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Randomized control trials

Tomato-based randomized controlled trial in prostate cancer patients: Effect on PSA

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SUMMARY

Background & aims: The effect of lycopene-containing foods in prostate cancer development remains undetermined. We tested whether a lycopene-rich tomato intervention could reduce the levels of prostate specific antigen (PSA) in prostate cancer patients.

Methods: Prior to their curative treatment, 79 patients with prostate cancer were randomized to a nutritional intervention with either 1) tomato products containing 30 mg lycopene per day; 2) tomato products plus selenium, omega-3 fatty acids, soy isoflavones, grape/pomegranate juice, and green/black tea (tomato-plus); or 3) control diet for 3 weeks.

Results: The main analysis, which included patients in all risk categories, did not reveal differences in changes of PSA-values between the intervention and control groups. Post-hoc, exploratory analyses within intermediate risk (n = 41) patients based on tumor classification and Gleason score post-surgery, revealed that median PSA decreased significantly in the tomato group as compared to controls (−2.9% and +6.5% respectively, p = 0.016). In separate post-hoc analyses, we observed that median PSA-values decreased by 1% in patients with the highest increases in plasma lycopene, selenium and C20:5 n-3 fatty acid, compared to an 8.5% increase in the patients with the lowest increase in lycopene, selenium and C20:5 n-3 fatty acid (p = 0.003). Also, PSA decreased in patients with the highest increase in lycopene alone (p = 0.009).

Conclusions: Three week nutritional interventions with tomato-products alone or in combination with selenium and n-3 fatty acids lower PSA in patients with non-metastatic prostate cancer. Our observation suggests that the effect may depend on both aggressiveness of the disease and the blood levels of lycopene, selenium and omega-3 fatty acids.

Abbreviations: C20:5 n-3, Eicosapentaenoic acid; FAME, Fatty acid methyl ester; FFQ, Food Frequency Questionnaire; HPLC, High Performance Liquid Chromatography; ICP-MS, Inductively Coupled Plasma Mass Spectrometry; PUFA, poly-unsaturated fatty acids; PSA, prostate specific antigen; RCT, randomized controlled trial; SHBG, sex hormone-binding globulin; SNP, Single Nucleotide Polymorphism; WCRF, World Cancer Research Fund.

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1. Introduction

Prostate cancer is the second most common cancer in men in the world with nearly 900 000 new cases diagnosed each year [1]. The incidence and mortality of prostate cancer show large geographical differences [2]. The epidemiological patterns suggest that lifestyle and dietary factors impact the occurrence of prostate cancer. Tomatoes have been identified as one possible candidate for reducing the risk of prostate cancer with lycopene as the major potential active component [3]. While the Second Expert Report by the World Cancer Research Fund (WCRF) [4] concluded that lycopene containing foods (mainly tomatoes) probably protect against prostate cancer, the recently released Continuous Update Project Report from WCRF states that no conclusion was possible due to limited evidence [5].

Prostate specific antigen (PSA) is an important biomarker used in clinical risk assessments, follow-ups and as part of risk stratification of prostate cancers patients [6,7]. The effect of tomatoes or lycopene-containing foods on PSA values in patients with established prostate cancer has not been well documented. In an uncontrolled trial, a tomato sauce intervention for 3 weeks reduced PSA in a subgroup of prostate cancer patients [8], while lycopene supplements or extracts seemed to be less effective in reducing PSA [9–11]. However, the lycopene contents of tomato-based foods, as well as lycopene bioavailability varies considerably [12] and may impact the efficacy of tomato-based interventions.

Several other foods or food components have also been suggested to protect against prostate cancers [5]. The WCRF concludes that there is still limited evidence for the association between prostate cancer and these dietary components. Thus a second intervention arm with selenium, omega-3 fatty acids, soy isoflavones, grapes, pomegranates, tea and tomato-products was included in our study to investigate possible additive effects on PSA.

The present 3-arm randomized controlled trial (RCT), aimed to test whether a tomato based lycopene-rich diet changed the kinetics of PSA in patients with non-metastatic prostate cancer during the three weeks period preceding the patients' curative treatment. Furthermore, we performed two sets of exploratory post-hoc analyses. First, we hypothesized that a lycopene-rich diet during this period leads to a more favorable PSA profile in intermediate risk patients as compared to a matched control group. Secondly, we tested whether the effects on PSA depend on bioavailability of the active treatment components.

2. Subjects and methods

2.1. Ethics statement

This study was approved by the regional ethics committee in Norway (REK Sør, no. S-06187). The study is registered in ClinicalTrials.gov with no. NCT00433797. All participants signed a letter of informed consent.

2.2. Subjects and study design

Subjects for this parallel group RCT were recruited from two clinical centers within the Oslo University Hospital, Oslo, Norway; the Norwegian Radium Hospital and Aker University Hospital between June of 2007 and March of 2012. Patients diagnosed with non-metastatic prostate cancer (N0 and M0 as confirmed by negative chest X-ray, bone scintigraphy and pelvic MRI or CT), and scheduled for either radical prostatectomy or high-dose radiotherapy consisting of a combination of high-dose rate brachytherapy and pelvic external beam radiotherapy [13] were considered eligible. Patients were invited to participate in the study by their counseling urologists, oncologist or study nurses. Exclusion criteria: White blood cells outside normal reference window; Hb < 11 g/dL; prior endocrine treatment; <5 year life-expectancy; ECOG score >1; incontinence/urinary retention; critical comorbidity (e.g. cardiovascular disease, chronic obstructive pulmonary disease, insulin dependent diabetes mellitus, vasculitis, inflammatory bowel syndrome or other conditions which could influence radiation therapy).

The risk classification of the prostate cancer patients includes PSA, pT-staging and Gleason score. Patients with low or intermediate risk according the D'Amico risk classification [14] were considered eligible for the study. In addition, 13 patients with Gleason score 8 and 9, pT3a-stage or PSA ≥20 μg/L (provided that they fulfilled all other inclusion and exclusion criteria) were also considered eligible after individual evaluation by their oncologist/urologist.

After surgery, the prostatectomy specimens enable new and more precise tumor description [15]. Taking into account this post-surgery information, we defined an adjusted/alternative risk classification for prostatectomized patients in part following the 2013 European guidelines [16]. This adjusted risk classification was based on pT category and the Gleason score in the prostatectomy specimen and included the pre-intervention PSA. Three risk groups emerged among the study group: Low risk (pT1c-pT2a, and PSA < 10 μg/L, and, Gleason score ≤ 6); Intermediate risk (pT2b-pT2c, and/or 10 μg/L ≤ PSA <20 μg/L, and/or Gleason score 7); or High risk (pT3, and/or ≥ PSA 20 μg/L, and/or Gleason score 8–10).

2.3. Randomization and blinding

At inclusion, patients were randomized to one of three arms; a control group, a tomato group, and a tomato-plus group. Randomization was computer generated real-time by the "Department of Clinical Research Support" at the Oslo University Hospital at time of inclusion. By assigning single digit numbers to the interventions, randomization was blinded for the investigators until after initial statistical analyses were performed.

2.4. Blood samples and handling

All blood samples were collected at the hospitals during routine clinical visits. Blood samples were drawn by venipuncture before the start of the diet-intervention, and at the end of the diet-intervention (i.e. shortly before surgery/radiotherapy). For plasma samples, blood samples (standard heparin, EDTA and citrate tubes) were centrifuged (1500*g* for 10 min) at 4 °C. Red blood cells were collected from centrifuged citrate blood samples after the collection of citrate plasma and removal of the buffy coat. All samples were stored at −70 °C until analysis.

2.5. Interventions

The tomato intervention included tomato products with a content of 30 mg lycopene per day (see Supplementary Table 1 for details). In addition to the same amount of tomato products, the tomato-plus intervention also included green tea (a cup made from 1 sachet) and black tea (a cup made from 1 sachet), pomegranate- and grape juice (330 mL of each), 200 mg soy isoflavones, 200 μg 1-selenomethionin and 3.13 g n-3 fatty acids per day (for details and producers see Supplementary Table 2). The patients in the control group were encouraged to continue their habitual diet.

In order to select tomato products to be included in the study, we measured lycopene contents (experimental procedure below) in 170 tomato products commercially available in Norway (Supplementary Table 3). This screening revealed large differences

(0.2–55.7 mg/100 g) in the lycopene content of the tomato products. Six products were selected for the study based on their high contents of lycopene per portion size (Supplementary Table 1). Participants were provided with tomato products for 25 days and were free to choose which of these products to consume, however the total dose was adjusted to give ~30 mg of lycopene per day.

Self-reported compliance to the two interventions was registered by the participants in provided compliance questionnaire. In both interventions combined, 85% of the patients completed all 21 days of their respective interventions, and the median (range) of intervention days were 21.5 (12.0, 24.0) for the tomato group, and 22.0 (20.0, 24.0) for the tomato-plus group. One patient in the tomato group completed only 12 days of intervention due to surgery on day 13. Otherwise all patients completed at least 18 days of intervention.

One patient in the tomato-plus group discontinued the intake of fish oil supplement only, due to regurgitation. No other side effects were reported.

2.6. Food and nutrient intakes

A validated food frequency questionnaire was used to assess habitual food and nutrient intakes prior to the intervention [17,18]. There were no differences in the intakes of energy, tomatoes, tea, grapes, pomegranate, tofu (soy), selenium, cod liver oil, omega-3- or other dietary supplements between the intervention groups (data not shown).

2.7. Lycopene in tomatoes and tomato products

All products were purchased from large grocery stores in the Oslo and Akershus area in Norway. When possible, at least three samples per product were purchased from three different stores. All products were stored as recommended by the producer. The contents of lycopene in the tomatoes and tomato products was measured using High Performance Liquid Chromatography (HPLC) as described in [19].

3. Clinical biochemical measurements

3.1. Prostate specific antigen

Levels of total PSA in heparin plasma were measured at the Dept. of Medical Biochemistry, Oslo University Hospital using the DELFIA PSA Free/Total kit on the AutoDELFIA automatic immunoassay system (Perkin Elmer, Boston, MA, USA). Samples were thawed and mixed, and analyzed in duplicates. To minimize analytical variation, all samples in the study were analyzed in one run. Three serum controls with PSA concentrations of 0.17, 3.8 and 18.1 μg/L (CV% of 7.8, 3.5 and 3.7, respectively) were analyzed at the start and end of every plate.

3.2. Carotenoids

Plasma carotenoids were detected using HPLC by Vitas (Oslo, Norway). In dark-colored vials, proteins were precipitated from 25 μL plasma and carotenoids extracted with isopropanol added internal standard (β-Apo-8-carotenal). The sample was mixed thoroughly, and thereafter centrifuged. Subsequently, an aliquot of the isopropanol phase was injected into an 1100-series HPLC with a 1260 diode array detector (453 nm) (Agilent Technologies, Palo Alto, CA). Separation was performed on a 3 μm column (YMC C30 (150 mm × 4.6 mm i.d.)) (YMC, Japan).

3.3. Fatty acids in red blood cells

Polyunsaturated fatty acids in red blood cells were markers for bioavailability and were measured by as Vitas (Oslo, Norway) using gas chromatography with flame ionization detector. Samples were methylated with 3N MeOH HCl. Fatty acid methyl esters (FAMEs) were extracted with hexane, and samples were neutralized with 3N KOH in water. After mixing and centrifuging the hexane phase was injected into the gas chromatography with flame ionization detector. Analyses were performed on a 7890A GC with a split/splitless injector, a 7683B automatic liquid sampler, and flame ionization detection (Agilent Technologies, Palo Alto, CA). Separations were performed on a SP-2380 (30 m × 0.25 mm i.d. × 0.25 μm film thickness) column from Supelco.

3.4. Selenium

Plasma selenium was measured by Fürst Medical Laboratory (Oslo, Norway) using a PerkinElmer Sciex, Elan® DRC™ II (Shelton, USA) Inductively Coupled Plasma Mass Spectrometry (ICP-MS) instrument was used. The 82Se isotope was measured in standard mode. External calibration was used and the standard was matched with sample-matrix by adding Selenium PerkinElmer Pure Atomic Spectroscopy Calibration Standard, Matrix 2% HNO3, 1000 μg/mL (Shelton, USA) to Autonorm™ (Billingstad, Norway). Samples, standard and quality controls were diluted 1:20 with Milli-Q™ deionized water (Millipore, Bedford, MA, USA) with 0.1% (vol:vol) Nitric acid (65% m/v, Suprapur®, Merck, Darmstadt, Germany) and 0.5% (vol:vol) 1-Butanol (74.12 g/mol pro analysis, Merck, Darmstadt, Germany). 10 μg/L Rhodium PerkinElmer Pure Atomic Spectroscopy Calibration Standard, Matrix 10% HCl, 1000 μg/mL (Shelton, USA) was added directly to the diluent and was used as an internal standard.

3.5. Hormones

Routine biochemical measures of testosterone and sex hormone-binding globulin (SHBG) were performed at the Department of Medical Biochemistry, Oslo University Hospital.

3.6. Power calculation

Power calculation was performed based on an anticipated change in mean PSA value in the tomato group or the tomato-plus group. The estimated effect size was set to a 20% decrease from the values in the control group. With a standard deviation of 3.5 μg/L, a power of 0.8 and a significance level of $\leq$0.05), an estimated 28 subjects in each group and 84 subjects in total, were needed. With an estimated 70% rate of initial consent and 20% drop out rate, a total of 160 patients were invited to take part in the study, of which 86 accepted and 79 completed the study (Fig. 1). The final initial consent rate was 54%, while the final drop out rate was 8%.

3.7. Statistical analysis

Primary endpoints were changes in PSA. Secondary endpoints were measures of plasma concentrations of carotenoids and selenium and RBC concentrations of fatty acids. The number of samples analyzed per endpoint differs slightly since some samples were not available for analysis.

Pre-intervention values and PSA changes during the intervention were not normally distributed and were thus tested statistically using Kruskal Wallis test and Mann–Whitney test. Fisher–Freeman–Halton test was used to identify statistical differences in categorical data and contingency tables. For tests of

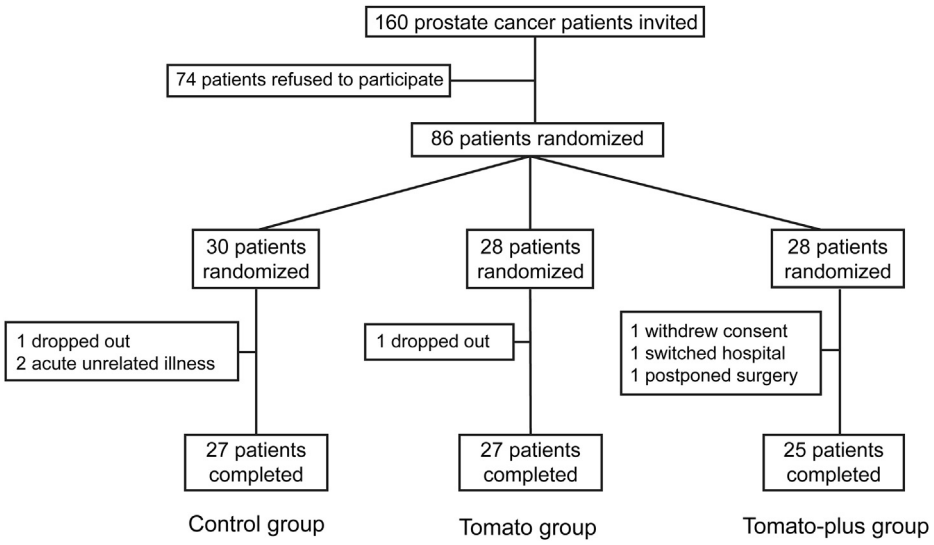

Fig. 1. Study design and patient flow.

correlation, Spearman's Correlation Coefficient was calculated. These statistical analyses were performed using IBM SPSS statistical software version 20 (SPSS Inc., Chicago, IL, USA).

4. Results

In this RCT, 86 men with localized prostate cancer were allocated to either tomato-, "tomato-plus-" or a control-group, and 79 subjects completed the trial (Fig. 1). Patient characteristics are presented in Table 1.

The main analyses, which included all patients, did not reveal differences in changes of PSA-values between the intervention and control groups (Table 2).

Next, we proceeded with two sets of exploratory post-hoc analyses. First we analyzed our data according to an alternative post-surgery risk classification (see Methods). As compared to the risk classification at the time of diagnosis, twenty four patients shifted from clinical low- or intermediate risk to our ad-hoc defined high risk due to changes in T-staging and/or Gleason score. Thus, of the 79 patients that completed the trial, 41 patients were reclassified as intermediate risk, while 1 and 37 patients were reclassified as low and high risk, respectively.

In those reclassified as intermediate risk patients based on our post-surgery risk group classification, the tomato based interventions significantly affected changes in PSA ($p = 0.041$) during the intervention (Table 3). Interestingly, median PSA decreased by 0.23 μg/mL in the tomato group as compared to a 0.45 μg/L increase in the control group ($p = 0.016$) during the three week intervention. Similar, however, non-significant change was observed in tomato-plus group ($p = 0.094$). For both intervention groups combined ($n = 27$), PSA was not changed (median change: 0.0 μg/L) for patients with intermediate risk, which was significantly different from the expected increase in the control group ($p = 0.014$ by Mann–Whitney Test).

Table 1
Patient characteristics prior to the intervention.

	Control (n = 27) Median (range)	Tomato (n = 27) Median (range)	Tomato-plus (n = 25) Median (range)	P-value
Age (y) [a]	64 (51, 74)	62.5 (48, 72)	64 (54, 75)	0.277
PSA (μg/L) [a]	9.3 (4.4, 55.0)	8.54 (1.5, 25.9)	10.6 (5.1, 31.5)	0.280
Treatment (Surgery/Radiotherapy) [b]	22/5	25/2	23/2	0.490
Pre-treatment risk classification [b] (Low/Intermediate/High)	6/17/4	7/18/2	2/16/7	0.220
BMI (kg/m^2) [a]	26.4 (22.4, 31.7)	25.5 (18.4, 33.5)	26.4 (20.4, 48.2)	0.197
Testosterone (nmol/L) [a]	13.6 (4.2, 28.1)	14.1 (6.6, 21.8)	13.4 (4.6, 34.4)	0.755
SHBG (nmol/L) [a]	44 (25, 67)	40 (19, 62)	38 (21, 106)	0.420
Free testosterone index [a] ((testosterone/SHBG)*100)	33.2 (8.1, 49.3)	35.7 (21.1, 54.4)	32.4 (19.2, 65.2)	0.356
Gleason score (6/7/8/9)[b]	(10/15/1/0)[c]	(13/13/0/1)	(16/8/1/0)	0.252

[a] Kruskal–Wallis tests was used to detect differences between groups.
[b] Counts are compared statistically using the Fisher–Freeman–Halton Test.
[c] One missing Gleason score prior to surgery.

Table 2
Baseline PSA values and changes during the intervention.

	Control n = 24 Median (range)		Tomato n = 26 Median (range)		Tomato-plus n = 25 Median (range)		P-values[a]	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
PSA [μg/L]	9.34 (4.42, 55.0)	0.41 (−8.53, 4.0)	8.54 (1.52, 25.90)	0.00 (−3.30, 2.40)	10.60 (5.10, 31.50)	0.14 (−12.40, 4.80)	0.280	0.416

[a] Kruskal–Wallis tests were used to detect differences between groups.

Table 3
Baseline PSA values and changes during intervention for post-surgery classified intermediate risk patients.

	Control (n = 13) Median (range)		Tomato (n = 17) Median (range)		Tomato-plus (n = 10) Median (range)		P-values[a]	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
PSA [μg/L]	6.91 (4.42,17.70)	0.45 (−0.26, 2.24)	7.98 (1.52, 18.00)	**−0.23** (−1.12, 1.90)	7.13 (5.10, 12.50)	**0.28** (−0.78, 1.20)	0.880	0.041[b,c]

Bold characters identify statistically significant differences.
[a] Kruskal–Wallis tests were used to detect differences between groups.
[b] Mann–Whitney Test p = 0.016 for difference control vs tomato.
[c] Mann–Whitney Test p = 0.094 for difference control vs tomato-plus.

In patients reclassified as high risk patients based on prostatectomy specimens (n = 37), no significant differences in any PSA measures were detected (data not shown).

The initial analyses between treatment groups did not take into account the response to the intervention in terms of changes in blood concentration for the intervention component (as a measure of compliance and absorption). Self-reported compliance (i.e. amount of products consumed) was 99% for the tomato intervention and 96–99% for the tomato-plus group. Thus, as per protocol, the potential effects of variation in response to the intervention were taken into account in the following post-hoc analysis.

To test the response to the interventions, we measured lycopene and selenium in plasma, and poly-unsaturated fatty acids (PUFAs) in red blood cells. Overall, lycopene in plasma was more than doubled in both intervention groups, and these changes were significantly different from controls for both interventions (tomato $p < 0.001$, tomato-plus $p < 0.001$) (Table 4). Plasma selenium values nearly doubled in the tomato-plus group during the intervention period, whereas no changes were detected in either the control- or the tomato-groups ($p < 0.001$ between the tomato-plus and the control group) (Table 4). The fatty acid profile of red blood cells reflects the dietary intake of fatty acids [20]. The level of n-3 PUFAs (C20:5 n-3, C22:5 n-3 and C22:6 n-3) all increased in the subjects in the tomato-plus group, as compared to the controls (Table 4) ($p < 0.001$ for all). A simultaneous decrease in several n-6 and n-9 fatty acids in the tomato-plus group (Table 4) confirms bioavailability of the supplement, and a shift in fatty acid profile in the tomato-plus subjects.

The absorption of active substances may vary considerably between individuals, and thus we also investigated the individual changes in lycopene, selenium and C20:5 n-3 (Fig. 2). For lycopene, there was a strikingly wide variation in the increases in plasma concentrations. Even though the self-reported compliance to the intake of tomato-products was 99%, 3 subjects in the tomato-group (Fig. 2E) and 1 subject in the tomato-plus group (Fig. 2F) displayed marked decreases in plasma lycopene concentrations. Furthermore, 2 subjects in the control group displayed high increases in plasma lycopene, indicating increased intake of tomatoes or lycopene supplementation during the intervention period. In the tomato-plus group, all subjects increased plasma selenium concentrations (Fig. 2C), while all but one increased C20:5 n-3 (Fig. 2I). None of the subjects in the tomato- or control group had large increases in the plasma selenium concentrations (Fig. 2B and A, respectively), while three subjects in the tomato group and six subjects in the control group displayed marked increases in C20:5 n-3 (Fig. 2H and G).

Due to large variations in responses to the intervention, we tested whether the PSA values were affected by individual changes in lycopene, selenium and C20:5 n-3. Independent of the allocated intervention group, we compared the PSA values for those patients with an above median increase in lycopene, selenium or C20:5 n-3 as well as those patients with an above median increase in all three biomarkers (Table 5). A statistically significant decrease in PSA ($p = 0.043$) was found in those patients who had a more than median increase in lycopene (n = 35), with no such decrease in men with a below median change (n = 35) (Table 5). The patients with a high increase in lycopene, selenium and C20:5 n-3 combined, had

Table 4
Biomarkers of the intervention.

Biomaker	Control Median (range)		Tomato Median (range)		Tomato-plus Median (range)		P-value	
	Baseline	Change	Baseline	Change	Baseline	Change	Baseline	Change
Carotenoid [μg/mL] control n = 23, tomato n = 26, tomato-plus n = 25								
Lutein	0.12 (0.04, 0.39)	0.01 (−0.06, 0.14)	0.12 (0.04, 0.29)	0.03 (−0.12, 0.09)	0.14 (0.04, 0.24)	0.01 (−0.06, 0.13)	0.498	0.272
Zeaxanthin	0.03 (0.01, 0.14)	0.00 (−0.05, 0.03)	0.03 (0.01, 0.05)	0.00 (−0.02, 0.02)	0.03 (0.02, 0.06)	−0.01 (−0.02, 0.02)	0.895	0.117
β-kryptoxantin	0.06 (0.01, 0.18)	0.01 (−0.04, 0.10)	0.05 (0.01, 0.21)	0.02 (−0.17, 0.10)	0.06 (0.03, 0.21)	0.01 (−0.04, 0.09)	0.345	0.088
α-carotene	0.05 (0.01, 0.24)	0.00 (−0.09, 0.09)	0.03 (0.01, 0.10)	0.00 (−0.05, 0.09)	0.04 (0.01, 0.16)	−0.00 (−0.08, 0.04)	0.358	0.399
β-carotene	0.18 (0.03, 0.50)	0.02 (−0.18, 0.18)	0.14 (0.03, 0.30)	0.06 (−0.16, 0.26)	0.13 (0.06, 0.59)	0.06 (−0.18, 0.18)	0.762	0.157
Lycopene	0.32 (0.13, 0.62)	−0.02 (−0.15, 0.53)	0.24 (0.03, 0.68)	**0.25** (−0.12, 0.68)	0.25 (0.09, 0.46)	**0.32** (−0.29, 0.75)	0.227	<0.001 [a,b]
Selenium [μmol/L] Control n = 21, tomato n = 24, tomato-plus n = 23								
Selenium	1.1 (0.8, 1.5)	0.0 (−0.3, 0.2)	1.2 (0.8, 1.8)	0.0 (−0.1, 0.2)	1.1 (0.9, 1.5)	**0.9** (0.6, 1.3)	0.449	<0.001 [a]
Fatty acid [% of total FAME weight] Control n = 22, tomato n = 24, tomato-plus n = 24								
C18:2 n-6	7.08 (5.31, 10.20)	0.26 (−2.32, 9.78)	7.24 (4.63,12.35)	−0.11 (−4.18, 1.43)	7.09 (5.05,13.71)	**−0.80** (−7.82, 1.22)	0.605	0.023 [b]
C20:1 n-9	0.18 (0.12, 0.24)	0.00 (−0.06, 0.03)	0.21 (0.16, 0.37)	−0.00 (−0.04, 0.03)	0.21 (0.14, 0.29)	**−0.01** (−0.07, 0.02)	0.034	0.009 [b]
C20:3 n-6	0.79 (0.61, 1.29)	0.01 (−0.21, 0.36)	0.88 (0.58, 2.02)	0.00 (−0.11, 0.21)	0.86 (0.54, 1.61)	**−0.13** (−0.57, 0.15)	0.287	<0.001 [b]
C20:4 n-6 & 22:1 n-9	9.91 (7.88,13.22)	0.07 (−3.14, 2.21)	9.74 (7.43,12.68)	0.26 (−0.60, 3.20)	10.15 (6.99,12.36)	**−0.41** (−1.30, 2.64)	0.909	<0.001 [b]
C20:5 n-3	1.41 (0.69, 2.15)	0.06 (−0.26, 0.73)	1.49 (0.43, 2.49)	0.02 (−0.29, 0.40)	1.51 (0.70, 3.74)	**0.98** (0.10, 1.72)	0.874	<0.001 [b]
C22:5 n-3	2.48 (1.83, 3.00)	0.03 (−1.02, 1.06)	2.46 (1.21, 3.27)	0.03 (−0.14, 0.90)	2.58 (1.36, 3.15)	**0.34** (−0.20, 1.04)	0.720	<0.001 [b]
C22:6 n-3	6.10 (4.26, 7.84)	0.02 (−2.10, 2.98)	6.02 (2.91, 8.08)	0.14 (−0.63, 1.91)	6.05 (3.51, 8.20)	**0.49** (−0.39, 2.15)	0.802	0.006 [b]

Carotenoids in plasma were measured by HPLC. Selenium in plasma was measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS). Fatty acids in red blood cells was measured as % of total Fatty acid methyl esters by Gas chromatography. Data is presented as median (range). Kruskal–Wallis tests were used to identify statistical differences within the dataset, and the Mann–Whitney test was used to compare groups. Bold characters identify statistically significant differences.
[a] Mann–Whitney Test p < 0.05 for difference control vs tomato.
[b] Mann–Whitney Test p < 0.05 for difference control vs tomato-plus.

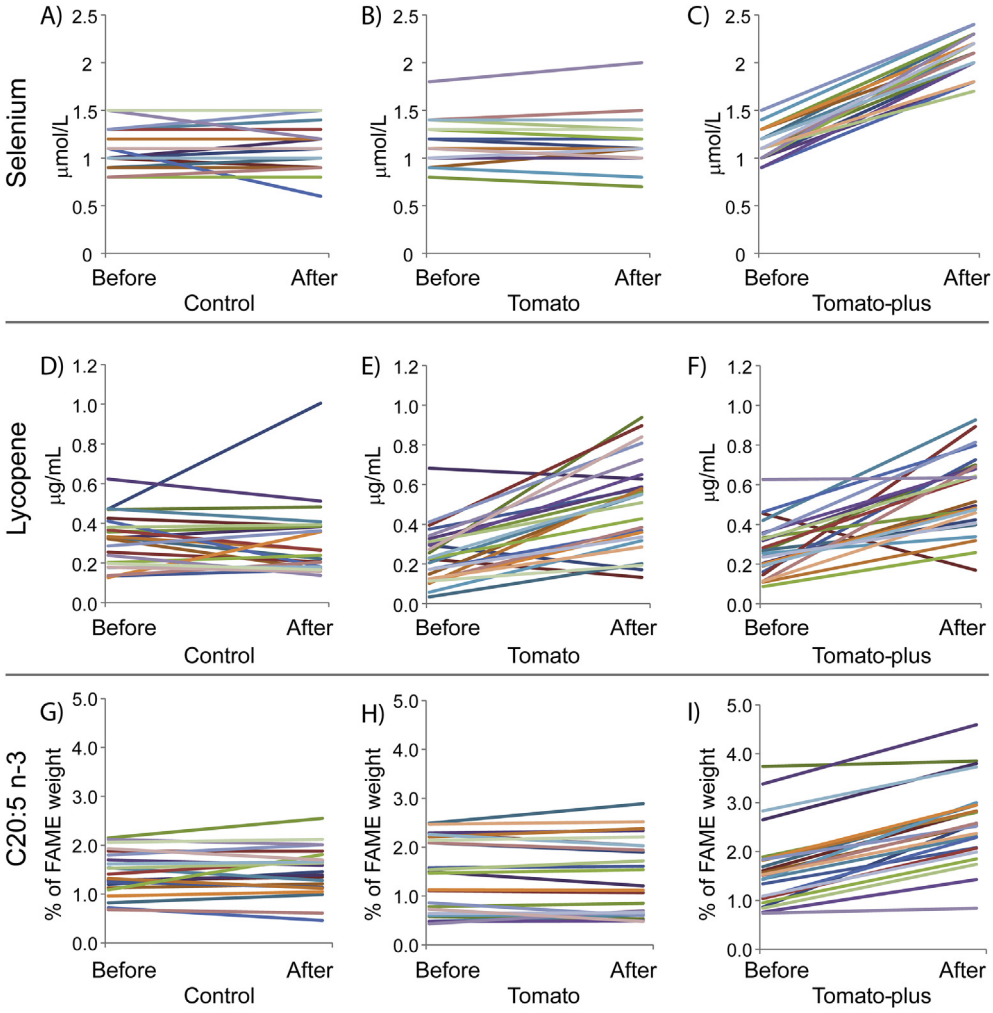

Fig. 2. Individual changes for selenium, lycopene and C20:5 n-3. A-I) Individual change in biomarkers for each intervention group. Changes from before to after intervention for each individual for A-C) selenium, D-F) lycopene, and G-I) C20:5 n-3 in red blood cells. Selenium in plasma was measured by Inductively Coupled Plasma Mass Spectrometry. Lycopene in plasma was measured by HPLC. C20:5 n-3 in red blood cells was measured as % of total Fatty acid methyl esters (FAME) by Gas chromatography.

an even more pronounced decrease in PSA ($p = 0.006$) compared to those patients with below-median increases (Table 5).

Furthermore, changes in PSA during the intervention were negatively correlated with the changes in lycopene ($r = -0.247$, $p = 0.034$) for all patients included in the study. Additionally, post-intervention PSA values were negatively correlated with the post-intervention lycopene concentration ($r = -0.400$, $p = 0.048$).

5. Discussion

Since tomato intake may protect against prostate cancer, and PSA is used in the clinical practice as a biomarker for prostate cancer, we have tested whether tomato-based interventions may dampen PSA increase in prostate cancer patients. In this RCT, 79 prostate cancer patients were treated with tomato products; a combination of selenium, omega-3 fatty acids, soy isoflavones, grape-and pomegranate juice, and green- and black tea in addition to the tomato products; or control diet. The patients received the diet intervention 3 weeks prior to prostatectomy or radiotherapy.

A crucial aspect of this food-based study is that we screened tomato products for their lycopene content and selected only those containing the highest levels of lycopene for use in the intervention. Without this screening, we would most likely also have included tomato products with low or intermediate lycopene content. The very large variation in lycopene contents in the products is noteworthy. It should also be noted that our screening is performed in products available in Norway, and thus might not completely reflect the lycopene content in products available in other countries.

The main analysis, which includes all patients, revealed no differences in PSA changes in intervention groups compared to the control group. However, it is possible that the study ended up underpowered for this primary analysis due to a lower than expected initial consent rate.

Borel et al. [12] reported large variations in the bioavailability of lycopene and such variations may help explain the wide variation in lycopene changes in our intervention groups. We also find large variations in the C20:5 n-3 changes in red blood cells after PUFA supplementation. Such variations in the response to omega-3 supplementation have also previously been reported [21], and we speculate that these variations may be related to Single Nucleotide Polymorphism (SNP)-related differences in fatty acid metabolism [22]. When the individual variations of response to the interventions were taken into account, we observed significantly lower PSA increases for patients with the highest plasma increases in lycopene alone, and lycopene, selenium and C20:5 n-3 combined. These data suggest that individuals with the highest increase in plasma lycopene have a more favorable PSA development than

Table 5
PSA values compared between groups based on changes in lycopene, selenium and C20:5 n-3.

	Low increase Median (range)		High increase Median (range)		P-value[a]	
	Baseline	Change	Baseline	Change	Baseline	Change
Changes in C20:5 n-3, selenium and lycopene (low increase n = 17, high increase n = 15) [b]						
PSA [μg/L]	8.44 (5.48,18.90)	0.72 (−1.63, 2.40)	9.03 (5.38, 30.20)	**−0.10** (−12.40, 1.47)	0.407	0.006*
Changes in lycopene (low increase n = 35, high increase n = 35) [b]						
PSA [μg/L]	9.74 (4.42, 31.50)	0.45 (−3.30,4.80)	8.12 (1.52, 30.20)	**−0.02** (−2.40, 1.70)	0.957	0.043*
Changes in C20:5 n-3 (low increase n = 34, high increase n = 33) [b]						
PSA [μg/L]	8.28 (1.52, 25.90)	0.29 (−8.53, 2.40)	9.72 (4.42, 31.50)	−0.13 (−12.40, 4.80)	0.144	0.178
Changes in selenium (low increase n = 32 high increase n = 27) [b]						
PSA [μg/L]	9.00 (1.52, 19.60)	0.025 (−8.53, 2.40)	11.20 (5.10, 31.50)	0.00 (−12.40, 4.80)	0.100	0.411

[a] Mann–Whitney test was used to compare groups.
[b] Groups are divided based on median change in plasma/red blood cell concentrations. Bold characters identify statistically significant differences, * = $p < 0.05$.

individuals with lower lycopene increases. In a separate post-hoc analysis, subjects with intermediate risk prostate cancers in the post-surgery risk reclassification had significant reduction in PSA in the tomato group as compared to the control group. Even though the changes in PSA in our post-hoc analyses are promising, our study is possibly underpowered for these analyses, and thus further RCTs are warranted in order to verify these findings.

Despite the widespread interest in the association between prostate cancers and intake of tomatoes, randomized controlled trials are limited both in number and quality, as also concluded by the WCRF [5]. The effect of tomato products has only been tested in one previous clinical trial in patients with established prostate cancers. In an uncontrolled clinical trial, Chen et al. [8] found that a 3 week tomato sauce intervention (containing 30 mg lycopene/day) reduced PSA about 18% in 32 patients with intermediate-risk prostate cancers.

RCTs with lycopene supplements (i.e. not tomato products) have been inconclusive. A recent Cochrane review concluded with no evidence of effect of lycopene on PSA, as only 3 RCT's were found eligible to be included in the review, and two of these three trials were viewed as being of high risk of bias [3]. In a RCT, patients with metastatic prostate cancer undergoing orchidectomy consumed 4 mg of lycopene daily [23]. For the patients consuming lycopene, significantly lower PSA values were seen after 2 years, compared to surgical treatment alone. A Phase II RCT by Kucuk et al. [24] treated patients with 30 mg of lycopene for a 3-week intervention prior to prostatectomy. A non-significant 18% decrease of PSA was seen in the intervention group. In a non-controlled clinical intervention trial Kumar et al. [9] explored the effect of 15, 30, and 45 mg of lycopene daily (no control group) for 30 days prior to prostatectomy. They found no significant differences in PSA-development within or between the groups. Another uncontrolled trial in patients with castration resistant prostate cancer showed no effect on PSA values by 30 mg of lycopene per day for 3 months [10]. The lack of proper control groups, make these results difficult to interpret.

A recent Cochrane review found no effect of selenium on the risk of prostate cancer [25]. Also, very few clinical trials have been performed with n-3 PUFA, soy, grapes, pomegranate, green- or black tea. In a recent RCT by Galet et al. [26], 4–6 weeks on a low fat and high fish oil diet decreased cell cycle progression in prostate cancer tissue, as compared to tissue from patients on a high fat diet. Furthermore, in a RCT with genistein supplements for three week prior to prostatectomy, a borderline significant reduction in PSA as compared to controls were observed [27]. Two clinical trials have found increases in PSA doubling time in prostate cancer patients with intake of pomegranate, however these results should be interpreted with caution as both studies lack control arms [28,29]. In experimental models of prostate cancer, mice receiving pomegranate extract displayed reduced tumor growth and lower PSA levels, as compared to controls [30].

Two trials have previously been conducted with combined diet interventions. However, these studies were performed either in men at high risk of prostate cancer or in patients with biochemical relapse. Fleshner et al. [31] found no effect of a combination of soy (40 g), vitamin E (800 U), and selenium (200 μg) on progression from high grade prostatic intraepithelial neoplasia to prostate cancer in a placebo controlled trial. A combined supplement of isoflavones, lycopene, silymarin, selenium, and several vitamins and minerals reduced PSA slope in patients with rising PSA levels after initial prostatectomy [32].

Our study suggests that daily consumption of lycopene-rich tomato products may affect PSA values in prostate cancer patients with intermediate risk profiles however this finding needs to be confirmed in RCTs. It is interesting to speculate that tomato-products may delay the progression of intermediate risk prostate cancer or keep indolent cancers in the highly differentiated stage. Our observation may also be applicable to active surveillance protocols for prostate cancer patients, since PSA screening detects many cases of prostate cancer who are referred to primary treatment with potentially unnecessary morbidity. If our data can be replicated in low risk cancer patients, lycopene-rich tomato products may be considered as part of an active surveillance in future protocols.

In conclusion, this is the first RCT where the effect of tomato-products has been tested on PSA development in patients with localized prostate cancers. Our findings support the importance of controlling for changes in blood concentrations in dietary interventions, as the largest reduction in PSA was detected in those patients who had the highest increase in lycopene, selenium and C20:5 n-3. Furthermore, our data suggest that daily consumption of tomato products containing 30 mg lycopene for 3 weeks may reduce PSA values in intermediate risk prostate cancer patients. The indication of reduced PSA increase in intermediate risk prostate cancer patients by tomato products warrants further investigation.

Conflict of interest

The following authors declare no competing interest; IP, WL, SKB, EH, WK, LV, KA, NB, TB, PL, KAT, AS, LME, BB, MHC, SDF, SSS, and AK. RB has interests in Vitas AS. Vitas AS was established by Oslo Innovation Center.

Authors contributions

RB, WL, AK, PL, SSS, SDF designed the research; IP, AK, WL, NB, EH, WK, LV, KA, KAT, LME, BB, AS, MHC conducted research; TB provided essential material; IP, SKB, EH, AK, PL, MHC analyzed data

and performed statistical analysis; IP, WL, SKB, EH, SDF, SSS, RB wrote the paper; IP and RB had primary responsibility for the final content. All authors read and approved the final manuscript.

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Clinical trial registration

This study was approved by the regional ethics committee in Norway (REK Sør, no. S-06187). The study is registered in ClinicalTrials.gov with no. NCT00433797.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.clnu.2016.06.014.

References

[1] Ferlay J, Shin H, Bray F, Forman D, Mathers C, Parkin D. GLOBOCAN 2008 v2.0, cancer incidence and mortality worldwide: IARC CancerBase No. 10. 2010. http://globocan.iarc.fr.
[2] Center MM, Jemal A, Lortet-Tieulent J, Ward E, Ferlay J, Brawley O, et al. International variation in prostate cancer incidence and mortality rates. Eur Urol 2012;61:1079–92.
[3] Ilic D, Forbes KM, Hassed C. Lycopene for the prevention of prostate cancer. Cochrane Database Syst Rev 2011:CD008007.
[4] World Cancer Research Fund. Food, nutrition, physical activity and the prevention of cancer: a global perspective. Washington DC: AICR; 2007.
[5] World Cancer Research Fund International/American Institute for Cancer Research Continuous Update Project Report. Diet, nutrition, physical activity, and prostate cancer. 2014.
[6] Abrahamsson PA, Lilja H, Oesterling JE. Molecular forms of serum prostate-specific antigen. The clinical value of percent free prostate-specific antigen. Urol Clin North Am 1997;24:353–65.
[7] Catalona WJ, Smith DS, Wolfert RL, Wang TJ, Rittenhouse HG, Ratliff TL, et al. Evaluation of percentage of free serum prostate-specific antigen to improve specificity of prostate cancer screening. JAMA 1995;274:1214–20.
[8] Chen L, Stacewicz-Sapuntzakis M, Duncan C, Sharifi R, Ghosh L, van Breemen R, et al. Oxidative DNA damage in prostate cancer patients consuming tomato sauce-based entrees as a whole-food intervention. J Natl Cancer Inst 2001;93:1872–9.
[9] Kumar NB, Besterman-Dahan K, Kang L, Pow-Sang J, Xu P, Allen K, et al. Results of a randomized clinical trial of the action of several doses of lycopene in localized prostate cancer: administration prior to radical prostatectomy. Clin Med Urol 2008;1:1–14.
[10] Jatoi A, Burch P, Hillman D, Vanyo JM, Dakhil S, Nikcevich D, et al. A tomato-based, lycopene-containing intervention for androgen-independent prostate cancer: results of a Phase II study from the North Central Cancer Treatment Group. Urology 2007;69:289–94.
[11] Bunker CH, McDonald AC, Evans RW, de la Rosa N, Boumosleh JM, Patrick AL. A randomized trial of lycopene supplementation in Tobago men with high prostate cancer risk. Nutr Cancer 2007;57:130–7.
[12] Borel P, Desmarchelier C, Nowicki M, Bott R. Lycopene bioavailability Is associated with a combination of genetic variants. Free Radic Biol Med 2015;83:238–44.
[13] Raabe NK, Lilleby W, Tafjord G, Astrom L. High dose rate brachytherapy in prostate cancer in Norway. Tidsskr Nor Laegeforen 2008;128:1275–8.
[14] D'Amico AV, Whittington R, Malkowicz SB, Schultz D, Blank K, Broderick GA, et al. Biochemical outcome after radical prostatectomy, external beam radiation therapy, or interstitial radiation therapy for clinically localized prostate cancer. JAMA 1998;280:969–74.
[15] Kvåle R, Møller B, Wahlqvist R, Fosså SD, Berner A, Busch C, et al. Concordance between Gleason scores of needle biopsies and radical prostatectomy specimens: a population-based study. BJU Int 2009;103:1647–54.
[16] Heidenreich A, Bastian PJ, Bellmunt J, Bolla M, Joniau S, van der Kwast T, et al. EAU guidelines on prostate cancer. part 1: screening, diagnosis, and local treatment with curative intent-update 2013. Eur Urol 2014;65:124–37.
[17] Carlsen MH, Lillegaard IT, Karlsen A, Blomhoff R, Drevon CA, Andersen LF. Evaluation of energy and dietary intake estimates from a food frequency questionnaire using independent energy expenditure measurement and weighed food records. Nutr J 2010;9:37.
[18] Carlsen MH, Karlsen A, Lillegaard IT, Gran JM, Drevon CA, Blomhoff R, et al. Relative validity of fruit and vegetable intake estimated from an FFQ, using carotenoid and flavonoid biomarkers and the method of triads. Br J Nutr 2011;105:1530–8.
[19] Kolberg M, Pedersen S, Bastani NE, Carlsen H, Blomhoff R, Paur I. Tomato paste alters NF-kappaB and cancer-related mRNA expression in prostate cancer cells, xenografts, and xenograft microenvironment. Nutr Cancer 2015;67:305–15.
[20] Andersen LF, Solvoll K, Drevon CA. Very-long-chain n-3 fatty acids as biomarkers for intake of fish and n-3 fatty acid concentrates. Am J Clin Nutr 1996;64:305–11.
[21] Nording ML, Yang J, Georgi K, Hegedus Karbowski C, German JB, Weiss RH, et al. Individual variation in lipidomic profiles of healthy subjects in response to omega-3 fatty acids. PLoS One 2013;8:e76575.
[22] Lattka E, Illig T, Koletzko B, Heinrich J. Genetic variants of the FADS1 FADS2 gene cluster as related to essential fatty acid metabolism. Curr Opin Lipidol 2010;21:64–9.
[23] Ansari MS, Gupta NP. A comparison of lycopene and orchidectomy vs orchidectomy alone in the management of advanced prostate cancer. BJU Int 2003;92:375–8.
[24] Kucuk O, Sarkar FH, Sakr W, Djuric Z, Pollak MN, Khachik F, et al. Phase II randomized clinical trial of lycopene supplementation before radical prostatectomy. Cancer Epidemiol Biomarkers Prev 2001;10:861–8.
[25] Dennert G, Zwahlen M, Brinkman M, Vinceti M, Zeegers MP, Horneber M. Selenium for preventing cancer. Cochrane Database Syst Rev 2011:CD005195.
[26] Galet C, Gollapudi K, Stepanian S, Byrd JB, Henning SM, Grogan T, et al. Effect of a low-fat fish oil diet on pro-inflammatory eicosanoids and cell cycle progression score in men undergoing radical prostatectomy. Cancer Prev Res (Phila) 2013;7:97–104.
[27] Lazarevic B, Boezelijn G, Diep LM, Kvernrod K, Ogren O, Ramberg H, et al. Efficacy and safety of short-term genistein intervention in patients with localized prostate cancer prior to radical prostatectomy: a randomized, placebo-controlled, double-blind Phase 2 clinical trial. Nutr Cancer 2011;63:889–98.
[28] Pantuck AJ, Leppert JT, Zomorodian N, Aronson W, Hong J, Barnard RJ, et al. Phase II study of pomegranate juice for men with rising prostate-specific antigen following surgery or radiation for prostate cancer. Clin Cancer Res 2006;12:4018–26.
[29] Paller CJ, Ye X, Wozniak PJ, Gillespie BK, Sieber PR, Greengold RH, et al. A randomized phase II study of pomegranate extract for men with rising PSA following initial therapy for localized prostate cancer. Prostate Cancer Prostatic Dis 2013;16:50–5.
[30] Malik A, Afaq F, Sarfaraz S, Adhami VM, Syed DN, Mukhtar H. Pomegranate fruit juice for chemoprevention and chemotherapy of prostate cancer. Proc Natl Acad Sci U. S. A 2005;102:14813–8.
[31] Fleshner NE, Kapusta L, Donnelly B, Tanguay S, Chin J, Hersey K, et al. Progression from high-grade prostatic intraepithelial neoplasia to cancer: a randomized trial of combination vitamin-E, soy, and selenium. J Clin Oncol 2011;29:2386–90.
[32] Schroder FH, Roobol MJ, Boeve ER, de Mutsert R, Zuijdgeest-van Leeuwen SD, Kersten I, et al. Randomized, double-blind, placebo-controlled crossover study in men with prostate cancer and rising PSA: effectiveness of a dietary supplement. Eur Urol 2005;48:922–30.