Plasma lycopene concentration as a dietary compliance biomarker – a pilot study

Version 3.1

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PROTOCOL TITLE *Plasma lycopene concentration as a dietary compliance biomarker – a pilot study.*

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

ABR	**General Assessment and Registration form (ABR form), the application form that is required for submission to the accredited Ethics Committee; in Dutch: Algemeen Beoordelings- en Registratieformulier (ABR-formulier)**
AE	**Adverse Event**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
CV	**Curriculum Vitae**
DSMB	**Data Safety Monitoring Board**
EU	**European Union**
EudraCT	**European drug regulatory affairs Clinical Trials**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IMP	**Investigational Medicinal Product**
IMPD	**Investigational Medicinal Product Dossier**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
(S)AE	**(Serious) Adverse Event**
SPC	**Summary of Product Characteristics; in Dutch: officiële productinformatie IB1-tekst**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: To determine the plasma lycopene concentration before and after an oral intake of lycopene in order to use this measurement as a life style compliance marker.
Objective: Primary objective: to determine if a plasma lycopene concentration can serve as a response parameter after a single dose of dietary lycopene.
Study design: Cross-over interventional pilot study.
Study population: Ten male healthy volunteers 18-75 years.
Intervention: Oral food supplement tablet 40 mg lycopene once, versus oral soup of cooked tomatoes equivalent to 40 mg lycopene content. In addition, the participants' habitual diet and actual food intake during the intervention will be measured using a food frequency questionnaire and a food diary.
Main study parameters/endpoints: Variation of plasma lycopene 1 hour before, and 1,3,6,12,24,48,72 hours after intervention.
Nature and extent of the burden and risks associated with participation, benefit and group relatedness: Eight blood samples of 6 ml full venous blood obtained by vena puncture per intervention per individual, 2 times in a cross-over pilot study, in which interventions are 3 weeks apart (so 2 x 8 samples in 10 volunteers). Risk of vena puncture is negligible, idemque the burden.

1. INTRODUCTION AND RATIONALE

Dietary study in Prostate cancer: Men with prostate cancer (PCa) often ask if the (composition of) their diet may contribute to the suppression of their tumor. There is indirect epidemiological evidence that diet, especially antioxidants like lycopene, are beneficial for an advantageous course of PCa [1]. Lycopene is a non-provitamin A carotenoid that exhibits several health benefits. Epidemiological data support a correlation between lycopene intake and the attenuation of several chronic diseases, including certain types of cancers and cardiovascular diseases. It is currently unknown whether the beneficial effects are from the native structure of lycopene or its metabolic derivatives [2].

Scientific proof for the impact of dietary interventions on PCa growth is extremely difficult to provide, as the natural course of PCa from the time of diagnosis is frequently over 20 years. In dietary studies many confounders are present, therefore dietary studies also need extreme large populations. Furthermore, the biochemical parameters needed to study lycopene concentrations in blood do not have defined normal values: SNPs in *BCO1* and other genes may modulate human plasma and prostate tissue responses to dietary lycopene intake. These individual genetic variants related to carotenoid metabolism may partially explain heterogeneous human blood and tissue responses and may be critical covariates for population studies and clinical trials [3]. On top of that, intestinal absorption is promoted by co-consumption of fat [2], and 10 gram of olive oil has been identified as an optimal dose for lycopene uptake. The way lycopene itself is offered appears to be less of importance, as there was no difference of plasma concentration observed between well processed dietary and artificial in-tablet servings of lycopene [4].

While the generally accepted opinion is that diets high in antioxidants are beneficial for suppressing and prevention of all cancers, and that they promote the overall health status of individuals, it may be difficult to change a life style that incorporates a healthy diet. Therefore in this study, we are searching for a parameter in body fluids that reflects the intake of healthy diet, and as such may motivate and sustain its continuous use (compliance marker). In case of prostate cancer we address lycopene as our target for reasons described above. Ideally, our lycopene related marker should reflect the short-term uptake of lycopene, and be based on a single dose consumption, in order to act as a direct feed-back to the consumers dietary action. Current methods of determination (see further) do not yet live up to point-of-care applications. There is, however, evidence that single dose consumption is reflected in the lycopene plasma concentration. In a trial in Italian consumers on lycopene kinetics on single dose consumption [5], a significant increase of plasma lycopene concentration of 40

nmol/l was observed above an individual base level (around 400 nmol/l), with a max concentration at 12 hours, and a normalisation after 48 hours after consumption of 15 mg of lycopene. Also a biomarker reflecting the adequate lycopene consumption of a medium long term (e.g. one week consumption) might provide a valuable asset for consumers. In a trial in Swiss male consumers on an 8-day intervention of 20 mg after lycopene depletion, the individual lycopene plasma concentration topped to a steady state of 1000 to 1500 nmol/l, while two weeks after the intervention (so during lycopene depletion) a plateau phase was obtained on a plasma concentration of 400-500 nmol/l [4], likely due to the release of lycopene from liver resources. We conclude that for consumer reasons the choice for a point-of-care testing of single dose lycopene is feasible, but restricted by current technology, and that feed-back on medium long term lycopene consumption is also feasible, as it is well reflected in plasma lycopene concentrations.

Study design: We have chosen lycopene as it is related to PCa growth, it can be used as a dietary intervention, and it is measurable reproducibly in food and in body fluids. Here we study the lycopene plasma concentration dynamics after a single dose consumption in volunteers in a Dutch setting (which differs from the geographic and environmental setting and life-style in the above mentioned studies). This is to obtain the necessary information on technology, logistics, and food products needed to conduct the follow-up study (METC 2017-389) in cancer patients. Once we have proven that a measurable response can be obtained in healthy volunteers in a Dutch population, that the assay is available and robust, and in line with food consumption as evaluated by validated food questionnaires, we will incorporate that parameter (lycopene) in the current randomised study for men with PCa and undergoing treatment or have undergone treatment in the past in which three dietary interventions are given and compliance to the intervention is currently measured by food intake questionnaires (METC 2017-389).

Here we test plasma lycopene as a candidate compliance marker for that purpose. This is based on the assumption that in Dutch participants the plasma level of lycopene might be comparable to the Swiss population literature, that a single dose response to 40 mg lycopene in combination with oil will provide a measurable response of plus 10 % in individuals, and that a concentration between 1000-1500 nmol/l is appreciated by patients and caregivers as the optimal lycopene plasma concentration to be accomplished long term (1-3 months).

Dietary interventions should always be analysed against the background of the normal individual diet. Therefore, to evaluate the lycopene intervention and related marker, it is

crucial to take the participant's habitual diet, as well as actual food intake during the intervention into account [6].

Research questions:

1) Can a plasma lycopene concentration serve as a response parameter after a single dose of dietary lycopene.

We will measure plasma carotenoid concentrations (including lycopene) in response to a single dose dietary intervention with lycopene-rich food or a lycopene tablet in a cross-over study design.

Plasma lycopene concentrations show variation between individuals, and within individuals. It is unknown if and how much a single dietary intervention with lycopene from lycopene-rich vegetables like tomatoes alters the plasma concentration, and therefore can be used as a compliance marker.

As the bioavailability and plasma kinetics of lycopene is known to a limited extend (see studies above), a scheme for plasma determinations is followed according to previous marker (GABA, also found in tomatoes, serving as an exemplary study design) research from the same Wageningen group, including a wash out period of at least two weeks between interventions in the cross-over design [7].

2) Is a tablet lycopene with equivalent lycopene content as effective as a single dose dietary intervention regarding the change of plasma lycopene concentration?

Previous studies suggest equivalence, which might be of interest for consumers/patients.

3) Is the prior food consumption of individuals, as measured by dietary questionnaires, related to the plasma lycopene levels before and after lycopene intervention?

If an existing individual high intake of lycopene as analysed by a validated questionnaire would result in a high standard plasma lycopene concentration, or little response on single dose lycopene consumption, the subject might exclude for further supplementary lycopene consumption and life style changes.

Intervention: We have chosen a single dose of 40 mg lycopene as an intervention based on the following. Previously, in a dose escalating study to 90 mg lycopene daily in men with

recurrent PCa after primary treatment, no effects were seen on the level of PSA, in contradiction to two studies conducted in men with metastatic PCa [8]. Also in high doses maintained for several weeks no toxicity was observed, while lycopene plasma levels corresponded with dietary dose. For chronic use, the daily dose of 30 mg is recommended based on a large review [9].

Lycopene belongs to the chemical family of carotenoids that can be found in intensely coloured fruits and vegetables, like tomatoes. The uptake from the alimentary tract is increased by heating the natural present lycopene, initiating a chemical conversion from a trans to a cis-form [10]. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans [10]. The addition of oils also are reported to have a beneficial influence [11]. Lycopenes are absorbed in the gut, and transported in chylomicrones because of their lipophylic nature [12]. Their exact biological action is still unknown, but regarded to be an antioxidant.

Various dietary interventions have been applied previously for the study on bioavailability of lycopene. The lycopene content in 200g of tomato soup equals ca.33 mg of lycopene. Most experimental studies aim to provide 40 mg lycopene whether in a carotene-rich diet, whether by pill-form (supplement), in combination with a minimum of 10 g of fat [2].

Assay: Standard liquid-chromatography-based technology for the analysis of carotenoids and similar structures including lycopene. Plasma carotenoids, including retinol, alpha- and beta-carotene, beta-cryptoxanthin, lutein, lycopene, tocopherols and zeaxanthin, are determined using ultra-pressure liquid chromatography coupled to diode array detection (UPLC-DAD). In short, 375 µL plasma is denatured using ethanol in the presence of the internal standard retinyl acetate. The carotenoids are subsequently extracted using 0.01% w/v butylated hydroxytoluene in hexane. The extracts are dried under nitrogen in a TurboVap evaporator (Biotage, Uppsala, Sweden) at 35 °C, reconstituted in acetonitrile, and transferred to a LC vial. LC analysis is performed on a Acquity H-class UPLC coupled to an Acquity PDA eLambda detector (Waters, Etten-Leur, the Netherlands) using an Acquity UPLC HSS T3 column (Waters). Gradient elution is performed using a mixture of acetonitrile-dichloromethane-methanol (ratio 85:5:10) containing 0.1% ammonium acetate (eluens A) and ULC-MS grade water (eluens B) with a constant flow of 400 µL/min and a runtime of 30 min. The detector is set at 292, 325 and 450 nm. Concentrations are calculated using a 6-point calibration curve. A pooled plasma sample is analyzed in duplicate in each analytical batch to monitor the quality of the analyses.

2. OBJECTIVES

Primary Objective:

To determine whether a plasma lycopene concentration can serve as a response parameter after a single dose of dietary lycopene.

Secondary Objectives:

1. To determine if a food supplement tablet lycopene with equivalent lycopene content compared to a single dose dietary intervention provides identical plasma lycopene concentration responses compared to the dietary intervention.

2. To determine the influence of prior food consumption, as measured by dietary questionnaires, on plasma lycopene levels before and after lycopene intervention.

3. STUDY DESIGN

Cross-over interventional pilot study.

Ten healthy individuals (male 18-75 years) are randomly divided in two groups (A and B) of 5 individuals. One week before the dietary intervention a daily food questionnaire will be completed.

One hour before the intervention a blood sample will be taken (all blood samples 6 ml EDTA plasma tube). All individuals will be given an infusion needle for 26 hours. Samples are spinned and plasma is pipetted and distributed over 1 ml Eppendorf cups for immediate freezing at -80 Celsius at the research lab.

Group A will take 40 mg lycopene (2,5 pills of food supplement Vitabiotics Ultra Lycopene 15mg, potent extract). Group B will take 250 ml of tomato soup (matching with 40 mg lycopene) with 20 ml olive oil. Blood sampling as described in all individuals from needle: 1,3,6,12,24 hours after intervention, and after 48 and 72 hours by direct venapunction. Wash out period of 3 weeks, after which the identical above procedure takes place including questionnaires, but group A and B changed (cross-over). All samples (n=160) will be frozen at -80, and transported on dry ice to Wageningen University for lycopene determination.

4. STUDY POPULATION

4.1 Population (base)

Potential subjects will be recruited via distribution of flyers and publication of the flyer on the intranet of Erasmus MC. .

4.2 Inclusion criteria

In order to be eligible to participate in this study, a subject must meet all of the following criteria:

- Male;
- Healthy;
- 18-75 yrs old.

4.3 Exclusion criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Allergic for tomatoes;
- Any gastrointestinal disorder within 3 months prior to the intervention;
- Recent medication or supplement use;
- Recent substantial change in weight;
- Adherence to a specific diet (for example the Moorman-diet);
- Using recreational drugs more than once a month;
- Smoking and excessive alcohol consumption (>10 standardized glasses a week).
- Risk of a dependency situation with the researchers.

4.4 Sample size calculation

Observational study obtaining individual lycopene concentration curves in plasma. It is anticipated that a measurable lycopene response will be seen in all participants. Based on the literature described in the introduction section it is hypothesized that in Dutch participants the plasma level of lycopene might be comparable to the Swiss population, that a single dose response to 40mg lycopene in combination with oil will provide a measurable response of plus 10% in all individuals. Therefore a sample size of two times five participants (10 participants in total) has been chosen.

Our pilot still needs to obtain an impression that responses are observed in all individuals, and to confirm that our HPLC-assay is an adequate technique. We will express that in

percentages of the group, such as: ‘In xx percent the lycopene concentration increased more than 10% of the T=0 within 48 hours after intervention A’.

5. TREATMENT OF SUBJECTS

5.1 Investigational product/treatment

Intervention 1: 40 mg lycopene (i.e. 2.5 food supplement pills of Vitabiotics Ultra Lycopene 15mg, potent extract). Tablets contain 430mg Tomato Extract which provides 15mg pure Lycopene, no artificial colours or preservatives, made by Vitabiotics, UK.

Intervention 2: 250 ml of tomato soup with 20 ml olive oil. Soup will be prepared from 5 kg freshly harvested tomatoes (Harvest of Health lycopene enriched tomato), blended, and frozen down at -20 C for max 1 week. These tomatoes have been characterised previously on their lycopene content which will be determined again at Wageningen University Human Nutrition Research Unit of Wageningen University and Research.

5.2 Use of co-intervention

Subjects are allowed to continue their normal diet without alterations.

5.3 Escape medication

Not applicable.

6. INVESTIGATIONAL PRODUCT

6.1 Name and description of investigational product(s)

Food supplement Vitabiotics Ultra Lycopene 15mg, potent extract. Tablets contain 430mg Tomato Extract which provides 15mg pure Lycopene, no artificial colours or preservatives, made by Vitabiotics, UK.

Tomatoes are a source of bioactive phytochemicals and are widely consumed. The phytochemical pattern of tomato products can be refined by choosing breeds with naturally higher nutrient levels that require more intense horticultural techniques and food processing [13]. Producing high-nutrient tomatoes is achieved through classic and conventional breeding techniques, done by professional vegetable breeding companies, as indicated in European Union Law. This process does not include genetic modification processes. In this study the Harvest of Health (Proudesse) tomato will be used. Per 100g this tomato contains 8,4mg lycopene, which is three times higher as ‘normal’ supermarket tomatoes. Unique characteristics of the Proudesse tomato are its preservability, shape, high concentration of lycopene and its taste.

Per subject 250 ml of tomato soup with 20 ml olive oil will be prepared from freshly harvested Harvest of Health lycopene enriched tomatoes, blended, and frozen down at -20 °C for max one week.

6.2 Summary of findings from non-clinical studies

Food supplement Vitabiotics Ultra Lycopene 15mg, potent extract: we are not aware of any performed non-clinical studies.

Harvest of health tomato: studies have been conducted measuring the nutrient levels over time, confirming that the Harvest of Health tomato contains almost 3x higher lycopene levels as compared to the normal supermarket tomato. This was recently confirmed by measurements performed by the Wageningen University.

6.3 Summary of findings from clinical studies

Food supplement Vitabiotics Ultra Lycopene, 15mg, potent extract: we are not aware of any performed clinical studies.

Harvest of Health tomato: to the knowledge of the research group, no clinical studies have been done with the Harvest of Health (Proudesse) tomato.

6.4 Summary of known and potential risks and benefits

Food supplement Vitabiotics Ultra Lycopene, 15mg, potent extract: according to the manufacturers' frequently asked questions page 'Ultra Lycopene has no known side-effects when taken as directed' (https://www.vitabiotics.com/products/ultra-lycopene-tablets).

Harvest of Health tomato: the Harvest of Health tomato contains 8,4mg lycopene per 100gr. The acceptable daily intake of lycopene is 0,5 mg/kg bodyweight. For an average male participant weighing 80kg this would result in consuming a maximum of 40mg lycopene per day, converting into a daily uptake of ±500gr of tomatoes. It is not likely that participants will eat more than 500gr of tomatoes per day and therefore the risk of consuming too much lycopene is low.

6.5 Description and justification of route of administration and dosage

Not applicable.

6.6 Dosages, dosage modifications and method of administration

Not applicable.

6.7 Preparation and labelling of Investigational Medicinal Product

Not applicable.

6.8 Drug accountability

The food supplement Vitabiotics Ultra Lycopene 15mg, potent extract supplement has been bought in store and stored dry and at room temperature.

Blended tomato fluids (soup) are frozen till consumption, and heated at normal soup temperature circa 60-80 degrees Celsius.

7. NON-INVESTIGATIONAL PRODUCT

7.1 Name and description of non-investigational product(s)

Not applicable.

7.2 Summary of findings from non-clinical studies

Not applicable.

7.3 Summary of findings from clinical studies

Not applicable.

7.4 Summary of known and potential risks and benefits

Not applicable.

7.5 Description and justification of route of administration and dosage

Not applicable.

7.6 Dosages, dosage modifications and method of administration

Not applicable.

7.7 Preparation and labelling of Non Investigational Medicinal Product

Not applicable.

7.8 Drug accountability

Not applicable.

8. METHODS

8.1 Study parameters/endpoints

8.1.1 Main study parameter/endpoint

Raise of plasma lycopene concentration in absolute (ng/ml) and relative (percentage of T0-value) after single lycopene intervention.

8.1.2 Secondary study parameters/endpoints

1. Optimal time point of lycopene concentration response after single dietary intervention (hours, days).

2. Influence of prior food consumption on plasma lycopene levels before and after lycopene interventions, as measured by dietary questionnaires.

8.1.3 Other study parameters

Clinical parameters at intake: age, body weight, comorbidity.
Dietary parameters: weekly lycopene food content estimated by weekly food questionnaires.

8.2 Randomisation, blinding and treatment allocation

10 study subjects randomised for study scheme AB versus BA (cross-over) by blinded draw of 10 tickets by an independent statistician. The 10 study subjects will be randomized at once, before the start of the study.

8.3 Study procedures

Participants will be randomized into the study scheme AB versus BA (cross-over). One week before the intervention a food frequency questionnaire will be distributed to participants. Group A will take 40mg lycopene (2,5 pills of food supplement Vitabiotics Ultra Lycopene 15mg, potent extract). Group B will take 250 ml of tomato soup (matching with 40mg lycopene) with 20ml olive oil. Wash out period of 3 weeks, after which the identical procedure takes place including questionnaires, but group A and B changed (cross-over).

Blood draws are taken on indicated time points (before intervention, 1, 3, 6, 12, 24, 48 and 72 hours after intervention) in standard vacutainers (BD Vacutainer; Becton, Dickinson and Company). For the first blood draws (before intervention and 1-24 hours

after the intervention) all participants will be given an infusion needle for 26 hours. Blood draws after 48 and 72 hours will be done by direct venapunction. Next, the tubes are centrifuged at 3000g for 10 minutes at 4 °C (Sigma 4-16K; Sigma-zentrifugen). Plasma samples are immediately put on dry ice for rapid freezing. At the end of each test day, the plasma samples are stored at −80 °C until further analysis.

Standard liquid-chromatography-based technology for the analysis of carotenoids and similar structures including lycopene. Plasma carotenoids, including retinol, alpha- and beta-carotene, beta-cryptoxanthin, lutein, lycopene, tocopherols and zeaxanthin, are determined using ultra-pressure liquid chromatography coupled to diode array detection (UPLC-DAD). In short, 375 µL plasma is denatured using ethanol in the presence of the internal standard retinyl acetate. The carotenoids are subsequently extracted using 0.01% w/v butylated hydroxytoluene in hexane. The extracts are dried under nitrogen in a TurboVap evaporator (Biotage, Uppsala, Sweden) at 35 °C, reconstituted in acetonitrile, and transferred to a LC vial. LC analysis is performed on a Acquity H-class UPLC coupled to an Acquity PDA eLambda detector (Waters, Etten-Leur, the Netherlands) using an Acquity UPLC HSS T3 column (Waters). Gradient elution is performed using a mixture of acetonitrile-dichloromethane-methanol (ratio 85:5:10) containing 0.1% ammonium acetate (eluens A) and ULC-MS grade water (eluens B) with a constant flow of 400 µL/min and a runtime of 30 min. The detector is set at 292, 325 and 450 nm. Concentrations are calculated using a 6-point calibration curve. A pooled plasma sample is analyzed in duplicate in each analytical batch to monitor the quality of the analyses.

An extensive Food Frequency Questionnaire (FFQ) will be used to assess the participants' habitual diet of the previous month. This extensive FFQ was validated for energy, macronutrients, and a number of vitamins [14-17] and administered online with the self-administered Dutch FFQ-tool™ [18], one week prior to the intervention. Participants indicate the frequency of consumed food items by selecting answers ranging from 'not consumed' to '7 days per week' and portion sizes can be estimated using natural portions and commonly used household measures. The Dutch Food Composition Database (i.e., NEVO) will be used to calculate energy and nutrient content of foods.

During the intervention, participants will report their food intake in a smartphone-based food diary on the first day of the intervention and the two following days. For this purpose, Traqq® will be used, an ecological momentary dietary assessment app [19]. In the morning, participants will receive a notification on their smartphone from Traqq®, inviting them to report their food intake during that day. Participants report their food intake by

clicking on the notification or opening the app. Next, participants can select consumed foods from an extensive food list based on the Dutch Food Composition Database. Subsequently, participants are prompted to report quantity and eating occasion, i.e., breakfast, lunch, dinner, snack. Quantity can be reported in household measures (e.g., spoon, cup), standard portion sizes (e.g., small, large) or amount in grams. Traqq® also contains a “My dishes” feature where participants can enter all ingredients of a recipe and the portion of the dish consumed, with yield and retention factors automatically being taken into account. The “My Dishes” feature can also be used to create frequently consumed product combinations (e.g., daily breakfast), which simplified reporting of these items and decreases (mis)calculation errors.

8.4 Withdrawal of individual subjects

Subjects can leave the study at any time for any reason if they wish to do so without any consequences. The investigator can decide to withdraw a subject from the study for urgent medical reasons.

8.4.1 Specific criteria for withdrawal

Not applicable.

8.5 Replacement of individual subjects after withdrawal

Only withdrawal of subjects before the first plasma determination (T0) will lead to replacement. After evaluation of results of 10 minus 3 subjects new participants will be reconsidered.

8.6 Follow-up of subjects withdrawn from treatment

Incomplete data will be used only with their consent, otherwise destroyed.

8.7 Premature termination of the study

There are no conditions defined for premature stopping the study. Data will be kept for 15 years as described on the patient information leaflet. Blood samples will be kept for 2 years to perform new analyses if needed. If, due to premature termination of the study, this will no longer be needed, the samples will be destroyed.

9. SAFETY REPORTING

9.1 Temporary halt for reasons of subject safety

In accordance to section 10, subsection 4, of the WMO, the sponsor will suspend the study if there is sufficient ground that continuation of the study will jeopardise subject health or safety. The sponsor will notify the accredited METC without undue delay of a temporary halt including the reason for such an action. The study will be suspended pending a further positive decision by the accredited METC. The investigator will take care that all subjects are kept informed.

9.2 AEs, SAEs and SUSARs

9.2.1 Adverse events (AEs)

Adverse events are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the investigational product / trial procedure/ the experimental intervention. All adverse events reported spontaneously within a period of 1 week after consuming the soup or taking the food supplement (including blood drawing afterwards) by the subject or observed by the investigator or her staff will be recorded.

9.2.2 Serious adverse events (SAEs)

A serious adverse event is any untoward medical occurrence or effect that

- results in death;
- is life threatening (at the time of the event);
- requires hospitalisation or prolongation of existing inpatients' hospitalisation;
- results in persistent or significant disability or incapacity;
- is a congenital anomaly or birth defect; or
- any other important medical event that did not result in any of the outcomes listed above due to medical or surgical intervention but could have been based upon appropriate judgement by the investigator.

An elective hospital admission will not be considered as a serious adverse event.

The investigator will report all SAEs to the sponsor without undue delay after obtaining knowledge of the events, except for the following SAEs: there are no conditions to be considered based on swallowing a food supplement or eating

tomato soup or flebotomy that are likely resulting in a SAE within one week of consumption.

The sponsor will report the SAEs through the web portal *Onderzoeksportaal* to the accredited METC that approved the protocol, within 7 days of first knowledge for SAEs that result in death or are life threatening followed by a period of maximum of 8 days to complete the initial preliminary report. All other SAEs will be reported within a period of maximum 15 days after the sponsor has first knowledge of the serious adverse events.

9.2.3 Suspected unexpected serious adverse reactions (SUSARs)

Not applicable.

9.3 Annual safety report

Not applicable.

9.4 Follow-up of adverse events

All AEs will be followed until they have abated, or until a stable situation has been reached. Depending on the event, follow up may require additional tests or medical procedures as indicated, and/or referral to the general physician or a medical specialist. SAEs need to be reported till end of study within the Netherlands, as defined in the protocol.

9.5 Data Safety Monitoring Board (DSMB) / Safety Committee

Not applicable.

10. STATISTICAL ANALYSIS

Lycopene plasma concentration curves over time will be depicted in tables and in graphs in absolute and relative (compared to T0) amounts. Trends in changes over time will be interpreted, if needed based on background information on confounding parameters. There are no statistical calculations planned.

10.1 Primary study parameter(s)

Lycopene plasma concentration curves over time will be depicted in tables and in graphs in absolute and relative (compared to T0) amounts. Trends in changes over time will be interpreted, if needed based on background information on confounding parameters. There are no statistical calculations planned.

10.2 Secondary study parameter(s)

Lycopene plasma concentration curves over time will be depicted in tables and in graphs in absolute and relative (compared to T0) amounts. Trends in changes over time will be interpreted, if needed based on background information on confounding parameters. There are no statistical calculations planned.

FFQ and food diary data will be reported descriptively.

10.3 Other study parameters

Lycopene plasma concentration curves over time will be depicted in tables and in graphs in absolute and relative (compared to T0) amounts. Trends in changes over time will be interpreted, if needed based on background information on confounding parameters. There are no statistical calculations planned.

10.4 Interim analysis

No interim analysis is planned.

11. ETHICAL CONSIDERATIONS

11.1 Regulation statement

The study will be conducted according to the principles of the Declaration of Helsinki and in accordance with the Medical Research Involving Human Subjects Act (WMO) and other relevant guidelines, regulations and Acts, such as the GDPR.

11.2 Recruitment and consent

After reading the flyer, interested volunteers can contact the principal investigator. Through email or mail the volunteer will receive a patient information leaflet. After careful consideration and time to ask questions, volunteers can decide to participate yes/no. Signed informed consent forms can be handed over or send back by mail to the principle investigator or a member of the research team. The dedicated member of the research team will document and store the signed informed consent forms.

11.3 Objection by minors or incapacitated subjects

Not applicable.

11.4 Benefits and risks assessment, group relatedness

Consuming tomato soup, taking the dietary supplement and completing the food frequency questionnaire and the food diary do not pose any significant risks. The blood drawings carry a small risk of infection and may be somewhat painful.

11.5 Compensation for injury

The sponsor/investigator has a liability insurance which is in accordance with article 7 of the WMO.

The sponsor (also) has an insurance which is in accordance with the legal requirements in the Netherlands (Article 7 WMO). This insurance provides cover for damage to research subjects through injury or death caused by the study.
The insurance applies to the damage that becomes apparent during the study or within 4 years after the end of the study.

11.6 Incentives

Participants will receive a remuneration of €50,- per visit cycle (cycle A, cycle B – or cycle B, cycle A), €100,- in total for participation in the study. Furthermore, participants will receive reimbursement of their travel costs.

12. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

12.1 Handling and storage of data and documents

After reading the flyer, interested volunteers can contact the principal investigator who will provide them with an informed consent form. Once men have returned a signed informed consent form they will be assigned a study number and be included in a study database (Castor). The designated researcher from Erasmus MC will have access to the identification list, all other databases shall only contain the study number assigned to an individual participant.

In communication with the Wageningen University, who is responsible for determining the lycopene level, analysing the outcomes of the FFQ and the food diary, the study number of participants will be used. The researchers at Erasmus MC will receive a file with the outcomes of the analysed lycopene levels of all participants as well as the results of the FFQ and the food diary.

Per blood draw, one tube of 6ml EDTA blood will be drawn from participants. Processed material will initially be stored at Erasmus MC. When all blood samples have been collected, half of the material per blood drawing will be send to the Wageningen University. The other half of the material will be stored at Erasmus MC as a back-up, and can be used in case something went wrong with transporting the material to the Wageningen University the first time.

12.2 Monitoring and Quality Assurance

Monitoring is part of a broader quality control system and an essential instrument for the quality assurance of research that is subject to WMO. It serves to verify that the rights and wellbeing of the research subjects are protected, that the study data are reported accurately and are fully verifiable in source documents, and that the conduct of the study is in accordance with the approved protocol/amendment (s), with ICH-GCP and with the relevant legal requirements.

Monitoring will be performed in accordance with applicable law and regulation, the NFU Guideline ‘Quality assurance of research involving human subjects’ and the NFU risk classification (negligible risk/‘verwaarloosbaar risico’). The monitor will have and independent role in relation to the study, i.e. he/she will not have any involvement in the set-up of the study, the conduct of the study and the interpretation of the results. In accordance with the ICH-GCP guidelines, the monitor must have direct access to the

investigator's study- and source documentation in order to verify compliance with the study protocol and check the data recorded in the CRFs for consistency.

Monitoring is executed according to Erasmus MC policy standard operating procedures for monitoring. At the beginning of the study a study specific monitoring plan will be written and approved by the sponsor. The investigator agrees to cooperate with the monitor to ensure that any problems detected in the course of these monitoring visits are resolved. Prior to subject screening an initiation meeting will be held with all participating sites to discuss the protocol and procedures and to train the site. After data collection is complete, all eCRF queries have been answered and closed, a close out visit will be conducted. The investigator should be made aware that trial – and source documents for this study should be readily made available to regulatory authority or health authority inspectors.

12.3 Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All substantial amendments will be notified to the METC and to the competent authority.

Non-substantial amendments will not be notified to the accredited METC and the competent authority, but will be recorded and filed by the sponsor.

12.4 Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

12.5 Temporary halt and (prematurely) end of study report

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the last participant's last visit.

The sponsor will notify the METC immediately of a temporary halt of the study, including the reason of such an action.

In case the study is ended prematurely, the sponsor will notify the accredited METC within 15 days, including the reasons for the premature termination.

Within one year after the end of the study, the investigator/sponsor will submit a final study report with the results of the study, including any publications/abstracts of the study, to the accredited METC.

12.6 Public disclosure and publication policy

Data are used to further conduct a previously started dietary study for prostate cancer (MEC-2017-389).

13. STRUCTURED RISK ANALYSIS

13.1 Potential issues of concern

There are no potential issues of concern.

a. Level of knowledge about mechanism of action

There is no adverse effect of lycopene known at a dose of 40 mg/day.

b. Previous exposure of human beings with the test product(s) and/or products with a similar biological mechanism

No adverse effects observed.

c. Can the primary or secondary mechanism be induced in animals and/or in *ex-vivo* human cell material?

Not in relation to life style compliance.

d. Selectivity of the mechanism to target tissue in animals and/or human beings

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e. Analysis of potential effect

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f. Pharmacokinetic considerations

Previously used pharmacokinetic schedule for plasma biomarkers being used.

g. Study population

Healthy subjects.

h. Interaction with other products

Unknown.

i. Predictability of effect

The changes in plasma carotenoid concentrations are unpredictable, and are potentially influenced by prior food consumption.

j. Can effects be managed?

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13.2 Synthesis

Not applicable.

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