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Limited antioxidant effect after consumption of a single dose of tomato sauce by young males, despite a rise in plasma lycopene

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Abstract

This study investigated the effect of a single dose of tomato sauce on healthy male volunteers in a randomized crossover study. Healthy male subjects ($n = 10$) were enrolled. Placebo (rice and olive oil) or tomato (tomato sauce, rice and olive oil) meals were provided to the volunteers. Blood and urine samples were taken before consumption of meal (0 h) and 2, 4, 6, 24 and 48 h after meal. Consumption of tomato sauce increased plasma lycopene level by 5–22%, with a maximum level at 24 h ($p < 0.01$) after the meal. Levels of plasma F_2-isoprostanes, hydroxyeicosatetraenoic acid products, allantoin and urinary 8-hydroxy-2′-deoxyguanosine did not change after either meal, but urinary F_2-isoprostanes ($p < 0.05$) significantly decreased at 48 h compared to 0 h after the tomato sauce meal. This study showed that a single dose of tomato sauce meal had only a limited antioxidant effect *in vivo*.

Keywords: *Tomato, lycopene, isoprostanes, HETEs, 8OHdG, oxidative stress*

Introduction

Interventional studies with dietary antioxidant supplements have often failed to show health benefits [1–3]. One of the flaws in such studies is that they almost invariably failed to determine whether there was indeed any human *in vivo* antioxidant effect of the administered compound or foodstuff and hence they did not really examine the hypothesis that antioxidant effects *in vivo* would produce beneficial health outcomes. In this study we administered a common dietary substance (tomato) thought to have antioxidant activity to healthy volunteers, to determine whether such antioxidant activity can be demonstrated in humans *in vivo*.

Tomato with its rich supply of lycopene is widely thought to improve health [4–6]. It is claimed that lycopene intake as well as serum lycopene levels are inversely related to cancer incidence, particularly prostate [7,8], cervical, ovarian and liver, and cardiovascular diseases [9]. Lycopene is a potent antioxidant in some *in vitro* assays, e.g. it can quench singlet oxygen more efficiently than β-carotene or α-tocopherol [10]. Intervention studies in disease models showed decreased oxidative DNA damage in prostate cancer patients [7,8] and other studies have shown lower levels of oxidized LDL in healthy subjects [11,12] or in type II diabetics [13] after consuming tomato products. Several intervention studies on healthy volunteers using tomato products claimed decreases in plasma TBARS [14] and LDL-TBARS [15], MDA [16], LDL oxidation [17] or oxidative DNA damage [18].

Although several oxidative stress-related biomarkers have been measured in healthy subjects after ingestion of tomato products, many are questionable,

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e.g. TBARS [1]. F_2-isoprostanes (F_2-IsoPs), products of peroxidation of arachidonic acid, are widely thought to be the most reliable marker for assessing *in vivo* oxidative stress related to lipid oxidation provided that measurements are made by mass spectrometric methods [19,20]. Increases in F_2-IsoPs are found in diabetic, CHD, stroke and thalassemia patients [20–24]. In diet intervention studies related to tomato ingestion, a decrease in urinary F_2-IsoPs measured by immunoassay was reported in healthy subjects after 21 days of daily consumption [17]. Arachidonic acid can also be oxidized by free radicals, lipoxygenase and cytochrome P450 enzymes to produce epoxyeicosatrienoic acid products (EETs) or hydroxyeicosatetraenoic acid products (HETEs) [25–27]. Many types of HETE isomers are found such as 5-, 8-, 9-, 11-, 12-, 15-, 20-HETE and some of these isomers have been linked with vascular function and cancer, e.g. 20-HETE is reported to be a vasoconstrictor in the cerebral circulation [28], increased 9-HETE was observed in coronary artery disease [29] and 5-, 8-, 12- and 15-HETE are reported to be involved in tumour development [30].

In this study we investigated the effect of a single dose of tomato sauce on multiple oxidative stress-related biomarkers [23,31] in a group of healthy subjects. Biomarkers related to lipid oxidation and oxidation of DNA and its precursor pool (8O HdG) were examined. We also examined levels of allantoin, a product of ROS attack on uric acid and a putative biomarker of oxidative damage [32].

Materials and methods

Study protocol

The randomized crossover study was conducted in a single centre. After signing the informed consent (Institutional Review Board, National University Hospital, Singapore) the 10 young Chinese male volunteers were randomized in an open-labelled manner to the two treatment sequences. The volunteers were asked to abstain from eating foods rich in lycopene (e.g. tomatoes, watermelons, pink grapefruits, apricots, pink guava and papaya) and other foods containing any form of processed tomato products (e.g. tomato juice, ketchup, paste, sauce or soup) for at least a week before their scheduled study days. The washout interval between the two feeding periods was 2 weeks. One day before the study day, subjects were asked to avoid any strenuous exercise and to fast for at least 8 h prior to the study visit. Subject enrolment was determined by medical history, physical examination, normal laboratory results and BMI (Table I).

Subjects in the placebo group were provided with 300 g of boiled plain rice with 5 ml olive oil (Bertoli, Italy). Subjects in the tomato group were given a single oral dose of 150 g of tomato sauce (Hunts, USA) heated at 100°C in a water bath for 30 min and then mixed with 5 ml olive oil (room temperature) and 150 g of boiled rice (Fragrant Thai Brand, Thailand). Laboratory analysis (described in Lee et al. [33]) indicated that 150 g of tomato sauce contained up to 30 mg of lycopene. This lycopene content was comparable to that used in other studies [34,35]. Heating the sauce for 1 h at 100°C increases the lycopene absorption [36] and lipophilic oil added is suggested to improve absorption of the lycopene from the diet [37]. Lycopene level was low in cooked rice (0.9 mg per 300 g) and olive oil (less than 0.05 mg per 5 ml).

Table I. Baseline status of healthy male volunteers.

Male	10
Age (years)	26 ± 1
BMI	21.1 ± 1.9
Waist-Hip-Ratio	0.86 ± 0.04
Total Cholesterol (mmol/L)	4.3 ± 0.7
Triglyceride (mmol/L)	0.8 ± 0.4
HDL (mmol/L)	1.3 ± 0.3
LDL (mmol/L)	2.6 ± 0.6
Glucose (mmol/L)	4.4 ± 0.3

All values are expressed as mean ± SD.

Sample preparation

Samples of blood and urine of healthy male volunteers were collected in the morning and at 2, 4, 6, 24 and 48 h after ingestion of their meal. Venous blood was collected into Na-EDTA blood tubes that were primed with 15 µl of 5 mM indomethacin dissolved in ethanol. Plasma was separated immediately by centrifugation and then placed into tubes with 20 µl per ml plasma of 2 mM BHT (in ethanol). Both plasma and urine were stored at −80°C and were analysed for plasma F_2-IsoPs, HETEs, allantoin and urate and urinary F_2-IsoPs and 8-hydroxy-2′-deoxyguanosine (8OHdG) within 6 months from sample collection.

Extraction and analysis of carotenoids

Plasma samples were prepared as described in Lee et al. [33]. In brief, in the presence of internal standard (lutein, zeaxanthin, β-cryptoxanthin, lycopene α- and β-carotene 0.2 mg/l), plasma samples were initially deproteinized and then extracted by organic solvent. Thereafter the extracted components were analysed by high pressure liquid chromatography (HPLC). Chromatographic separation was achieved by isocratic elution and detected by photodiode array. Data acquisition and peak purity tests were performed with Waters Empower software.

Extraction and analysis of F_2-IsoPs and HETEs

Before analysis, the plasma samples were thawed at room temperature. Mixed heavy isotopes of F_2-IsoPs and HETEs all prepared in ethanol were added to

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plasma and mixed. To measure [31] the total (free + esterified) form of oxidized lipids (F_2-IsoPs and HETEs) and total arachidonate, 1 ml plasma was hydrolysed at 37°C for 30 min with 1 ml of 1 M potassium hydroxide prepared in methanol for the release of esterified lipids. Afterwards, 0.5 ml methanol, 0.2 ml of 5 M HCl and 2.5 ml of 40 mM formic acid (pH 4.6) were further added and mixed. For measurement of free forms in plasma and urine [23,31] for F_2-IsoPs and in plasma for HETEs, 1 ml of formic acid (40 mM, pH 4.5) was added to 1 ml of sample, mixed and then immediately processed by SPE. For standardizing the dilution of urine, creatinine levels were measured using the Sigma (USA) diagnostic kit.

The prepared samples were extracted using anionic solid phase extraction (SPE) and derivatized [31] for gas chromatography-mass spectrometry (GC-MS) analysis. F_2-IsoPs, HETEs and arachidonate were analysed by a mass selective detector (Hewlett-Packard 5973N, Agilent Technologies, USA) connected to a gas chromatograph (Hewlett-Packard 6890, Agilent Technologies, USA), fitted with an automatic sampler and a computer workstation [31]. The mass spectrometer was used in the negative chemical ionization (NCI) mode set at selective ion monitoring (SIM) and chromatographic separations were carried out on a fused silica capillary column coated with cross-linked 5% phenylmethylsiloxane (HP-5, Agilent Technologies, USA). Quantitation was achieved by relating the peak area of the total and free forms of F_2-IsoPs or HETEs and total arachidonate with their respective deuterated internal standard peaks [31].

Extraction and analysis of 8OHdG

To the urine sample, internal standard [$^{15}N_5$] 8OHdG (50 pmol) was added and then acidified with formic acid (10%) [38]. Thereafter, the prepared samples were cleaned by SPE using HLB Vac cartridges (Waters, USA) and then freeze-dried for derivatization for GC-MS analysis. Separations were carried out on a fused silica capillary column (30 m) coated with cross-linked 5% phenylmethylsiloxane (Ultra 2, Agilent, J&W, USA) and the temperature settings were programmed according to Lin et al. [38]. The detector was set at electron ionization (EI) mode and measurement was performed by SIM. Quantification of 8OHdG was calculated by comparing the peak area of each compound with the internal standard.

Extraction and analysis of allantoin and urate

These were measured as described by Gruber et al. [32]. Briefly, 25 μl plasma was centrifuged using Nanosep filter (10 kDa) and to the filtrate, 25 μl of 4 μM ^{15}N allantoin and 100 μl acetonitrile were added, mixed and then dried under nitrogen gas. The dried sample was then derivatised with 50 μl N-(butyl-dimethyl-silyl)-2,2,2-trifluoro-N-methyl-acetamide (MTBSTFA) in pyridine (1:1 v/v) at 50°C for 2 h. Allantoin was analysed by GC-MS. Separations were carried out on a fused silica capillary column (12 m × 0.2 mm i.d.) coated with cross-linked 5% phenylmethylsiloxane (film thickness 0.33 μm) (Ultra2, Agilent, J&W). Derivatized allantoin samples (1 μl) were injected into the GC injection port (100°C). Column temperature was increased from 100°C to 150°C at 40°C/min, then 150°C to 198°C at 4°C/min and then to 300°C and held for 1 min. Allantoin was monitored by selected ion monitoring using m/z 398 as target ion and m/z 400 for internal standard. Quantification of allantoin was calculated by comparison with the heavy isotope.

For urate analysis, 80 μl water was added to 20 μl plasma, mixed well and then centrifuged using Nanosep filters (10 kDa). The filtrate was then injected into an HPLC connected to a UV detector set at 293 nm (Agilent Technologies, USA). For calibration, plasma samples were prepared by adding 250 μM and 500 μM pure uric acid. Chromatographic separation was achieved using 250 mm Zorbax SB-C8 (5 μm) columns under isocratic condition where 2 mM $NH_4H_2PO_4$ (pH 2.95) was used for the mobile phase. The area of the eluted uric acid peak was measured and the concentration was determined against the linear calibration curve constructed with the spiked samples.

Statistics

Statistical analysis was performed using by GraphPad Prism version 5.0 for Macintosh (GraphPad Software, CA). All values are expressed as mean ± SD. Paired Student's *t*-test was performed between post-dose time points and 0 h. Any significant changes found by Student's *t*-test were confirmed by two-way ANOVA (mixed model) with repeated measures for the effect of tomato sauce and placebo meals over time and the values were also corrected with the baseline values. Significance of area under curve (AUC) was tested for the change of plasma lycopene concentration over time course after tomato feed. $p < 0.05$ was taken as significant.

Results

All volunteers (young males) enrolled into the study fulfilled the entry criteria and all completed the study (Table I). The baseline levels (0 h) of the plasma carotenoids were similar between the two feeding periods (Table II). Both placebo and tomato sauce meal showed no effect on plasma lutein, zeaxanthin, β-cryptoxanthin, α-carotene and β-carotene (Table II). As shown in Figure 1, plasma lycopene levels

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Table II. Concentration of plasma carotenoids after placebo and tomato sauce meal.

		Time (h)					
		0	2	4	6	24	48
Lutein (μg/ml)	Placebo	0.22 ± 0.06	0.24 ± 0.07	0.20 ± 0.05	0.25 ± 0.10	0.22 ± 0.10	0.22 ± 0.07
	Tomato	0.28 ± 0.15	0.26 ± 0.09	0.25 ± 0.13	0.23 ± 0.09	0.25 ± 0.14	0.22 ± 0.07
Zeaxanthin (μg/ml)	Placebo	0.07 ± 0.05	0.08 ± 0.06	0.06 ± 0.03	0.10 ± 0.07	0.06 ± 0.03	0.08 ± 0.07
	Tomato	0.08 ± 0.05	0.07 ± 0.04	0.08 ± 0.05	0.07 ± 0.05	0.06 ± 0.04	0.06 ± 0.03
β-Cryptoxanthin (μg/ml)	Placebo	0.10 ± 0.03	0.11 ± 0.05	0.10 ± 0.03	0.11 ± 0.04	0.11 ± 0.04	0.11 ± 0.04
	Tomato	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 0.03	0.11 ± 003	0.11 ± 0.03
α-Carotene (μg/ml)	Placebo	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.02	0.02 ± 0.01	0.02 ± 0.02	0.02 ± 0.02
	Tomato	0.02 ± 0.03	0.02 ± 0.03	0.02 ± 0.03	0.02 ± 0.03	0.02 ± 0.03	0.02 ± 0.03
β-Carotene (μg/ml)	Placebo	0.19 ± 0.12	0.19 ± 0.14	0.21 ± 0.13	0.19 ± 0.15	0.21 ± 0.14	0.19 ± 0.14
	Tomato	0.23 ± 0.15	0.22 ± 0.15	0.23 ± 0.16	0.23 ± 0.16	0.23 ± 0.13	0.23 ± 0.15

All values are expressed as mean ± SD. No significant changes were observed. 0 h indicates plasma concentration before consumption of meal.

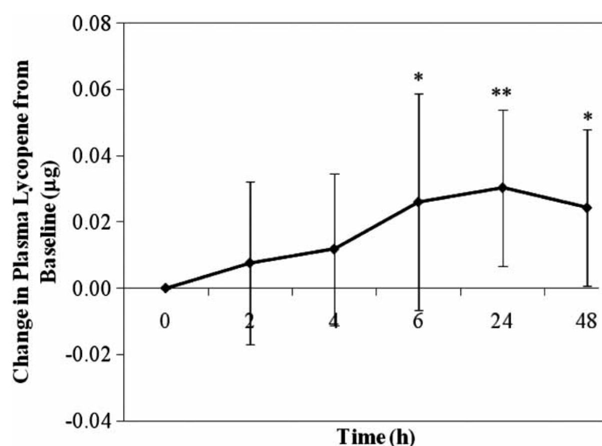

Figure 1. Change in lycopene concentration from baseline (0 h) after consumption of tomato sauce. All values are expressed as mean ± SD. *p < 0.05 and **p < 0.01 AUC.

significantly increased from 6 h ($p < 0.05$) post-tomato sauce feeding, reached a maximum at 24 h ($p < 0.01$) and began to decrease by 48 h. No change in plasma lycopene was found after placebo meal.

The baseline (0 h) levels for all F_2-IsoPs and HETEs, 8OHdG, allantoin and urate were not different between the two feeding periods (Tables II– V).

Plasma free F_2-IsoPs did not show any significant change after placebo or tomato sauce meal. Total and esterified F_2-IsoPs increased 4 and 6 h after placebo or tomato sauce meal (Table III) and decreased after 24 h; the rate of decrease was greater ($p < 0.05$) after tomato sauce meal than placebo meal (18% vs 4%) from 6 h to 24 h. However, no significant change from 0 h was found when total and esterified F_2-IsoPs levels were standardized with arachidonate. Levels of plasma arachidonate did not

Table III. Concentration of F_2-IsoPs and arachidonate in plasma and urinary F_2-IsoPs and 8-hydroxy-2′-deoxyguanosine and after placebo and tomato sauce meal.

		Time (h)					
		0	2	4	6	24	48
Free F_2-IsoPs (ng/ml)	Placebo	0.06 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02	0.06 ± 0.02
	Tomato	0.05 ± 0.02	0.05 ± 0.02	0.05 ± 0.02	0.06 ± 0.02	0.05 ± 0.02	0.05 ± 0.02
Esterified F_2-IsoPs (ng/ml)	Placebo	0.52 ± 0.24	0.58 ± 0.31	0.61 ± 0.27*	0.64 ± 0.32*	0.60 ± 0.15	0.56 ± 0.25
	Tomato	0.43 ± 0.17	0.55 ± 0.32	0.53 ± 0.27*	0.61 ± 0.27**	0.50 ± 0.20+	0.53 ± 0.18
Total F_2-IsoPs (ng/ml)	Placebo	0.58 ± 0.25	0.63 ± 0.32	0.66 ± 0.29*	0.70 ± 0.33*	0.67 ± 0.15	0.62 ± 0.27
	Tomato	0.48 ± 0.19	0.60 ± 0.33	0.60 ± 0.28*	0.67 ± 0.28**	0.55 ± 0.21+	0.57 ± 0.20
Arachidonate (ng/ml)	Placebo	88 ± 13	92 ± 12	94 ± 14	91 ± 9	91 ± 13	90 ± 15
	Tomato	83 ± 28	89 ± 26	88 ± 24	92 ± 25	96 ± 28*	93 ± 30
Esterified F_2-IsoPs/AA (pg/mg)	Placebo	6.2 ± 3.7	6.7 ± 4.4	6.7 ± 3.6	7.0 ± 3.9	6.7 ± 2.0	6.4 ± 3.4
	Tomato	6.0 ± 2.8	6.4 ± 3.2	6.1 ± 2.4	6.9 ± 2.1	5.7 ± 2.0	5.9 ± 2.3
Total F_2-IsoPs/AA (pg/mg)	Placebo	6.8 ± 3.8	7.3 ± 4.6	7.3 ± 3.8	7.7 ± 4.0	7.4 ± 2.1	7.2 ± 3.6
	Tomato	6.6 ± 2.9	7.0 ± 3.2	6.8 ± 2.5	7.5 ± 2.2	6.3 ± 2.1	6.4 ± 2.4
Urinary F_2-IsoPs (ng/mg Cr)	Placebo	1.29 ± 0.78	1.25 ± 0.54	1.33 ± 0.60	1.30 ± 0.73	0.87 ± 0.41	0.84 ± 0.57*
	Tomato	1.14 ± 0.86	1.55 ± 0.78	1.29 ± 0.79	1.38 ± 1.02	0.92 ± 0.53	0.55 ± 0.15* ++
Urinary 8-OHdG (μM/M Cr)	Placebo	1.6 ± 0.6	1.7 ± 0.6	1.4 ± 0.5	1.5 ± 0.5	1.6 ± 0.4	1.8 ± 0.6
	Tomato	2.0 ± 0.6	2.1 ± 1.0	1.9 ± 0.9	1.7 ± 0.6	1.9 ± 0.4	1.5 ± 0.7

All values are expressed as mean ± SD. 0 h: plasma concentration before consumption of meal; AA: Arachidonate.
*p < 0.05 0 h vs respective time; **p < 0.01 0 h vs respective time. +p < 0.05 24 h placebo vs 24 h tomato; ++p < 0.01 48 h placebo vs 48 h tomato.

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Table IV. Concentration of plasma HETEs after placebo and tomato sauce meal.

		Time (h)					
		0	2	4	6	24	48
Free HETEs (ng/ml)	Placebo	3.1±2.2	3.4±2.5	2.6±2.0	4.4±2.5	3.9±2.0	2.9±1.8
	Tomato	2.2±1.6	1.7±0.9	1.9±1.2	3.2±1.9	2.1±0.9	1.4±1.4
Esterified HETEs (ng/ml)	Placebo	12.9±6.6	14.7±5.5	11.3±5.4	10.3±3.9	8.4±3.1	9.6±3.7
	Tomato	11.3±4.6	12.0±4.1	11.7±4.3	11.5±3.7	11.0±5.1	14.4±6.9
Total HETEs (ng/ml)	Placebo	16.1±5.3	18.1±4.5	13.9±5.0	14.6±3.3	12.3±2.6	12.4±3.1
	Tomato	13.6±4.1	13.7±4.1	13.6±4.5	14.7±3.9	13.1±5.1	15.8±7.0
Esterified HETEs/AA (ng/mg)	Placebo	0.15±0.09	0.17±0.08	0.13±0.08	0.11±0.04	0.09±0.03	0.11±0.05
	Tomato	0.16±0.10	0.14±0.06	0.14±0.05	0.13±0.04	0.12±0.06	0.17±0.11
Total HETEs/AA (ng/mg)	Placebo	0.19±0.08	0.20±0.07	0.15±0.07	0.16±0.04	0.13±0.03	0.14±0.04
	Tomato	0.19±0.10	0.16±0.06	0.16±0.05	0.16±0.05	0.14±0.07	0.19±0.11

All values are expressed as mean±SD. No significant changes were observed. 0 h: plasma concentration before consumption of meal. AA: Arachidonate.

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change after placebo meal, whereas they tended to increase with the tomato meal and the increase was significant ($p < 0.05$) at 24 h after the tomato meal (Table III).

A significant decrease ($p < 0.05$) in urinary F_2-IsoPs was found after placebo meal or tomato sauce meal at 48 h compared to 0 h, but the degree of decrease was significantly greater ($p < 0.01$) after tomato sauce meal than placebo meal (57% vs 29%) (Table III). Our study showed no effect on urinary 8OHdG of the volunteers after placebo or tomato meals (Table III). Levels of free, esterified, total HETEs and total HETEs/arachidonate did not change over the study period after placebo or tomato sauce meal (Table IV).

Measurement of plasma allantoin, an oxidation product of uric acid, showed no change after placebo or tomato sauce meal (Table V). Plasma urate levels did not change after placebo meal, but increased significantly ($p < 0.01$) at 48 h compared to 0 h after tomato sauce meal.

Discussion

Several studies have been conducted on tomato products and oxidative stress, yet many are inconclusive. Even though our study involves only a single dose of tomato sauce, it indicated an increase in lycopene that was maximal at 24 h, consistent with a previous report [36]. Nevertheless, our findings did not indicate a strong antioxidant effect *in vivo* in healthy young male subjects after a tomato sauce meal.

Mixed reports are available on the level of F_2-IsoPs in healthy volunteers where they were lower in urine [17] or unaffected in the circulation [39,40] after consumption of tomato products. It may be that a longer feeding period is required to show an effect, since both the studies reported [17,40] provided tomato meals for 21 days. Our data indicate the need for feeding controls, since both the placebo diet and the tomato sauce meal produced changes in plasma F_2-IsoPs, an effect observed previously in a study with soy sauce [41]. However the drop in urinary F_2-IsoPs was significantly greater with tomato sauce than placebo meal.

Several reports are available on the effect of tomato products *in vivo* on the levels of damage adducts, on the levels of DNA damage adducts, namely 8-hydroxy-2′deoxyguanosine (8OHdG). DNA damage by free radicals is associated with progression of carcinogenesis [42] and lycopene in tomato products is hypothesized to lower DNA damage in prostate cancer patients [7,8,39,43]. In healthy volunteers, consumption of tomato products enhances the protection of DNA damage in the lymphocytes [18,44]. However tomato product consumption has been reported not to show any effect in urinary 8OHdG

Table V. Concentration of plasma allantoin and urate after placebo and tomato sauce meal.

		Time (h)					
		0	2	4	6	24	48
Allantoin (μM)	Placebo	1.5±0.3	1.9±0.6	1.8±0.4	1.6±0.5	1.6±0.5	1.7±0.6
	Tomato	1.7±0.6	2.1±0.5	2.2±0.6	1.8±0.4	1.7±0.4	1.7±0.5
Urate (μM)	Placebo	399±83	371±65	369±108	359±70	359±70	393±122
	Tomato	348±55	386±96	341±89	386±61	375±53	394±40**

All values are expressed as mean±SD. 0 h: plasma concentration before consumption of meal.
**$p < 0.01$ 0 h vs time point.

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in healthy volunteers in Thomson et al. [40], as also found in our study.

Urate is considered to be a reactive oxygen species (ROS) scavenger [1]. In the process of scavenging ROS, urate degrades to produce allantoin, augmented levels of which have been measured in ROS-related diseases such as Wilson's, Down's syndrome and haemochromatosis [1]. Our results showed no change in allantoin after the tomato sauce meal.

In summary, our study indicated that a single tomato sauce meal showed only weak antioxidant effects (a small but significant drop in urinary F_2-IsoPs but no significant change in plasma allantoin, HETEs or F_2-IsoPs or urinary 8OHdG) in healthy male volunteers. In order to ascertain whether there is a potential antioxidative effect longer periods of feeding may be required, with appropriate feeding controls. Thus, a rise in plasma lycopene *per se* does not necessarily equate to increased antioxidant effects *in vivo*.

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