Transgenic Flavonoid Tomato Intake Reduces C-Reactive Protein in Human C-Reactive Protein Transgenic Mice More Than Wild-Type Tomato

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Abstract

The increased consumption of fruits and vegetables is associated with reduced cardiovascular disease. The molecular basis of this health effect is not fully understood, yet dietary flavonoids are thought to play an important role. Genetic engineering has enabled us to overexpress specific flavonoids (flavones and flavonols) in tomato fruit. Human C-reactive protein transgenic (CRPtg) mice express markers of cardiovascular risk that allow us to study of the putative health effects of wild-type tomato (wtTom) and flavonoid-enriched tomato (flTom). In this study, we analyzed whether consumption of wtTom, at a dose achievable with a human diet, has beneficial effects on cardiovascular risk markers and whether flTom may enhance such effects. CRPtg mice were fed a diet containing 4 g/kg wtTom, flTom peel, vehicle, or 1 g/kg fenofibrate, which reportedly reduces cardiovascular risk, for 7 wk. Markers of general health (bodyweight, food intake, and plasma alanine aminotransferase activities) and of cardiovascular risk (plasma CRP, fibrinogen, E-selectin, and cholesterol levels) were analyzed. All groups had comparable food intakes and body-weight gains. Plasma alanine aminotransferase activities increased significantly in vehicle and fenofibrate-treated mice. Compared with baseline, wtTom and flTom significantly reduced basal human CRP concentrations by 43 and 56%, respectively. The CRP-lowering effect of flTom significantly exceeded that of wtTom. The effects of flTom on CRP were reversed within a 2-wk washout period. WtTom and flTom did not affect fibrinogen, but comparably repressed E-selectin expression and upregulated HDL cholesterol. Tomato peel consumption improved cardiovascular risk factors in CRPtg mice, a beneficial effect that was further enhanced by enrichment of the flavonoid content.


Introduction

Large prospective cohort studies demonstrate an inverse relation between the consumption of fruits and vegetables and the development of cardiovascular disease and stroke in both males and females (1, 2). However, the biological mechanisms by which fruit and vegetables exert their beneficial effects on human health are not fully understood and are likely to be multiple (3). One possible explanation may be the high content of bioactive flavonoids and flavonoid derivatives that are found in these foods (4). A growing body of recent experimental evidence supports the view that dietary constituents of the flavonoid class exert important vasculoprotective effects, among which are lowered blood pressure, antithrombotic, antioxidative, and anti-inflammatory effects (5–7). The flavonoid quercetin, for example, attenuates blood pressure in hypertensive rats (8), and the flavone luteolin is reported to mitigate adhesion molecule expression by quenching the proinflammatory master regulator, nuclear factor-kappa B (NF-κB), in human endothelial cells (9).

During the past few years, several crops have been genetically modified to increase specific constituents considered to be beneficial for human health. For example, eicosapentaenoic acid and docosahexaenoic acid have been introduced to Brassica juncea (10) and resveratrol to Brassica napus seed (11). Other valuable nutrients that have been modified include the introduction of β-carotene to tomato fruit (12) and zeaxanthin to potato tubers (13). In the present study, genetic engineering technology was used to increase the flavonoid content of tomato peel by stimulating the endogenous flavonoid pathway and by introducing a new biosynthetic branch [described in more detail in (14)]. These modifications resulted in tomatoes with high levels of flavonols.
(e.g., quercetin, kaempferol, and glycosides thereof) and flavonoids (e.g., luteolin and luteolin-7-glucoside) in their peel tissue.

Because most genetically modified products claiming to enhance health have not yet been approved for human consumption, it is not certain whether they do indeed exert beneficial effects and whether, for example, they improve surrogate biomarkers of disease. In vitro human cell models are often inadequate because many food constituents, such as flavonoids, undergo significant metabolic modification by hydrolysis and conjugation during absorption in the small intestine, the colon, and the liver (15). As an alternative, the efficacy of these products could be evaluated in animal models that mimic the situation in humans.

Our study, therefore, used a humanized animal model, the human C-reactive protein transgenic (CRPtg) mouse (16,17). Human CRP is a highly sensitive inflammation marker and constitutes a strong independent predictor of future cardiovascular events in humans (18). Dietary treatment of CRPtg mice with wild-type tomato (wtTom) fruit and genetically modified, flavonoid-enriched tomato (ftTom) fruit, enabled us to study putative health effects of tomato fruit in general and of specific flavonoids in particular.

The tomato quantity fed to CRPtg mice, on an energy basis, equals 2.3 g peel, or ~230 g fresh tomato/d in humans (human energy intake, 10 MJ/d), and thus constitutes a portion that is achievable in a human diet. The effect of tomato fruit in CRPtg mice was compared to treatment with fenofibrate, an established hypolipidemic, anti-inflammatory drug known to decrease markers of cardiovascular risk, including CRP, which reduces coronary heart disease (19). In addition to human CRP, we analyzed markers that reflect the general health status [body-weight, fruit intake, and alanine aminotransferase (ALT)] and markers that predict future cardiovascular risk independent from CRP (fibrinogen, E-selectin and cholesterol).

Materials and Methods

Generation and characterization of wild-type and flavonoid-enriched tomato peel. Gene cloning and tomato transformation was carried out as previously described in detail (14). Briefly, full-length cDNA sequences encoding Petunia chalcone isomerase (CHI) and Gerbera hybrida flavone synthase-II (FNS) were used to create the double gene construct Pd3SS-Chi-Tnos-Pd3SS-FnsII-Tnos. This construct was subsequently cloned into the binary vector BBC50 (20) followed by transfer to Agrobacterium tumefaciens. To obtain transgenic tomato plants (Lycopersicon esculentum cv. Moneymaker), hypocotyls were used for Agrobacterium-mediated transformation. Kanamycin-resistant shoots were grown on rock-wool plugs under controlled greenhouse conditions. Wild-type tomato plants (n = 3), as well as plants (n = 3) derived from the transgenic tomato line with the highest levels of flavones and flavonols, were grown to harvest ripe fruits (>50 of each variety). Fruits were peeled and the peel was immediately frozen in liquid nitrogen and stored at ~80°C. Flavonoid levels and composition of the pooled peel tissues of each variety were determined after extraction in 75% aqueous methanol with 15 min of sonication. Compounds were separated on a C18 reverse phase HPLC column (Luna C18(2), 3 μm, 150 × 4 mm, Phenomenex) at 40°C, and analyzed by photodiode array detection (type 996, Waters). A gradient of 5–50% acetonitrile in 0.1% trifluoro acetic acid was used as the mobile phase. Absorbance spectra (240–600 nm) and retention times of eluting compounds were used for identification purposes in comparing with authentic flavonoid standards (Apin Chemicals). Both wild-type and transgenic tomato peel tissue were lyophilized separately (Sneders Scientific freeze dryer), stored at ~80°C, and analyzed for flavonoids by HPLC before adding to experimental diets. Tomato peel constituted ~100 g/kg of fresh tomato weight. Lyophilization reduced the peel weight another ~90%.

Mice and diets. All animal experiments described in this report conformed to the rules and regulations set forth by the Netherlands Law on Animal Experiments and were approved by the Institutional Animal Care and Use Committee of TNO. Male CRPtg mice were used for the study. CRPtg mice carry a 31-kb human DNA fragment containing the human CRP gene, including all known cis-acting regulatory elements, i.e., the entire human CRP promoter. The human-like pattern of expression of CRP in CRPtg mice has been described (16,17). CRPtg mice of the specific pathogen-free breeding stock of TNO were screened for inheritance of the CRP transgene using a human CRP-specific PCR reaction. Mice were housed in Macrolon cages at 21°C, with 50–60% relative humidity, a 12-h light cycle (0600–1800), free access to a commercial standard rodent diet (Smiff R/M-H, Specialdien GmbH; crude nutrients in g/kg dry matter included protein, 216; N-free extract, 617; fat, 38; fiber, 56; ash, 73; and was supplemented with vitamins and minerals; 16.0 MJ/kg metabolizable energy), and free access to tap water until the start of the experimental treatment (t = 0 or baseline).

At baseline, mice were matched into 4 experimental groups on the basis of their basal plasma CRP concentration. From t = 0 onward, mice had free access to a commercial experimental rodent diet (AM-III Hope Farms; crude nutrients in g/kg dry matter included protein, 272; carbohydrates, 561; fat, 72; fiber, 41; ash, 54; and was supplemented with vitamins and trace minerals; 17.6 MJ/kg metabolizable energy), and tap water. To test wtTom and ftTom with respect to putative benefits in vivo, equal amounts of either wtTom or ftTom peel were separately mixed into this experimental diet. Specifically, the diet was supplemented with vehicle (Con; Con group; n = 10), or 4 g/kg dried wild-type tomato peel (wtTom group; n = 10), or 4 g/kg flavonoid-enriched tomato peel (ftTom group; n = 8). An additional control group was treated with 1 g/kg fenofibrate (FF group; n = 10), a hypolipidemic drug known to reduce cardiovascular risk and to lower plasma inflammation markers such as CRP and fibrinogen in CRPtg mice (17) and humans (21). Body weight and food intake were determined weekly during the experimental period of 7 wk. In wk 6, an inflammatory response was induced in all mice by intraperitoneal injection of 85,000 scps/μl interleukin-1β (IL-1β; Sanvertech/mouse as described (17). Plasma CRP was measured in tail blood collected before (i.e., at the wk 6 sampling point) and 10 h after IL-1β injection. After mice had recovered and CRP concentrations had returned to baseline levels (17), the mice were subjected to a washout follow-up study from wk 7 to wk 9. At wk 7, experimental diet treatment was stopped, and all mice received a standard maintenance diet for 2 wk. Then plasma was collected and mice were killed by CO2 asphyxiation, and their livers were weighed.

Analysis of plasma inflammation markers. The plasma concentrations of the systemic inflammation markers, CRP and fibrinogen, were determined in tail blood samples by ELISA (22). Plasma concentrations of E-selectin and ALAT were quantified by ELISA (R&D Systems Europe) and kit 745138 (Roche Diagnostics), respectively. Because mice were matched on the basis of their baseline plasma CRP concentrations at t = 0, baseline levels of other plasma markers such as fibrinogen and E-selectin showed some variation between groups. Therefore, to allow for a direct comparison, the initial plasma concentrations of fibrinogen and E-selectin concentrations at baseline were set at 100% and treatment-dependent changes were expressed as a percentage thereof. The absolute concentrations of plasma fibrinogen and E-selectin at baseline, i.e., the control value used for normalization, are given in the legend of Figure 2.

Lipid and lipoprotein analysis. Plasma total cholesterol levels were measured using kits1489437 (Roche Diagnostics). Lipoprotein profiles were obtained by SMART analysis using the AKTA FPLC system (Pharmacia) as previously described (23). We determined the triglyceride content of the lipoprotein fractions using kit 337-B (Sigma Aldrich Chemie BV).

Statistical analysis. Results in the text are expressed as means ± SD. Changes over time within a group were analyzed by repeated measures ANOVA in combination with Dunnett post-hoc analysis (InStat software package, GraphPad), and significant changes compared with t = 0 (baseline). Differences between groups at a particular time point were
analyzed by 1-way ANOVA followed by a least significant difference (LSD) post-hoc test (SPSS, version 11.5). The stimulating effect of IL-1β on CRP was analyzed by 2-way ANOVA in combination with a Bonferroni post-hoc analysis. Differences were considered significant at $P < 0.05$.

**Results**

**Generation and characterization of wild-type and flavonoid-enriched tomato peel.** WtTom peel flavonoids mainly consisted of naringenin chalcone and moderate amounts of quercetin-3-rutinoside (Table 1). Overexpression of CHI and FNS resulted in a strong decrease of naringenin chalcone in flTom (3% compared with wtTom) because CHI and FNS use the naringenin chalcone pool for the production of flavonols and flavones, respectively. Overexpression of CHI and FNS in flTom was accompanied by a strong increase of the flavonols quercetin-3-rutinoside (18-fold of wtTom) and kaempferol-rutinoside (>36-fold of wtTom). Furthermore, quercetin-3-glucoside and kaempferol-3-glucoside were detected (not quantified) in the fruit peel of flTom. WtTom do not contain flavones because the biochemical branch leading to this flavonoid class is dependent on the presence of FNS, an enzyme absent in wtTom. The introduction of the transgenes (in flTom) also resulted in the occurrence of the flavones luteolin-7-glucoside and luteolin. Compounds other than flavonoids (for example, the content of chlorogenic acid, a precursor of the flavonoids) were comparably expressed in wtTom and flTom (56 ± 5 and 68 ± 8 mg/kg dry weight, respectively).

**General health markers in CRPtg mice.** The food intake during the treatment period was comparable in all groups and was, overall, 4.5 ± 0.5 g · d$^{-1}$ · mouse$^{-1}$. Compared with baseline, body weights increased significantly and comparably in all groups except the FF group from wk 4 onward (data not shown). In the FF group, significant increases in body weight occurred from wk 6 onward. The total gain in body weight during the experimental period did not differ among the groups and was, overall, 2.6 ± 2.2 g/mouse.

Plasma ALAT activities increased slightly in the Con group at the end of the 6-wk treatment period (45.8 ± 15.2 IU/L, $P < 0.05$)

tabulated with those at baseline (27.7 ± 5.6 IU/L). This increase in ALAT activity was not observed in the tomato peel–treated wtTom and flTom groups, both of which displayed plasma ALAT activities comparable to the initial values at baseline (wtTom, 23.1 ± 2.4 IU/L; flTom; 20.7 ± 3.0 IU/L). In the FF group, plasma ALAT concentration was strongly elevated (117 ± 29.9 IU/L; $P < 0.05$). The liver weight to body weight ratios, as assessed after termination at 9 wk, were comparable among the Con group (0.051 ± 0.02), the wtTom group (0.052 ± 0.006), and the flTom group (0.054 ± 0.004), whereas a slight increase was found in the FF group (0.057 ± 0.004 g; $P < 0.05$ vs. Con).

**Effect of tomato peel-feeding and its withdrawal on plasma CRP.** All groups had comparable plasma CRP concentrations at baseline (mean 9.7 mg/L), and the CRP levels of the Con group remained at this level during the complete experimental period (Fig. 1A). Treatment with wild-type tomato peel reduced CRP after 2 wk, with a maximal reduction after 4 wk of treatment (43% reduction compared with baseline, $P < 0.05$). CRP levels remained low in the wtTom group for the remainder of the treatment period. The CRP-lowering effect in the flTom was even more pronounced (by 56% reduction compared with baseline, $P < 0.05$), and plasma CRP concentrations in this group were constantly lower than in the wtTom group at all

**TABLE 1** Flavonoid composition of lyophilized tomato peel tissue from wild-type or genetically engineered flavonoid tomatoes$^{1,2}$

<table>
<thead>
<tr>
<th>Flavonoid precursor</th>
<th>wtTom</th>
<th>flTom</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonones</td>
<td>mg/kg dry peel</td>
<td>mg/kg dry peel</td>
</tr>
<tr>
<td>Naringenin chalcone</td>
<td>3707 ± 505</td>
<td>115 ± 2</td>
</tr>
<tr>
<td>Quercetin aglycon</td>
<td>ND$^3$</td>
<td>275 ± 12</td>
</tr>
<tr>
<td>Luteolin aglycon</td>
<td>630 ± 50</td>
<td>12120 ± 760</td>
</tr>
<tr>
<td>Flavones</td>
<td>≤53</td>
<td>1985 ± 118</td>
</tr>
<tr>
<td>Quercetin-3-rutinoside (rutin)</td>
<td>12120 ± 760</td>
<td>12120 ± 760</td>
</tr>
<tr>
<td>Kaempferol-3-glucoside</td>
<td>≤53</td>
<td>1985 ± 118</td>
</tr>
<tr>
<td>Flavonoids</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Luteolin-7-glucoside</td>
<td>3343 ± 243</td>
<td>3343 ± 243</td>
</tr>
<tr>
<td>Luteolin-7-glucoside</td>
<td>1547 ± 131</td>
<td>1547 ± 131</td>
</tr>
<tr>
<td>Flavonon precursors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chlorogenic acid</td>
<td>56 ± 5</td>
<td>68 ± 8</td>
</tr>
</tbody>
</table>

$^1$ Values are means ± SD, $n = 3$.

$^2$ Expressed as dry weight. Tomato peel constituted ~100 g/kg of fresh tomato wt.

$^3$ Not detected.

**Figure 1** Basal CRP expression (A) and interleukin-1β (IL-1β)-induced CRP expression (B) in CRPtg mice administered wild-type tomato peel (wtTom), flavonoid-enriched tomato peel (flTom), fenofibrate (FF), or vehicle control (Con). Values are means ± SEM; $n = 10$ (Con, wtTom, FF), $n = 8$ (flTom). The unstimulated CRP value in (B) is equivalent to the basal CRP value in (A) at 6 wk.

*Different from baseline, $P < 0.05$. Means at a time without a common letter differ, $P < 0.05$. *Different from unstimulated, $P < 0.05$. 

Tomato flavonoids reduce risk markers 2333
Treatment time points. The additional CRP-reducing effect in the flTom group compared with the wtTom group became significant at the end of the treatment period, i.e., at 6 wk and 7 wk. CRP concentrations declined rapidly in the FF group and more notably than in both tomato peel–fed groups (93% reduction compared with baseline at 2 wk; P < 0.05). CRP concentrations remained at this low level during the period that mice received FF. After 6 wk of experimental dietary treatment the mice were challenged with intraperitoneally injected acute inflammatory stimulus, IL-1β. Analysis of plasma CRP concentrations before and 10 h after stimulation with IL-1β showed that induction of CRP expression was comparable in all groups, except for the FF group, in which the IL-1β-dependent induction of CRP was almost fully suppressed (Fig. 1B; Of note, IL-1β-induced CRP values are not shown in Fig. 1A.)

When dietary treatment with wtTom and flTom was stopped and mice received maintenance diet again (Fig. 1A, 7–9 wk, washout), CRP concentrations in the wtTom group were still lower at 9 wk than at baseline, whereas the flTom group returned to a level comparable to baseline. A similar effect was seen in the FF group, which also returned to the initial level, indicating that the decrease in CRP in the flTom and FF groups was a specific treatment effect.

**Effect of tomato peel-feeding on cardiovascular risk factors other than CRP.** The plasma concentrations of fibrinogen (a hepatic acute phase reactant involved in coagulation), E-selectin (a sensitive marker of vascular inflammation), and total cholesterol at baseline, 4 wk, and 7 wk were determined (Fig. 2). Because mice were matched to groups on the basis of their baseline CRP concentrations, the initial concentrations of fibrinogen, E-selectin, and cholesterol varied among groups (cf. absolute concentrations in legend of Fig. 2). Therefore, initial concentrations were set at 100% and treatment-dependent changes were expressed relative to those.

There was no significant change in plasma fibrinogen in the Con group nor in the 2 tomato peel–treated groups (data not shown). In contrast, strongly reduced plasma fibrinogen concentrations (31% reduction compared with baseline; P < 0.05) were found with FF treatment in wk 4 and wk 7 (20% reduction; P < 0.05). During the experimental period, plasma E-selectin concentrations increased in the Con group at wk 4 (63%, P < 0.05) and wk 7 (40%, P < 0.05) compared with baseline (Fig. 2A). This increase in E-selectin was not observed in the wtTom and flTom groups, both of which displayed E-selectin concentrations that were comparable to baseline. Fenofibrate had a similar quenching effect, with plasma E-selectin concentrations also remaining at baseline.

In the Con group, the plasma cholesterol level did not significantly change over time, i.e., comparable levels were observed at the beginning and at the end of the experimental period (Fig. 2B). In both tomato peel–treated groups, total plasma cholesterol concentrations increased slightly, and, after 7 wk of treatment, a comparable increase was observed (7% in the wtTom and 5% in the flTom). Analysis of the lipoprotein profile at this time point revealed that the cholesterol-elevating effect of tomato fruit could mainly be ascribed to an increase in HDL cholesterol (Fig. 2C). A similar HDL-increasing effect was observed in the FF group, with total cholesterol being significantly increased by 11% at 7 wk.

**Discussion**

Several epidemiological studies demonstrate that a high intake of fruit and vegetables correlates with a decreased risk for cardiovascular pathologies (1–3). Among the nutrients considered to be responsible for this protective effect are the dietary flavonoids. In recent years, genetic engineering technologies have allowed the generation of transgenic plants, which accumulate specific nutrients, including flavonoids (14,20,24), with
potentially favorable effects (25). High flavonoid transgenic plants have not been tested in clinical studies so far. It thus remains unclear whether their consumption may improve human health or surrogate markers thereof. In this study, we used human CRPtg mice (16,17,23) to examine the in vivo effects of wtTom and transgenic flavone- and flavanol-rich, but flavanone-reduced tomato, flTom, on markers of general health and cardiovascular risk. The diet fed to CRPtg mice reflected a human intake of ~2.3 g tomato peel or 230 g fresh fruit (~3 medium tomatoes)/d. Our results show that 1) consumption of wtTom peel has beneficial effects on the plasma concentrations of human CRP, E-selectin, and HDL cholesterol, and 2) consumption of flTom additionally improves the beneficial effects of wtTom by further lowering human CRP levels. To our knowledge, this is the first time that a specific fruit has been demonstrated to reduce human CRP and that transgenic overexpression of specific flavonoids results in a further reduction of this important cardiovascular risk marker. Most importantly, these findings were obtained in a humanized in vivo model.

A growing body of epidemiological evidence supports the view that flavonoids, and in particular, the subclasses of flavonols and flavones, exert cardioprotective health effects (26). Among the fruits and vegetables rich in flavonols (mostly quercetin) are the following [in mg/kg fresh weight (FW)]: tomatoes, 5–30; onions, 150–500; brewed black and green tea, 40–50; apples, 15–70; and red grapes, 30–40. Flavones (mg/kg FW) occur mainly in: celery, 60; hot peppers, 50–100; and herbs such as parsley, 3000; and peppermint, 200; but hardly occur in tomatoes (27,28).

The transgenic tomatoes used in this study were engineered to overexpress CHI, resulting in a high accumulation of flavonols, predominantly quercetin and kaempferol, with their respective glucosides and rutinosides. Similarly, the expression of the gene construct introduced luteolin and its glucosides into tomato. The flavonol and flavone concentrations achieved in fresh tomato peel (flavonols, 1400 mg/kg; and flavones, almost 500 mg/kg) indicate high concentrations in the fruit. Tomato skin contains >95% of the fruit flavonoids (29). In addition to flavonoids, carotenoids are substantial secondary metabolites of tomato peel. The major carotenoids found in tomato peel extracts were lycopene and β-carotene, contributing 90–95% and 5–10% of total carotenoid content, respectively. Measurements of these carotenoids in the freeze-dried tomato peel of wtTom and flTom showed similar concentrations (data not shown). Thus, through genetic engineering, tomato fruits were generated that rival those edible food plants with the highest flavonol and flavone concentrations. A similar upregulation of flavonols in tomato plants has recently been reported after overexpression of petunia CHI (20).

Increased consumption of dietary fat, as during experimental diet feeding, results in an upregulation of hepatic factors associated with stress and organ damage [reviewed in (30)]. Plasma ALAT activities in the Con group increase within 6 wk after the switch from a maintenance to an experimental diet containing ~90% more fat. Our data demonstrate that tomato peel mixed into the experimental diet prevented an experimental diet–induced upregulation of plasma ALAT activities, a serum marker of stress, and possible tissue damage (31). In contrast to the beneficial effects of tomato peel on the liver, fenofibrate treatment strongly increased plasma ALAT activities and also increased the liver weight to body weight ratio. Elevation of plasma ALAT activities by fibrates occurs in a peroxisome proliferator-activated receptor-α (PPARα)-dependent way in rodents (32). This effect may be related to the well-documented enlargement of rodent livers by fibrates and the adverse (carcinogenic) effects of PPARα-activators in rodents (33,34).

A major finding of this study is the suppression of CRP expression by tomato peel, an effect even more pronounced with transgenic flavonoid tomato peel (24% further reduction; P < 0.05). To our knowledge, results from this study demonstrate for the first time that a genetically engineered fruit with enhanced flavonoids levels can have anti-inflammatory effects that exceed the effects of its wild-type counterpart. An important regulator of CRP gene expression is NFκB (22). NFκB is a proinflammatory, redox-sensitive transcription factor that is induced by cytokines such as IL-1β and plays an important role in the development of cardiovascular pathologies (35). Other genes that are regulated by NFκB include vascular cell adhesion molecule-1 and endothelial cell adhesion molecule (E-selectin) (30).

Tomato peel contains several components that suppress NFκB signaling. Among the components that potentially interfere with the NFκB signaling route are the carotenoids lycopene and β-carotene, both of which were present in wtTom and flTom at similar concentrations. For example, lycopene inhibits mitogen-activated NFκB in a murine dendritic cell model (36) and β-carotene blocks the lipopolysaccharide-induced nuclear translocation of NFκB in macrophages (37). In line with this, epidemiological data show a positive correlation between high β-carotene and lycopene plasma concentrations and low CRP expression (38,39).

Among the components overexpressed in flTom are the flavonoids quercetin and luteolin, and their conjugates. Clinical studies suggest that flavonols and flavones affect antioxidant biomarkers. For instance, dietary quercetin reduces lymphocyte DNA strand breakage, decreases urinary 8-hydroxy-2′-deoxyguanosine concentrations, increases plasma antioxidant capacity, and improves the oxidative resistance of LDL (28,40). However, the effects of dietary quercetin on inflammatory markers in humans are not consistent (40), and evidence for anti-inflammatory effects of flavonols and flavones comes predominantly from cell culture studies. For example, the flavonol quercetin inhibits NFκB activation in cultured human synovial cells (41) and rat aortic smooth muscle cells (42) and reduces IL-1β-induced NFκB activation in rat hepatocytes (43). The flavones luteolin and apigenin interfere with the NFκB pathway thereby inhibiting the production of proinflammatory cytokines (44) and circulating inflammatory markers (45) and repressing the induction of cell adhesion molecules (9,46). We cannot exclude the fact that a reduction of the flavone content (i.e., naringenin chalcone) in genetically engineered flTom might have contributed to the effects observed in the flTom group (e.g., CRP-lowering). Naringenin chalcone inhibits histamine release and acts as an antiallergin (47), but potential direct proinflammatory effects have so far not been described. Neutral or mild anti-inflammatory properties, such as the inhibition of lipopolysaccharide-induced NO synthase expression in RAW 264.7 cells, have been reported in the naringenin chalcone isomer, naringenin, (48). Together, the above findings demonstrate that predominantly flavonols and flavones exert anti-inflammatory activities on NFκB-regulated genes, which supports our finding that the consumption of flavonoid-rich flTom reduces human CRP concentrations beyond those achieved with wtTom.

Our data show that tomato peel effectively lowers baseline human CRP, i.e., a chronic low-grade inflammatory state, but does not suppress an acute inflammatory stimulus, i.e., an intraperitoneal injection of IL-1β resulting in a subsequent elevation of human CRP plasma concentrations. This is in contrast to the effects seen with the more potent anti-inflammatory,
hypolipidemic drug, fenofibrate. Fibrates strongly suppress the upregulation of CRP by IL-1β in CRPtg mice and in primary human hepatocytes (17,22,23). In addition to lowering plasma CRP, fenofibrate also reduces plasma fibrinogen concentrations, as was shown recently in mice and humans (21). WtTom and flTom had no effect on fibrinogen in CRPtg mice, further underscoring the fact that wtTom and flTom are less potent with regard to their anti-inflammatory activity at the concentrations used.

We observed that total plasma cholesterol concentrations increased slightly and comparably in both the wtTom group and the flTom group. Plasma cholesterol in mice was mainly confined to the HDL fraction and only to a minor extent to VLDL and LDL/intermediate density lipoprotein (IDL) particles. Although VLDL and LDL are atherogenic, HDL is atheroprotective. Increased HDL levels correlate with a reduced incidence of cardiovascular events (49). Our plasma lipoprotein analysis showed that consuming wtTom and flTom increases the amount of HDL and decreases the amount of VLDL and LDL particles. These findings accord with the inverse association between fruit and vegetable intake and plasma LDL levels as reported in some, though not all, clinical studies (50).

Similar to the favorable effects of flavonoid-enriched tomato peel in this study are other dietary ingredients such as plant sterols (51) and very long chain (n-3) fatty acids (52) that are also known to affect human cardiovascular risk factors in a beneficial way. A combination of such active dietary ingredients may thus, by additive or even synergistic effect, exert effects resembling those of established pharmacological drugs (53). Genetic enhancement of valuable dietary components in plant foods, such as specific flavonoids in tomatoes, may allow us to optimize human food composition and may help to reduce the burden of cardiovascular disease.

**Literature Cited**


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