis); 10 control (healthy)	TNF-α ↓		Marotta et al., 2007
Mangosteen juice (180, 360, and 540 mL)	56	Healthy	Placebo-controlled parallel	10 × 4	(dose-dependent effect) IL-12 ↓		Udani et al., 2009
Orange and blackcurrant juice. (500 mL)	28	Peripheral arterial disease	Placebo-controlled crossover	24 juice, 23 control	IL-6 ↔		Dalgård et al., 2009
Strawberry juice (94.66 mg total phenols)	42	Overweight	Placebo-controlled parallel	12 juice, 12 control	TNF-α, IL-1β, IL-6 ↔		Ellis et al., 2011
Vegetable soup (500 ml)	14	Healthy	Uncontrolled	12	TNF-α, IL-1β, IL-6 ↔		Sánchez-Moreno et al., 2004
Vegetable soup (500 mL)	14	Healthy	Uncontrolled	12	TNF-α, IL-1β, IL-6 ↔		Sánchez-Moreno, et al., 2006

↔ No change; ↑ increase; ↓ decrease.

Table 4 Overview of the reviewed intervention studies in humans providing quercetin and isoquercetin: characteristics and results for circulating cytokines and flavonoids

Treatment (daily dose)	Days	Subjects	Study design	*n*	Effect on cytokines	Effect on flavonoids	References
Isoquercitin (200 mg)	56	Japanese cedar pollinosis	Placebo-controlled parallel	10 × 2	IL-4, IL-5, IL-12, IL-13, IFN-γ, IgE ↔	Plasma quercetin ↔	Kawai et al., 2009
Quercetin (1 g)	21	Healthy	Placebo-controlled parallel	20 × 2	TNF-α, IL-1, IL-6, IL-8, IL-10 ↔	Plasma quercetin ↑ (1000 μg/L)	Nieman et al., 2007
Quercetin (150 mg)	42	Overweight	Placebo-controlled crossover	93 × 2	TNF-α ↓	Plasma quercetin ↑ (200 nM)	Egert et al., 2009
Quercetin (150 mg)	42	Overweight/obese	Placebo-controlled crossover	Apo E 3 (60) Apo E 4 (26)	TNF-α ↓ Apo E 3 TNF-α ↓ Apo E 4		Egert et al., 2010
Quercetin (498 mg) + vitamin C (399 mg)	28	Rheumatoid arthritis	Placebo-controlled crossover	20	TNF-α, IL-1β, and IL-6 ↔		Bae et al., 2009
Quercetin (50 mg, 100 mg, 150 mg)	14	Healthy	Uncontrolled	11, 12, 12	TNF-α ↔	Plasma quercetin ↑ (92.5, 172.1, and 315.8 nM) isorhamnetin ↑ (5.2, 11.8, and 22.2 nM)	Egert et al., 2008
Quercetin (500 mg, 1000 mg)	84	Healthy	Placebo-controlled parallel	38, 40, 42 control	TNF-α and IL-6 ↔	Plasma quercetin ↑ (300–500 μg/L)	Heinz et al., 2010
Quercetin (500 mg, 1000 mg)	84	Healthy	Placebo-controlled parallel	316, 309, 312 control	TNF and IL10 ↔ IL6 ↓(quercetin 1000 mg)	Plasma quercetin ↑ (400–600 μg/L)	Knab et al., 2011
Quercetin (1000 mg) Quercetin (1000 mg) + Epigallocatechin gallate (120 mg) + isoquercetin (400 mg)	14	Healthy	Placebo-controlled	13 Quercetin 14 quercetin+EGCG + isoquercetin 400 mg 12 control	IL-6 ↔ quercetin+EGCG IL-10 ↓ IL-1 ↔ TNF-α ↔	Plasma quercetin ↑ (600–800 μg/L)	Nieman et al., 2009

↔ No change; ↑ increase; ↓ decrease.

other authors failed to find any effect. In healthy subjects, 4-week administration of black or green tea had no effect on IL-6, IL1-β and TNF-α (de Maat et al., 2000). Same results were obtained for 2 different green tea extracts, which resulted to be ineffective on both TNF-α (de Maat et al., 2000) and IL-6 levels (de Maat et al., 2000; Eichenberger et al., 2010), no matter the higher or lower catechin content (2490 and 159 mg of catechins, respectively). Also in diabetic subjects, tea consumption did not affect circulating cytokine concentrations. Both IL-6 and TNF-α were unaffected by a daily supplementation of three cups of either green or black tea (Mukamal et al., 2007). Same results were obtained after consumption of green tea (900 mL) on circulatory levels of IL-6 (Ryu et al., 2006). Recently, Basu et al. (2011), in an intervention involving obese subjects with metabolic syndrome, did not record significant changes in IL-6 and IL-1β after 8-week daily consumption of a green tea or green tea extract providing 928 and 870 mg of catechins, respectively. None of these studies evaluated circulating catechin levels.

More effective results were obtained by interventions that used wine or grape extracts as source of flavonoids (Table 1). One year of red wine consumption decreased TNF-α, IL-6, and IL-18 in diabetic patients, more than Mediterranean diet alone (Marfella et al., 2006). Estruch and co-workers (2004) found reduced levels of IL-1α after 4-weeks of red wine consumption and this effect was in association with increased epicatechin gallate plasma concentrations. Interestingly, the authors recorded the same effect after gin consumption, although it was not correlated to higher circulating levels of flavonoids (Estruch et al., 2004) (Table 1). Vázquez-Agell (2007) found a decrease in IL-6 after 28 days consumption of Cava, the Catalan version of Champagne. However, opposite findings were also reported by other authors. Djurovic et al. (2007) found no effect on IL-6 and TNF-α levels after 3 weeks of daily ingestion of 150 mL of red wine, while Zern et al. (2005) reported a partial effect by a grape extract providing 5.8 g/kg of total polyphenols (4 weeks), showing an inhibitory effect on TNF-α production, but not on IL-6, in both pre- and postmenopausal women.

Only one study tested cocoa consumption, failing to display an effect on IL-6 circulating levels in subjects at high risk of cardiovascular disease (Monagas et al., 2009).

Long-term intervention studies using soy as source of bioactive molecules are reported in Table 2. Twelve studies agreed with eligible criteria and were included in the review. Among these, 10 interventions failed to demonstrate an effect of soy or soy products on either TNF-α (Jenkins et al., 2002; Hermansen et al., 2005; Ryan-Borchers et al., 2006; Beavers et al., 2009; Charles et al., 2009; Llaneza et al., 2011) or IL-6 levels (Hilpert et al., 2005; Azadbakht et al., 2007; Nasca et al., 2008; Beavers et al., 2009; Charles et al., 2009; Maskarinec et al., 2009) and 5 displayed increases of TNF-α (Zemel et al., 2010) or IL-6 concentrations (Jenkins et al., 2002; Huang et al., 2005; Zemel et al., 2010). Huang et al. (2005) described an increase in circulating levels of IL-6 and a decrease of TNF-α and IL-1α after soymilk consumption. Recently Zemel et al. (2010), in an intervention study providing 10 g of soy proteins, reported increased levels of TNF-α in both obese and overweight subjects and increased levels of IL-6 in obese only. When flavonoid plasma concentrations after intervention were also measured (2 studies out of 12), increases in total isoflavones (Azadbakht et al., 2007) or genistein and equol (Ryan-Borchers et al., 2006) levels induced by long term soy consumption were associated with both cytokine reductions (Azadbakht et al., 2007) and lack of effect (Ryan-Borchers et al., 2006).

Table 3 summarizes studies conducted with fruit and vegetable. We collected nine studies, providing fruit and vegetables in form of juices or vegetable soup. After supplementation, four studies reported a reduction of cytokine levels, but opposite results were also found by other authors. Udani et al. (2009) recorded a dose response decrease in IL-12 levels after three different doses of Mangosteen juice. Bilberry juice chronic consumption (Karlsen et al., 2010) decreased IL-6 and IL-15 levels and increased TNF-α, concurrently with increases of plasma quercetin and p-coumaric acid concentration. Contrarily, Boots et al. (2008) showed no effect on TNF-α after 4-weeks consumption of blueberry–apple juice despite an increase in quercetin circulating levels. Also IL-6 levels were unaffected by orange and blackcurrant juice consumption in peripheral arterial disease patients (Dalgård et al., 2009). On the other hand, fruit Tropical camu-juice reduced both IL-6 and IL-8 in smoker subjects (Inoue et al., 2008). Patients with liver cirrhosis showed an elevated serum level of TNF-α (about 4 pg/mL) versus healthy controls (Marotta et al., 2007). Long-term consumption of a fermented papaya preparation significantly lowered TNF-α values (about 2 pg/mL) (Marotta et al., 2007). More recently, Ellis et al. (2011) reported unchanged levels of IL-6, IL-1β, and TNF-α after 6 weeks of strawberry beverage consumption. Two trials from the same group tested the effects of vegetable soup, showing no effect on TNF-α, IL-1β, and IL-6 levels after 2-week supplementation (Sánchez-Moreno et al., 2004, 2006).

Based on our review, quercetin, and its metabolite isoquercetin, is the only pure molecule studied in 12 different interventions in humans (Table 4). The data seem to indicate a scarce impact on cytokine levels, with only four interventions reporting reduced cytokine levels after supplementation. The inflammatory cytokines IL-8 (Nieman et al., 2007a, 2007b), IL-6 (Nieman et al., 2007a, 2007b; Bae et al., 2009; Heinz et al., 2010), and TNF-α (Nieman et al., 2007a, 2007b; Egert et al., 2008; Bae et al., 2009; Egert et al., 2009; Nieman et al., 2009) were unaffected by quercetin supplementation in healthy subjects (Nieman et al., 2007a, , 2007b; Egert et al., 2008, 2009; Nieman et al., 2009; Heinz et al., 2010) or in patients with arthritis, when taken in combination with vitamin C (Bae et al., 2009). Quercetin in association with epigallocatechin 3-gallate was ineffective on IL-6 (Nieman et al., 2009), while quercetin alone decreased TNF-α in subjects with apolipoprotein E (ApoE) genotype ApoE3 and ApoE4 (Egert et al., 2010). Quercetin absorption was evaluated in 8 out of 12 studies. One study failed to record plasma quercetin increments after supplementation with 200 mg of isoquercetin (Kawai et al., 2009). Among the others, Knab et al. (2011) reported concomitant

reduced levels of IL-6 and increased concentrations of plasma quercetin. Also Egert et al. (2008) reported decreased TNF-α levels in parallel to plasma quercetin absorption. However, the absorption of quercetin into the mainstream was not related to any reduction in circulating cytokines by six of eight studies (Nieman et al., 2007a, , 2007b; Egert et al., 2009; Heinz et al., 2010). Finally, the only study measuring the Th2 cytokines after isoquercitin supplementation did not found any changes in IL-4 and IL-5 concentration as well as in quercetin levels (Kawai et al., 2009).

EFFECT OF FLAVONOIDS-RICH FOODS ON EX VIVO MARKERS OF IMMUNE FUNCTION

Markers of immune function have been investigated ex vivo for their response to flavonoid-rich foods or galenics supplementation, as summarized in Table 5. We collected 17 works, among which 8 addressed lymphocyte proliferation, 7 monocytes or neutrophils activity, and 6 NK activity and 8 ex vivo induced cytokine production.

Only a scarce variety of flavonoid-rich foods were tested, including red wine, mixed fruit juices, tomato, and carrot juices, different number of servings of fresh vegetable and fruits and dark chocolate. In addition, capsules containing fruit and vegetable concentrate, soy supplements, and pure quercetin were also tested. Despite some evidences of an immune modulating effect were found, nine experiments out of 17 failed to demonstrate an impact on ex vivo markers of immune function (Mathur et al., 2002; Bub et al., 2003; Watzl et al., 2004, 2005; Fanti et al., 2006; Nieman et al., 2007; Boots et al., 2008; Henson et al., 2008; Heinz et al., 2010).

Chronic (14 days) intake of 500 mL of red wine had no effect on phagocytic activity, lymphocyte proliferation, cytokines production and NK activity in healthy men (Watzl et al., 2004). Also a 6 weeks consumption of 200 mL of red wine or 175 mL of dealcoholized red wine had no effect on phagocytosis and burst of neutrophils, but reduced oxidative burst of monocytes (Ellinger et al., 2008). Accordingly, 3 weeks consumption of dealcoholized red wine reduced ex vivo monocyte migration (Imhof et al., 2008).

When fresh fruits and vegetables were tested, consumption of 2, 5, or 8 servings/day for 4 weeks did not modify cytokine production, lymphocyte proliferation and NK activity (Watzl et al., 2005). However, in hemodialyzed patients consumption of red grape juice (50 mL twice/day) alone or in combination with vitamin E (800 IU) for 2 weeks decreased neutrophils oxidative burst (Castilla et al., 2008). Recently, Rowe et al. (2011) observed a reduction in $\gamma\delta$ T-cell proliferation after 9 weeks of grape juice consumption. In agreement, consumption of fruit juice for 16 weeks increased phytohemagglutinin (PHA)-induced proliferation in both HIV and healthy patients (Winkler et al., 2004). Watzl, in two consecutive studies, in which 330 mL of tomato juice were administered to elderly (Watzl et al., 2000) subjects or healthy adults (Watzl et al., 2003), reported opposite effects: lymphocyte proliferation was unaffected in elderly and increased in healthy adults, while IL-2 production was reduced in elderly and increased in healthy adults. Also results for NK activity resulted to be different in relation to subject age, and both increased (Watzl et al., 2003) or unchanged (Watzl et al., 2000) activities were observed for healthy adults and elderly, respectively.

When fruit and vegetables were administered in form of capsules containing fruit and vegetable concentrate, 77 days supplementation led to a decrease of IFN-γ production from Phorbol myristate acetate-stimulated lymphocytes, but had no effects on IL-4 and IL-6 (Nantz et al., 2006).

Long-term (42 days) consumption of dark chocolate (36.9 g) and cocoa powder drink (30.95 g) unaffected lipopolysaccharide (LPS)-activated whole blood production of IL-1β, IL-6, and TNF-α (Mathur et al., 2002).

Twelve weeks supplementation with 500 or 1000 mg/d of quercetin did not impact granulocyte respiratory burst (Heinz et al., 2010), as well as did 3 weeks supplementation with 1000 mg/d (Nieman et al., 2007; Henson et al., 2008). Quercetin also failed to have an effect on PHA-stimulated lymphocyte proliferation and NK activity (Nieman et al., 2007).

Only 4 studies of 17 concomitantly evaluated flavonoid absorption from flavonoid-rich food or supplements and their effects on ex vivo markers of immune function. After intervention with supplement of isoflavone-containing soy in end-stage renal disease patients on chronic hemodialysis, blood isoflavone levels were 5- to 10-folds higher in the soy group than in the control group, but TNF-α or IL-6 ex vivo production were unaffected (Fanti et al., 2006). Similarly, a 4-week blueberry–apple juice (providing 97 mg/L quercetin) ingestion (Boots et al., 2008) and 12 weeks supplementation with quercetin at 500 or 1000 mg/d (Heinz et al., 2010) resulted in significant increases in plasma quercetin concentrations without affecting ex vivo LPS-induced TNF-α levels ((Boots et al., 2008) nor granulocyte respiratory burst (Heinz et al., 2010). Contrarily, 14 days of supplementation with a juice rich in flavonoids (330 mL/day) enhanced ex vivo lymphocyte proliferation and NK activity in parallel to an increased urinary excretion of total polyphenols (Bub et al., 2003).

DISCUSSION

In recent years attention of the scientific community has been focused on the understanding of the biological role of flavonoids, due to the evidences coming from large epidemiological studies, highlighting the association between flavonoid-rich food intake and reduced risk for degenerative diseases (Knekt et al., 1996; Go et al., 2003; Surh, 2003; Mente et al., 2009). Thus, an intense research on cell cultures, finalized to understand the mechanisms of action of flavonoids and their implication in body mechanisms of defenses was undertaken in the last decades. Beside their conventional antioxidant properties (Serafini et al., 2011), there is evidence by in vitro experiments that these molecules may

Table 5 Overview of the reviewed intervention studies in humans providing flavonoid-rich foods and quercetin supplements: characteristics and results for ex vivo markers of immune function and flavonoid concentrations

Treatment (daily dose)	Days	Subjects	Study design	*n*	Effect on ex vivo markers	Effect on flavonoids	References
Capsules containing fruit and vegetable concentrate	77	Healthy	Placebo-controlled parallel	31, 28 control	IFN-γ production ↓ IL-4 and IL-6↔		Nantz et al., 2006
Carotenoid-rich vegetables and fruit (2 servings/d, 5 servings/d, or 8 servings/d)	28	Healthy	Placebo-controlled parallel	21/group	Lymphocyte proliferation ↔ IL-2, IL-13, IFN-γ, IL-12, TNF-α production ↔ Lytic activity of NK ↔		Watzl et al., 2005
Tomato juice (330 mL)	56	Elderly	Placebo-controlled parallel	29, 21 control	Lymphocyte proliferation ↔ IL-2 production ↓ IL-4 production ↔ TNF-α production ↑ lytic activity of NK ↔		Watzl et al., 2000
Tomato juice, carrot juice (330 mL)	14	Healthy	Uncontrolled crossover	11/group	IL-2 ↑, IL-4 ↔, TNFα ↑ production Lymphocyte proliferation ↓ lytic activity of NK ↑		Watzl et al., 2003
Dark chocolate (168.26 mg procyanidins) and cocoa powder drink (482.82 mg procyanidins)	42	Healthy	Uncontrolled	25	IL-1β, IL-6 and TNF-α production ↔		Mathur et al., 2002
Isoflavone-containing soy-based nutritional supplements	56	Hemodialysis	Placebo-controlled parallel	15, 10 control	TNF-α, IL-6 production ↔	Isoflavone ↑ 300 nM	Fanti et al., 2006
Juice A anthocyanin- rich (330 mL) Juice B flavanol-rich (330 mL)	14	Healthy	Uncontrolled crossover	27	lymphocyte proliferation ↑ IL-2 production ↑ IL-4 production ↔ Lytic activity of NK ↑	urinary total polyphenols ↑4.53 mM A	Bub et al. 2003
Blueberry–apple juice (15 mg quercetin)	28	Healthy	Uncontrolled	7	TNF-α production ↔	Quercetin ↑ (50 nM)	Boots et al., 2008
Concord grape juice	63	Healthy middle-aged	Placebo-controlled parallel	40/38 control	lymphocyte proliferation ↓		Rowe et al., 2011
Fruit juice (13.1 mg quercetin) or fruit-vegetable concentrate (3.7 mg quercetin)	112	HIV and healthy	Uncontrolled	HIV: 12 juice, 8 concentrate, healthy 13 juice, 4 concentrate	lymphocyte proliferation ↑ in HIV with both treatment in healthy only with fruit juice		Winkler et al., 2004
Red grape juice (100 mL) ± vitamin E (800 IU)	14	Hemodialysis	Placebo-controlled parallel	8/group	neutrophils oxidative burst ↓		Castilla et al., 2008
Red wine (200 mL), dealcholized red wine (175 mL)	42	Healthy	Placebo-controlled parallel	24, 25, 25 control	phagocytosis ↔ burst of neutrophils ↔ burst of monocytes ↓		Ellinger et al. 2008
Red wine, dealcoholized red wine, beer, dealcoholized beer	21	Healthy	Placebo-controlled parallel	6-8/group	ex vivo monocyte migration ↓ (ethanol or dealcoholized red wine)		Imhof et al., 2008
Red wine (85.55 mg anthocyanins and 31.7 mg catechins), dealcoholized red wine (72.4 mg anthocyanins and 23.2 mg catechins), red grape juice (169.3 mg anthocyanins and 32.15 mg catechins)	14	Healthy	Placebo-controlled crossover	24	phagocytic activity ↔ lymphocyte proliferation ↔ IL-2, IL-4, TNF-α, TGF-β production ↔ Lytic activity of NK ↔		Watzl et al., 2004
Quercetin at 500 or 1000 mg/d	84	Healthy	Placebo-controlled parallel	38, 40, 42 control	Granulocyte respiratory burst ↔	Plasma quercetin ↑ (300–500 μg/L)	Heinz et al., 2010
Quercetin 1000 mg	21	Healthy	Placebo-controlled parallel	18, 21 control	Granulocyte respiratory burst ↔		Henson et al. 2008
Quercetin 1000 mg	21	Healthy	Placebo-controlled parallel	20/group	Granulocyte respiratory burst ↔ lymphocyte proliferation ↔ Lytic activity of NK ↔		Nieman et al., 2007

↔ No change; ↑ increase; ↓ decrease.

modulate immune responses, through the inhibition of Th1 cytokine production (Miles et al., 2005). In contradiction, according to the available literature, the results of the present review indicate that in vivo chronic supplementation of flavonoid-rich foods or quercetin pure molecule has a scarce effect on immunity in humans. Considering both anti-inflammatory in vivo actions and ex vivo effects on markers of immune function, scarce activity was recorded for tea (either black or green tea), cocoa powder, soy and soy products, vegetables (both in form of fresh vegetables and vegetable-soups or juices), and pure quercetin. Although the number of studies is still to scarce for drawing any firm conclusion, promising results were obtained with fruit juices, grape extract, and derivatives such as wine.

With respect to the most studied cytokines, decreased circulating concentrations of IL-6 were found in only the 13% (5/38) of the interventions, while reduced levels of TNF-α in the 20% (8/40). More effective seemed to be the action on marker of immune function, with the 36% of the interventions (9 of 25) reporting reductions of either ex vivo induced cytokine production, lymphocyte proliferation or phagocytic activity. However, the human immune system is a very interactive network of cells and their products, so as there is no single immune marker that accurately reflects an individual's immune competence (Calder, 2007). Thus, combining markers of systemic inflammation, such as circulating cytokines, with a panel of ex vivo markers of immune function is the best approach to measure immunomodulation in human nutrition intervention studies. In the present review, only two studies concomitantly performed the two types of measures, failing to detect any relevant effect on either cytokine circulating levels and their ex vivo induced production, or respiratory burst (Boots et al., 2008; Heinz et al., 2010).

In order to be able to associate flavonoid intake to modulation of immune system, we tried to extrapolate those studies that found a concomitant immunomodulatory effect and changes in circulating levels of flavonoids or their metabolites. In the present work, we found that most of the interventions lack an assessment of flavonoid absorption. With respect to the studies that used foods as sources of flavonoids, only 9 of 60 interventions (15%) reported measurements of flavonoids bioavailability. Among these, four failed to associate the two events, despite increases in flavonoid circulating levels were recorded. When pure quercetin was utilized as supplements, a plasma concentration was measured in 13 of 17 interventions. Among these, when an increase of quercetin plasma levels was observed, 10 interventions reported no changes in markers of immune function.

Based on these considerations, our results highlight a discrepancy between what has been reported by in vitro experiments and the in vivo and ex vivo evidence on the capacity of flavonoid-rich food or supplements to modulate human immune system. Although it is fundamental to examine the causal mechanisms for biological change induced by molecules, leading to a better knowledge of actions that potentially may occur inside human body, in vitro results cannot always be readily extrapolated to humans. In case of flavonoids, some considerations must be taken into account. First of all, the biological effect of flavonoids depends on their bioavailability, which results to be low in humans (Crozier et al., 2009). Secondly, once ingested, they are extensively metabolized into molecules with different chemical structure and activity compared to the ones originally present in the food (Crozier et al., 2009). The low extent of bioavailability of flavonoids and the extensive metabolic activity they undergo during absorption, lead to very low plasma concentrations and to the presence in the blood stream of a wide variety of known and less-known metabolites (Manach and Donovan, 2004). Thus, the large amount of in vitro evidence has been obtained for flavonoid compounds, which are present in plant foods, but may not be found in vivo. Moreover, the concentrations to which cells have been exposed have often been far higher than those measured in biological fluids, increasing the chance of misinterpretation of the results. It is interesting to note that the few available studies involving biological metabolites, and not flavonoid aglycones, indicate that, at concentrations close to postingestion circulating levels (Merfort et al., 1996; Suri et al., 2008; Monagas et al., 2009) (10^{-6} M), these compounds are more active than the original ones in reducing oxidative burst (Merfort et al., 1996; Suri et al., 2008) and inflammatory cytokines secretion (Monagas et al., 2009).

Interesting results were obtained when studies involving healthy subjects, healthy subjects with risk factors or subjects affected by diseases were considered separately as described in Figure 1. While no great difference was observed for what concern the effect on IL-6 between the two groups, the effect on TNF-α resulted to be completely different in relation to subject's health status. None of the intervention studies (0/21) conducted in healthy subjects was effective in reducing levels of TNF-α after ingestion of flavonoid-rich foods or supplements (Figure 1). On the other hand, in cases of subjects characterized by risk factors for CVD, flavonoids decreased TNF-α in almost 30% of the interventions (5/17) (Figure 1). The effect is more pronounced if we restrict the field to patient affected by different diseases: despite the scarce number of available studies, the 60% of the interventions (4/7) were effective in reducing TNF-α values after supplementation with either a papaya preparation (Marotta et al., 2007), soy products (Azadbakht et al., 2007), green tea extracts (Hsu et al., 2007), and 1 year adherence to Mediterranean diet (Marfella et al., 2006) (Figure 1). Despite

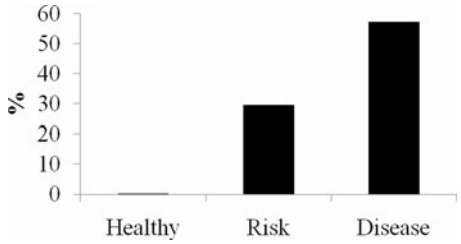

Figure 1 Dietary interventions trials with flavonoid-rich foods or quercetin and TNF-α levels in healthy subjects ($n = 21$), subjects characterized by risk factors for CVD ($n = 17$) and subjects with disease ($n = 7$). Data are expressed as percentage (%) of successful studies.

the effect is limited to TNF-α only, these data suggest that the influence of flavonoids-rich foods on immunity might be more effective in subjects, where, for the presence of CVD risk factors or pathologies, immune system is more challenged respect to healthy people with an apparently low degree of inflammation.

In conclusion, the role of flavonoid in the modulation of human immune system is not substantiated by the data from available human intervention studies. However, the immunomodulatory effect showed by flavonoid-rich foods on TNF-α in subjects with inflammatory stress but not in healthy people might partially explain our findings. More evidences in humans are needed in order to clarify if flavonoids represent ancillary ingredients or focal molecules involved in the immunomodulatory properties of foods of vegetable origins.

REFERENCES

Aherne, S. A. and O'Brien, N. M. (2002). Dietary flavonols: Chemistry, food content, and metabolism. *Nutrition.* **18**(1):75–81.S.

Azadbakht, L., Kimiagar, M., Mehrabi, Y., Esmaillzadeh, A., Hu, F. B., and Willett, W. C. (2007). Soy consumption, markers of inflammation, and endothelial function: A cross-over study in postmenopausal women with the metabolic syndrome. *Diabetes Care.* **30**(4):967–973.

Bae, S. C., Jung, W. J., Lee, E. J., Yu, R., and Sung, M. K. (2009). Effects of antioxidant supplements intervention on the level of plasma inflammatory molecules and disease severity of rheumatoid arthritis patients. *J. Am. Coll. Nutr.* **28**(1):56–62.

Basu, A., Du, M., Sanchez, K., Leyva, M. J., Betts, N. M., Blevins, S., Wu, M., Aston, C. E., and Lyons, T. J. (2011). Green tea minimally affects biomarkers of inflammation in obese subjects with metabolic syndrome. *Nutrition*. **27**(2):206–213.

Beavers, K. M., Serra, M. C., Beavers, D. P., Cooke, M. B., and Willoughby, D. S. (2009). Soymilk supplementation does not alter plasma markers of inflammation and oxidative stress in postmenopausal women. *Nutr. Res.* **29**(9):616–622.

Boots, A. W., Wilms, L. C., Swennen, E. L., Kleinjans, J. C., Bast, A., and Haenen, G. R. (2008). In vitro and ex vivo anti-inflammatory activity of quercetin in healthy volunteers. *Nutrition*. **24**(7–8):703–710.

Bruckdorfer, K. R. (2008). Antioxidants and CVD. *Proc. Nutr. Soc.* **67**:214–222.

Bub, A., Watzl, B., Blockhaus, M., Briviba, K., Liegibel, U., Müller, H., Pool-Zobel, B. L., and Rechkemmer, G. (2003). Fruit juice consumption modulates antioxidative status, immune status and DNA damage. *J. Nutr. Biochem.* **14**(2):90–98.

Calder, P. C. (2007). Immunological parameters: What do they mean? *J. Nutr.* **137**:773S–780S.

Carrero, P., Ortega, H., Martínez-Botas, J., Gómez-Coronado, D., and Lasunción, M. A. (1998). Flavonoid-induced ability of minimally modified low-density lipoproteins to support lymphocyte proliferation. *Biochem. Pharmacol.* **55**(7):1125–1129.

Castilla, P., Dávalos, A., Teruel, J. L., Cerrato, F., Fernández-Lucas, M., Merino, J. L., Sánchez-Martín, C. C., Ortuño, J., and Lasunción, M. A. (2008). Comparative effects of dietary supplementation with red grape juice and vitamin E on production of superoxide by circulating neutrophil NADPH oxidase in hemodialysis patients. *Am. J. Clin. Nutr.* **87**(4):1053–1061.

Cevenini, E., Caruso, C., Candore, G., Capri, M., Nuzzo, D., Duro, G., Rizzo, C., Colonna-Romano, G., Lio, D., Di Carlo, D., Palmas, M. G., Scurti, M., Pini, E., Franceschi, C., and Vasto, S. (2010). Age-related inflammation: The contribution of different organs, *tissues* and systems. How to face it for therapeutic approaches. *Curr. Pharm. Des.* **16**(6):609–618.

Charles, C., Yuskavage, J., Carlson, O., John, M., Tagalicud, A. S., Maggio, M., Muller, D. C., Egan, J., and Basaria, S. (2009). Effects of high-dose isoflavones on metabolic and inflammatory markers in healthy postmenopausal women. *Menopause*. **16**(2):395–400.

Crozier, A., Jaganath, I. B., and Clifford, M. N. (2009). Dietary phenolics: Chemistry, bioavailability and effects on health. *Nat. Prod. Rep.* **26**(8):1001–1043.

Dalgård, C., Nielsen, F., Morrow, J. D., Enghusen-Poulsen, H., Jonung, T., Hørder, M., and de Maat, M. P. (2009). Supplementation with orange and blackcurrant juice, but not vitamin E, improves inflammatory markers in patients with peripheral arterial disease. *Br. J. Nutr.* **101**(2):263–269.

de Maat, M. P., Pijl, H., Kluft, C., and Princen, H. M. (2000). Consumption of black and green tea had no effect on inflammation, haemostasis and endothelial markers in smoking healthy individuals. *Eur. J. Clin. Nutr.* **54**(10):757–763.

Dewas, C., Dang, P. M., Gougerot-Pocidalo, M. A., and El-Benna, J. (2003). TNF-α induces phosphorylation of p47phox in human neutrophils: Partial phosphorylation of p47phox Is a common event of priming of human neutrophils by TNF-α and granulocyte-macrophage colony-stimulating factor. *J. Immunol.* **171**(8):4392–4398.

Djurovic, S., Berge, K. E., Birkenes, B., Braaten, Ø. and Retterstøl, L. (2007). The effect of red wine on plasma leptin levels and vasoactive factors from adipose tissue: A randomized crossover trial. *Alcohol. Alcohol.* **42**(6):525–528.

Egert, S., Boesch-Saadatmandi, C., Wolffram, S., Rimbach, G., and Müller, M. J. (2010). Serum lipid and blood pressure responses to quercetin vary in overweight patients by apolipoprotein E genotype. *J. Nutr.* **140**(2):278–284.

Egert, S., Bosy-Westphal, A., Seiberl, J., Kürbitz, C., Settler, U., Plachta-Danielzik, S., Wagner, A. E., Frank, J., Schrezenmeir, J., Rimbach, G., Wolffram, S., and Müller, M. J. (2009). Quercetin reduces systolic blood pressure and plasma oxidised low-density lipoprotein concentrations in overweight subjects with a high-cardiovascular disease risk phenotype: A double-blinded, placebo-controlled cross-over study. *Br. J. Nutr.* **102**(7):1065–1074.

Egert, S., Wolffram, S., Bosy-Westphal, A., Boesch-Saadatmandi, C., Wagner, A. E., Frank, J., Rimbach, G., and Mueller, M. J. (2008). Daily quercetin supplementation dose-dependently increases plasma quercetin concentrations in healthy humans. *J. Nutr.* **138**(9):1615–1621.

Eichenberger, P., Mettler, S., Arnold, M., and Colombani, P. C. (2010). No effects of three-week consumption of a green tea extract on time trial performance in endurance-trained men. *Int. J. Vitam. Nutr. Res.* **80**(1):54–64.

Ellinger, S., Arendt, B. M., Fimmers, R., Stehle, P., Spengler, U., and Goerlich, R. (2008). Bolus ingestion but not regular consumption of native or dealcoholized red wine modulates selected immunological functions of leukocytes in healthy volunteers. *Ann. Nutr. Metab.* **52**(4):288–295.

Ellis, C. L., Edirisinghe, I., Kappagoda, T., and Burton-Freeman, B. (2011). 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.* **18**(4):318–327.

Estruch, R., Sacanella, E., Badia, E., Antúnez, E., Nicolás, J. M., Fernández-Solá, J., Rotilio, D., de Gaetano, G., Rubin, E., and Urbano-Márquez, A. (2004). Different effects of red wine and gin consumption on inflammatory biomarkers of atherosclerosis: A prospective randomized crossover trial. Effects of wine on inflammatory markers. *Atherosclerosis*. **175**(1):117–123.

Fanti, P., Asmis, R., Stephenson, T. J., Sawaya, B. P., and Franke, A. A. (2006). Positive effect of dietary soy in ESRD patients with systemic inflammation–correlation between blood levels of the soy isoflavones and the acute-phase reactants. *Nephrol. Dial Transplant.* **21**(8):2239–2246.

Go, V. L., Butrum, R. R., and Wong, D. A. (2003). Diet, nutrition, and cancer prevention: The postgenomic era. *J. Nutr.* **133**(11 Suppl. 1):3830S–3836S.

Greenrod, W. and Fenech, M. (2003). The principal phenolic and alcoholic components of wine protect human lymphocytes against hydrogen peroxide- and ionizing radiation-induced DNA damage in vitro. *Mutagenesis*. **18**(2):119–126.

Heinz, S. A., Henson, D. A., Nieman, D. C., Austin, M. D., and Jin, F. (2010). A 12-week supplementation with quercetin does not affect natural killer cell activity, granulocyte oxidative burst activity or granulocyte phagocytosis in female human subjects. *Br. J. Nutr.* **104**(6):849–857.

Henson, D., Nieman, D., Davis, J. M., Dumke, C., Gross, S., Murphy, A., Carmichael, M., Jenkins, D. P., Quindry, J., McAnulty, S., McAnulty, L., Utter, A., and Mayer, E. (2008). Post-160-km race illness rates and decreases in granulocyte respiratory burst and salivary IgA output are not countered by quercetin ingestion. *Int. J. Sports Med.* **29**(10):856–863.

Hermansen, K., Hansen, B., Jacobsen, R., Clausen, P., Dalgaard, M., Dinesen, B., Holst, J. J., Pedersen, E., and Astrup, A. (2005). Effects of soy supplementation on blood lipids and arterial function in hypercholesterolaemic subjects. *Eur. J. Clin. Nutr.* **59**(7):843–850.

Hilpert, K. F., Kris-Etherton, P. M., and West, S. G. (2005). Lipid response to a low-fat diet with or without soy is modified by C-reactive protein status in moderately hypercholesterolemic adults. *J. Nutr.* **135**(5):1075–1079.

Hsu, S. P., Wu, M. S., Yang, C. C., Huang, K. C., Liou, S. Y., Hsu, S. M., and Chien, C. T. (2007). Chronic green tea extract supplementation reduces hemodialysis-enhanced production of hydrogen peroxide and hypochlorous acid, atherosclerotic factors, and proinflammatory cytokines. *Am. J. Clin. Nutr.* **86**(5):1539–1547.

Huang, Y., Cao, S., Nagamani, M., Anderson, K. E., Grady, J. J., and Lu, L. J. (2005). Decreased circulating levels of tumor necrosis factor-alpha in postmenopausal women during consumption of soy-containing isoflavones. *J. Clin. Endocrinol. Metab.* **90**(7):3956–3962.

Imhof, A., Blagieva, R., Marx, N., and Koenig, W. (2008). Drinking modulates monocyte migration in healthy subjects: A randomised intervention study of water, ethanol, red wine and beer with or without alcohol. *Diab. Vasc. Dis. Res.* **5**(1):48–53.

Inoue, T., Komoda, H., Uchida, T., and Node, K. (2008). Tropical fruit camucamu (Myrciaria dubia) has anti-oxidative and anti-inflammatory properties. *J. Cardiol.* **52**(2):127–132.

Jenkins, D. J., Kendall, C. W., Connelly, P. W., Jackson, C. J., Parker, T., Faulkner, D., and Vidgen, E. (2002). Effects of high- and low-isoflavone (phytoestrogen) soy foods on inflammatory biomarkers and proinflammatory cytokines in middle-aged men and women. *Metabolism*. **51**(7):919–924.

Karlsen, A., Paur, I., Bøhn, S. K., Sakhi, A. K., Borge, G. I., Serafini, M., Erlund, I., Laake, P., Tonstad, S., and Blomhoff, R. (2010). Bilberry juice modulates plasma concentration of NF-kappaB related inflammatory markers in subjects at increased risk of CVD. *Eur. J. Nutr.* **49**(6):345–355.

Kawai, M., Hirano, T., Arimitsu, J., Higa, S., Kuwahara, Y., Hagihara, K., Shima, Y., Narazaki, M., Ogata, A., Koyanagi, M., Kai, T., Shimizu, R., Moriwaki, M., Suzuki, Y., Ogino, S., Kawase, I., and Tanaka, T. (2009). Effect of enzymatically modified isoquercitrin, a flavonoid, on symptoms of Japanese cedar pollinosis: A randomized double-blind placebo-controlled trial. *Int. Arch. Allergy Immunol.* **149**(4):359–368.

Kim, H. P., Son, K. H., Chang, H. W., and Kang, S. S. (2004). Anti-inflammatory plant flavonoids and cellular action mechanisms. *J. Pharmacol. Sci.* **96**(3):229–245.

Knab, A. M., Shanely, R. A., Henson, D. A., Jin, F., Heinz, S. A., Austin, M. D., and Nieman, D. C. (2011). Influence of quercetin supplementation on disease risk factors in community-dwelling adults. *J. Am. Diet Assoc.* **111**(4):542–549.

Knekt, P., Jarvinen, R., Reunanen, A., and Maatela, J. (1996). Flavonoid intake and coronary mortality in Finland: A cohort study. *BMJ.* **312**(7029):478–481.

Kolb, H., and Mandrup-Poulsen, T. (2010). The global diabetes epidemic as a consequence of lifestyle-induced low-grade inflammation. *Diabetologia*. **53**(1):10–20.

Lampe, J. W. (1999). Health effects of vegetables and fruit: Assessing mechanisms of action in human experimental studies. *Am. J. Clin. Nut.* **70**:475S–490S.

Lee, J. K., Kim, S. Y., Kim, Y. S., Lee, W. H., Hwang, D. H., and Lee, J. Y. (2009). Suppression of the TRIF-dependent signaling pathway of Toll-like receptors by luteolin. *Biochem. Pharmacol.* **77**(8):1391–1400.

Liu, R. H. (2003). Health benefits of fruit and vegetables are from additive and synergistic combinations of phytochemicals. *Am. J. Clin. Nutr.* **78**(3 Suppl.):517S–520S.

Llaneza, P., González, C., Fernandez-Iñarrea, J., Alonso, A., Diaz, F., Arnott, I., and Ferrer-Barriendos, J. (2011). Soy isoflavones, diet and physical exercise modify serum cytokines in healthy obese postmenopausal women. *Phytomedicine.* **18**(4):245–250.

López-Posadas, R., Ballester, I., Abadía-Molina, A. C., Suárez, M. D., Zarzuelo, A., Martínez-Augustin, O., and Sánchez de Medina, F. (2008). Effect of flavonoids on rat splenocytes, a structure-activity relationship study. *Biochem. Pharmacol.* **76**(4):495–506.

Manach, C., and Donovan, J. L. (2004). Pharmacokinetics and metabolism of dietary flavonoids in humans. *Free Radic. Res.* **38**:771–785.

Marfella, R., Cacciapuoti, F., Siniscalchi, M., Sasso, F. C., Marchese, F., Cinone, F., Musacchio, E., Marfella, M. A., Ruggiero, L., Chiorazzo, G., Liberti, D., Chiorazzo, G., Nicoletti, G. F., Saron, C., D'Andrea, F., Ammendola, C., Verza, M., and Coppola, L. (2006). Effect of moderate red wine intake on cardiac prognosis after recent acute myocardial infarction of subjects with Type 2 diabetes mellitus. *Diabet. Med.* **23**(9):974–981.

Marotta, F., Yoshida, C., Barreto, R., Naito, Y., and Packer, L. (2007). Oxidative-inflammatory damage in cirrhosis: Effect of vitamin E and a fermented papaya preparation. *J. Gastroenterol. Hepatol.* **22**(5):697–703.

Maskarinec, G., Steude, J. S., Franke, A. A., and Cooney, R. V. (2009). Inflammatory markers in a 2-year soy intervention among premenopausal women. *J. Inflamm. (Lond).* doi: 10.1186/1476-9255-6-9.

Mathur, S., Devaraj, S., Grundy, S. M., and Jialal, I. (2002). Cocoa products decrease low density lipoprotein oxidative susceptibility but do not affect biomarkers of inflammation in humans. *J. Nutr.* **132**(12):3663–3667.

Mente, A., de Koning, L., Shannon, H. S., and Anand, S. S. (2009). A systematic review of the evidence supporting a causal link between dietary factors and coronary heart disease. *Arch. Intern. Med.* **169**(7):659–669.

Merfort, I., Heilmann, J., Weiss, M., Pietta, P., and Gardana, C. (1996). Radical scavenger activity of three flavonoid metabolites studied by inhibition of chemiluminescence in human PMNs. *Planta Med*. **62**(4):289–292.

Miles, E. A., Zoubouli, P., and Calder, P. C. (2005). Effects of polyphenols on human Th1 and Th2 cytokine production. *Clin. Nutr.* **24**(5):780–784.

Monagas, M., Khan, N., Andres-Lacueva, C., Casas, R., Urpí-Sardà, M., Llorach, R., Lamuela-Raventós, R. M., and Estruch, R. (2009). Effect of cocoa powder on the modulation of inflammatory biomarkers in patients at high risk of cardiovascular disease. *Am. J. Clin. Nutr.* **90**(5):1144–1150.

Monagas, M., Khan, N., Andrés-Lacueva, C., Urpí-Sardá, M., Vázquez-Agell, M., Lamuela-Raventós, R. M., and Estruch, R. (2009). Dihydroxylated phenolic acids derived from microbial metabolism reduce lipopolysaccharide-stimulated cytokine secretion by human peripheral blood mononuclear cells. *Br. J. Nutr.* **102**(2):201–206.

Mukamal, K. J., MacDermott, K., Vinson, J. A., Oyama, N., Manning, W. J., and Mittleman, M. A. (2007). A 6-month randomized pilot study of black tea and cardiovascular risk factors. *Am. Heart J.* **154**(4):724.e1–6.

Murr, C., Schroecksnadel, K., Winkler, C., Ledochowski, M., and Fuchs, D. (2005). Antioxidants may increase the probability of developing allergic diseases and asthma. *Med. Hypotheses.* **64**(5):973–977.

Nair, M. P., Mahajan, S., Reynolds, J. L., Aalinkeel, R., Nair, H., Schwartz, S. A., and Kandaswami, C. (2006). The flavonoid quercetin inhibits proinflammatory cytokine (tumor necrosis factor alpha) gene expression in normal peripheral blood mononuclear cells via modulation of the NF-kappa beta system. *Clin. Vaccine Immunol.* **13**(3):319–328.

Nantz, M. P., Rowe, C. A., Nieves, C. Jr, and Percival, S. S. (2006). Immunity and antioxidant capacity in humans is enhanced by consumption of a dried, encapsulated fruit and vegetable juice concentrate. *J. Nutr.* **136**(10):2606–2610.

Nasca, M. M., Zhou, J. R., and Welty, F. K. (2008). Effect of soy nuts on adhesion molecules and markers of inflammation in hypertensive and normotensive postmenopausal women. *Am. J. Cardiol.* **102**(1):84–86.

Pérez-Jiménez, J., Neveu, V., Vos, F., and Scalbert, A. (2010). Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: an application of the phenol-explorer database. *J Agric Food Chem.* **58**(8):4959–4969.

Nieman, D. C., Henson, D. A., Davis, J. M., Angela Murphy, E., Jenkins, D. P., Gross, S. J., Carmichael, M. D., Quindry, J. C., Dumke, C. L., Utter, A. C., McAnulty, S. R., McAnulty, L. S., Triplett, N. T., and Mayer, E. P. (2007a). Quercetin's influence on exercise-induced changes in plasma cytokines and muscle and leukocyte cytokine mRNA. *J. Appl. Physiol.* **103**(5):1728–1735.

Nieman, D. C., Henson, D. A., Davis, J. M., Dumke, C. L., Gross, S. J., Jenkins, D. P., Murphy, E. A., Carmichael, M. D., Quindry, J. C., McAnulty, S. R., McAnulty, L. S., Utter, A. C., and Mayer, E. P. (2007b). Quercetin ingestion does not alter cytokine changes in athletes competing in the Western States Endurance Run. *J. Interferon Cytokine Res.* **27**(12):1003–1011.

Nieman, D. C., Henson, D. A., Gross, S. J., Jenkins, D. P., Davis, J. M., Murphy, E. A., Carmichael, M. D., Dumke, C. L., Utter, A. C., McAnulty, S. R., McAnulty, L. S., and Mayer, E. P. (2007). Quercetin reduces illness but not immune perturbations after intensive exercise. *Med. Sci. Sports Exerc.* **39**(9):1561–1569.

Nieman, D. C., Henson, D. A., Maxwell, K. R., Williams, A. S., McAnulty, S. R., Jin, F., Shanely, R. A., and Lines, T. C. (2009). Effects of quercetin and EGCG on mitochondrial biogenesis and immunity. *Med. Sci. Sports Exerc.* **41**(7):1467–1475.

Pérez-Jiménez, J., Neveu, V., Vos, F., and Scalbert, A. (2010). Systematic analysis of the content of 502 polyphenols in 452 foods and beverages: An application of the Phenol-Explorer database. *J. Agric. Food Chem.* **58**(8):4959–4969.

Rahman, I., Biswas, S. K., and Kirkham, P. A. (2006). Regulation of inflammation and redox signaling by dietary polyphenols. *Biochem. Pharmacol.* **72**(11):1439–1452.

Rowe, C. A., Nantz, M. P., Nieves, C. Jr, West, R. L. and Percival, S. S. (2011). Regular consumption of concord grape juice benefits human immunity. *J. Med. Food.* **14**(1–2):69–78.

Ryan-Borchers, T. A., Park, J. S., Chew, B. P., McGuire, M. K., Fournier, L. R., and Beerman, K. A. (2006). Soy isoflavones modulate immune function in healthy postmenopausal women. *Am. J. Clin. Nutr.* **83**(5):1118–1125.

Ryu, O. H., Lee, J., Lee, K. W., Kim, H. Y., Seo, J. A., Kim, S. G., Kim, N. H., Baik, S. H., Choi, D. S., and Choi, K. M. (2006). Effects of green tea consumption on inflammation, insulin resistance and pulse wave velocity in type 2 diabetes patients. *Diabetes Res. Clin. Pract.* **71**(3):356–358.

Sanbongi, C., Suzuki, N., and Sakane, T. (1997). Polyphenols in chocolate, which have antioxidant activity, modulate immune functions in humans in vitro. *Cell Immunol*. **177**(2):129–136.

Sánchez-Moreno, C., Cano, M. P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., and Martín, A. (2004). Consumption of high-pressurized vegetable soup increases plasma vitamin C and decreases oxidative stress and inflammatory biomarkers in healthy humans. *J. Nutr.* **134**(11):3021–3025.

Sánchez-Moreno, C., Cano, M. P., de Ancos, B., Plaza, L., Olmedilla, B., Granado, F., and Martín, A. (2006). Mediterranean vegetable soup consumption increases plasma vitamin C and decreases F2-isoprostanes, prostaglandin E2 and monocyte chemotactic protein-1 in healthy humans. *J. Nutr. Biochem.* **17**(3):183–189.

Sanderson, P., Elsom, R. L., Kirkpatrick, V., Calder, P. C., Woodside, J. V., Williams, E. A., Rink, L., Fairweather-Tait, S., Ivory, K., Cantorna, M., Watzl, B., and Stone, E. M. (2010). UK food standards agency workshop report: Diet and immune function. *Br. J. Nutr.* **103**(11):1684–1687.

Serafini, M., Miglio, C., Peluso, I., and Petrosino, T. (2011). Modulation of plasma non enzimatic antioxidant capacity (NEAC) by plant foods: The role of polyphenols. *Curr. Top Med. Chem.* **11**(14):1821–1846.

Serafini, M., Peluso, I., and Raguzzini, A. (2010). Flavonoids as anti-inflammatory agents. *Proc. Nutr. Soc.* **69**(3):273–278.

Sofi, F. (2008). Adherence to Mediterranean diet and health status: Meta-analysis. *BMJ.* **337**:1344–1350.

Surh, Y. J. (2003). Cancer chemoprevention with dietary phytochemicals. *Nat. Rev. Cancer.* **3**(10):768–780.

Suri, S., Taylor, M. A., Verity, A., Tribolo, S., Needs, P. W., Kroon, P. A., Hughes, D. A., and Wilson, V. G. (2008). A comparative study of the effects of quercetin and its glucuronide and sulfate metabolites on human neutrophil function in vitro. *Biochem. Pharmacol.* **76**(5):645–653.

Tominaga, T., Kawaguchi, K., Kanesaka, M., Kawauchi, H., Jirillo, E., and Kumazawa, Y. (2010). Suppression of type-I allergic responses by oral administration of grape marc fermented with Lactobacillus plantarum. *Immunopharmacol. Immunotoxicol.* **32**(4):593–599. Epub 2010 Feb 8.

Trichopoulou, A., Bamia, C., and Trichopoulos, D. (2009). Anatomy of health effects of Mediterranean diet: Greek EPIC prospective cohort study. *BMJ*. **23**:338.

Udani, J. K., Singh, B. B., Barrett, M. L., and Singh, V. J. (2009). Evaluation of Mangosteen juice blend on biomarkers of inflammation in obese subjects: A pilot, dose finding study. *Nutr. J.*, doi: 10.1186/1475-2891-8-48.

Vasto, S., Carruba, G., Lio, D., Colonna-Romano, G., Di Bona, D., Candore, G., and Caruso, C. (2009). Inflammation, ageing and cancer. *Mech. Ageing Dev.* **130**(1–2):40–45.

Vázquez-Agell, M., Sacanella, E., Tobias, E., Monagas, M., Antúnez, E., Zamora-Ros, R., Andrés-Lacueva, C., Lamuela-Raventós, R. M., Fernández-Solá, J., Nicolás, J. M., and Estruch, R. (2007). Inflammatory markers of atherosclerosis are decreased after moderate consumption of cava (sparkling wine) in men with low cardiovascular risk. *J. Nutr.* **137**(10):2279–84.2007 e.

Verbeek, R., Plomp, A. C., van Tol, E. A., and van Noort, J. M. (2004). The flavones luteolin and apigenin inhibit in vitro antigen-specific proliferation and interferon-gamma production by murine and human autoimmune T cells. *Biochem. Pharmacol.* **68**(4):621–629.

Watson, J. L., Vicario, M., Wang, A., Moreto, M., and McKay, D. M. (2005). Immune cell activation and subsequent epithelial dysfunction by Staphylococcus enterotoxin B is attenuated by the green tea polyphenol (−)-epigallocatechin gallate. *Cell Immunol*. **237**(1):7–16.

Watzl, B., Bub, A., Blockhaus, M., Herbert, B. M., Lührmann, P. M., Neuhäuser-Berthold, M., and Rechkemmer, G. (2000). Prolonged tomato juice consumption has no effect on cell-mediated immunity of well-nourished elderly men and women. *J. Nutr.* **130**(7):1719–1723.

Watzl, B., Bub, A., Briviba, K., and Rechkemmer, G. (2003). Supplementation of a low-carotenoid diet with tomato or carrot juice modulates immune functions in healthy men. *Ann. Nutr. Metab.* **47**(6):255–261.

Watzl, B., Bub, A., Pretzer, G., Roser, S., Barth, S. W., and Rechkemmer, G. (2004). Daily moderate amounts of red wine or alcohol have no effect on the immune system of healthy men. *Eur. J. Clin. Nutr.* **58**(1):40–45.

Watzl, B., Kulling, S. E., Möseneder, J., Barth, S. W., and Bub, A. (2005). A 4-wk intervention with high intake of carotenoid-rich vegetables and fruit reduces plasma C-reactive protein in healthy, nonsmoking men. *Am. J. Clin. Nutr.* **82**(5):1052–1058.

Winkler, P., Ellinger, S., Boetzer, A. M., Arendt, B. M., Berthold, H. K., Rockstroh, J. K., Spengler, U., and Goerlich, R. (2004). Lymphocyte proliferation and apoptosis in HIV-seropositive and healthy subjects during long-term ingestion of fruit juices or a fruit-vegetable-concentrate rich in polyphenols and antioxidant vitamins. *Eur. J. Clin. Nutr.* **58**(2):317–325.

Xu, L., Zhang, L., Bertucci, A. M., Pope, R. M., and Datta, S. K. (2008). Apigenin, a dietary flavonoid, sensitizes human T cells for activation-induced cell death by inhibiting PKB/Akt and NF-kappaB activation pathway. *Immunol. Lett.* **121**(1):74–83.

Xu, M., Mizoguchi, I., Morishima, N., Chiba, Y., Mizuguchi, J., and Yoshimoto, T. (2010). Regulation of antitumor immune responses by the IL-12 family cytokines, IL-12, IL-23, and IL-27. *Clin. Dev. Immunol.* pii:832454.

Zemel, M. B., Sun, X., Sobhani, T., and Wilson, B. (2010). Effects of dairy compared with soy on oxidative and inflammatory stress in overweight and obese subjects. *Am. J. Clin. Nutr.* **91**(1):16–22.

Zern, T. L., Wood, R. J., Greene, C., West, K. L., Liu, Y., Aggarwal, D., Shachter, N. S., and Fernandez, M. L. (2005). Grape polyphenols exert a cardioprotective effect in pre- and postmenopausal women by lowering plasma lipids and reducing oxidative stress. *J. Nutr.* **135**(8):1911–1917.