



## Efficacy of a tomato pomace extract for inhibiting platelet aggregation

### STUDY PROTOCOL

PHASE 1 – Ascending dose human tolerance study of a tomato pomace extract

PHASE 2 – The Effects of ingesting a tomato pomace extract on platelet aggregation

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## Protocol

### Efficacy of a tomato pomace extract for inhibiting platelet aggregation

#### **1. Summary**

Evidence from human intervention trials and mechanistic studies suggests that tomato and tomato based products are associated with a reduction in CVD risk. The mechanism by which this protective effect occurs is not clearly understood but research has focused on its potential to modulate platelet function. In a single-blind, randomized, parallel design human intervention trial (3-groups), we aim to recruit 99 participants (33/group) to ingest an orange flavoured beverage containing different doses of a tomato pomace extract (1.0 and 2.5 g,) or placebo control over a 5-day period. The acute and longer term effects of the treatments on platelet aggregation will be evaluated at the start and end of the intervention period. Before implementing this large scale human intervention trial we will also conduct a preliminary 'ascending dose human tolerance study' in a small group of participants across a ~ 3 week period.

Dr Ivan Palomo is Chief Investigator (CI) for this trial. The trial will be conducted at the University of Talca by Adriana Vásquez (Research nurse), Dr Iván Palomo (CI; Researcher), Dr Eduardo Fuentes (Researcher) and other members of the research group may be required to assist. Statistical analysis of the data will be undertaken by Dr Henri Tapp (statistician; Institute of Food Research, UK). Tomato pomace will be processed and supplied by Centro de Estudios en Alimentos Procesados (CEAP). This study is funded by the Newton Picarte Fund. This fund supports joint projects between Chilean and British researchers.

#### **2. Scientific Background**

Epidemiological evidence supports the concept that a diet rich in fruit and vegetables affords protection against the onset of cardiovascular disease (CVD) [1]. The aetiology of CVD is multi-factorial, but it has been recognized for some time that platelet function is related to the risk of developing atherosclerosis [2], a precursor to CVD. Activated platelets play a key role in the development of atherosclerosis by contributing to plaque formation within blood vessels during the early stages of atherogenesis. Platelets become activated after binding with von Willibrand (vWF) factor and collagen, which are secreted in response to damaged endothelial cells [3]. Subsequently, collagen and vWF bind to glycoprotein receptors (GPIIb/IIIa complex) situated on the platelet surface [4]. The activated platelets in turn synthesize and secrete thromboxane A<sub>2</sub> and intracellular ADP which, combined with conformational changes in the glycoprotein complex, result in platelet aggregation. Platelet hyper-aggregation is known to be apparent in disease states such as CVD and several anti-platelet therapies such as aspirin and glycoprotein IIb/IIIa inhibitors significantly reduce the incidence of coronary events [5]. In addition, healthy people (middle aged and older) and people under stress can also display impaired platelet activity. Optimal platelet function is therefore an important tool in the prevention of CVD and dietary solutions may be considered as one approach to maintaining cardiovascular health.

Tomatoes and tomato based products are a rich dietary source of vitamins (C & E), carotenoids ( $\beta$ -carotene, lycopene), polyphenols (naringenin, quercetin, kaempferol) and folates. Evidence from human intervention trials and mechanistic studies suggest that tomato and tomato based products are associated with a reduction in CVD risk.

Whilst some reports have attributed these beneficial effects to lycopene [6, 7], others report that lycopene may not be associated with CVD risk [8, 9], indicating that other components of tomato may be responsible for the cardio-protective effects observed. The cardio-protective properties of tomatoes and tomato based products in humans include the ability to improve lipid profiles [10-12] as well as induce anti-inflammatory [12] and anti-platelet activities [13, 14].

Industrial processing of tomatoes into sauces, purees, pastes and juices result in the accumulation of tomato by-products, or pomace. Historically, tomato pomace has been viewed as a waste product and is either disposed of or used as animal feed. The tomato pomace is about 5% of the processed tomato (by weight) and consists of tomato peel and pulp (78%) and crushed seeds (22%). The tomato pomace is composed of fibre (60%), sugar (27%), protein (19%), pectin (8 %), fat (6%) and minerals (4%). Research already conducted at the University of Talca evaluating the *in vitro*, *ex vivo* and *In vivo* effects of aqueous extracts of fresh tomatoes and tomato pomace on platelet function, has demonstrated marked decreases in platelet aggregation [15-17]. Moreover, the inhibitory effect on platelet aggregation was shown to be greater in tomato pomace extracts when compared with fresh tomato extracts [15, 17]. This is an important finding because the data indicate that industrial processing of tomato into pomace, may not adversely affect the anti-platelet activity observed in other studies and may therefore, be an important tool in the prevention of CVD.

It is our intention to investigate the acute and longer term effects of ingesting tomato pomace extracts on platelet function in humans. Since the tomato pomace extract is not ordinarily consumed by the wider population in this processed format, we firstly propose to conduct a preliminary ascending dose human tolerance study in a small group of people. In short, the purpose of this submission is to seek ethical approval to conduct a two phase study.

- Phase 1: A preliminary 'ascending dose' human tolerance study of ingesting a tomato pomace extract; leading onto
- Phase 2: A single-blind, randomized, parallel design, human intervention trial to investigate the effects of ingesting different doses of the tomato pomace extract on platelet aggregation.

Each phase of the study is described in detail below.

### **3. PHASE 1 – Ascending dose human tolerance study of a tomato pomace extract**

#### ***3.1 Study objectives***

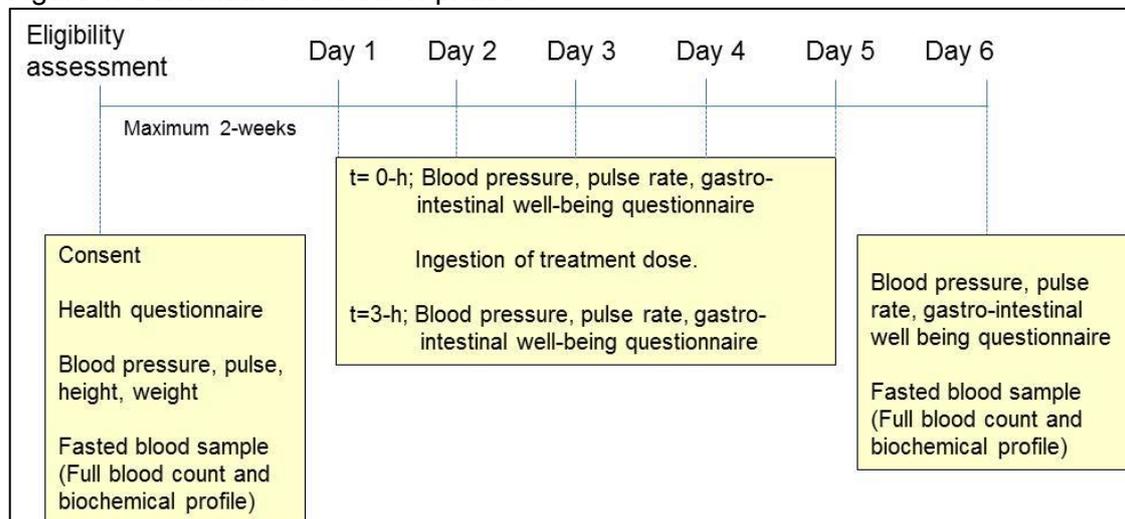
The objective of this single ascending dose study is to assess the safety and tolerability of the ingestion of tomato pomace extracts in humans.

#### ***3.2 Study Design***

Male participants (n=15) will be recruited to ingest a tomato pomace extract once daily for 5-days across three different doses. In week one, five participants will ingest 1.0 g of extract per day for 5 days. In week two, the next five participants will ingest 2.5 g of extract per day, for five days. In week three, the next five participants will ingest 10 g extract per day, for five days. The 1.0 and 2.5 g doses of extract are the same doses we intend to investigate in phase 2. Tolerability of the tomato pomace ingestion will be assessed by physical measurements (blood pressure and pulse rate) and a gastro-intestinal well-being assessment before ingestion, and then again

3-h after ingestion of the tomato pomace extract on each of the 5 treatment days. The blood test for full blood count and biochemical profile undertaken at clinical screening (section 3.6) will form part of the baseline assessment profile. The same blood tests will be repeated 24-h after the last treatment on day 5. Figure 1 is a schematic overview of the study.

Figure 1. Schematic overview of phase 1



### 3.3 Inclusion criteria

- Apparently healthy men aged between 18 and 26 years
- BMI >19.5 and < 26.0

### 3.4 Exclusion criteria

- **Known tomato allergy**
- Chronic medical conditions requiring active treatment (e.g. Cardiovascular disease, diabetes, asthma)
- Medically or self-prescribed medication
- Kidney or liver disease/disorders
- Gastro-intestinal disease/disorders
- Smokers
- Parallel participation in another research project which involves dietary intervention
- Depressed or elevated blood pressure measurements (<90/50 or 95/55 if symptomatic or ≥160/100 (mmHg))
- Any person related to or living with any member of the study team

### 3.5 Recruitment strategy

The study population will comprise apparently healthy men aged 18 – 26 years. A total of 15 participants are required to complete this phase of the study.

Recruitment of participants to this study will be achieved through poster and E-mail advertisements (annex 1) placed across the University of Talca, inviting anyone who is interested in receiving information about the study to contact named researchers. Those expressing an interest will be provided with a participant information sheet (annex 2). After reading the information sheet, those wishing to take part in the study will be invited to the research facility for an informal meeting with a member of the research team who will explain the study fully, focusing on the participant's

involvement. Participants will be encouraged to ask questions at this stage prior to making any commitment. At the end of the meeting, participants will be given ample opportunity to consider whether they wish to take part in the study. If, following the consideration period they wish to participate in the study, they will be asked to contact a member of the research team to arrange an appointment at the research facility for the pre-study clinical screening.

### *3.6 Clinical screening*

Participants will attend the research facility following a 10-hour overnight fast but will be advised that they should drink water during the fasting period. On arrival at the research facility a member of the research team will go through the consent form (annex 3) with the participant and answer any questions that may arise at this stage. He will then be asked to sign a consent form agreeing to participate in the study. A copy of this form will be given to the participant to keep. A research nurse will then complete a basic health screening questionnaire (annex 4), measure and record blood pressure, pulse, height and weight and calculate body mass index. A 10 mL blood sample will be obtained for full blood count (hemogram) and biochemical profile. The biochemical profile will include: glucose, urea nitrogen, urea, total cholesterol, uric acid, total proteins, albumin, globulins, total bilirubin, transaminase GOT, GPT transaminase, GGT, lactate dehydrogenase, alkaline phosphatase, total calcium and phosphorous. The results of the full blood count and biochemical profile will be reviewed by a medical doctor (Álvaro Valdéz MD and personnel of the Laboratorio Talca, Centro Diagnóstico Diagonal). In the event that the medical doctor deems the results of the blood test to be indicative of a health problem (that may compromise the well-being of the participant should he take part), then he will be excluded from the study. In these circumstances, the participant will be advised to contact a medical practitioner of his choice for further treatment/investigation.

Those participants meeting the basic inclusion/exclusion criteria (assessed using the health screening questionnaire) and with normal results of the clinical laboratory tests (full blood count, biochemical profile) will be eligible to participate in this study. Participation will occur no later than 2 weeks after clinical screening.

### *3.7 Preparation of tomato pomace drinks.*

Tomato pomace will be processed and supplied by CEAP. In brief, chopped fresh tomatoes are heated to produce a pulp. The pulp is passed through a 'pulper' and a 'finisher' before being extracted through a turbo press to produce a pomace. The tomato pomace is concentrated to dryness by evaporation. The pomace is then extracted and dried to form a powder extract. A certificate of analysis for pesticide and microbial safety tests on the final product is provided by CEAP (annex 5).

Immediately prior to ingestion, 'drinks' containing the appropriate mass of tomato pomace extract will be prepared at the research facility as described below. The water used to dilute the extract will be flavoured using a commercially available orange flavoured powder (Kool aid) which will be diluted according to the manufacturer's instructions before adding to the tomato pomace extract. Flavoured water is necessary because this is how we intend to blind the participants to the test product in phase 2.

- 1) Low dose pomace extract – 1 g extract will be accurately weighed before dissolving in flavoured water to a final mass of 100 g
- 2) Medium dose pomace extract – 2.5 g extract will be accurately weighed before dissolving in flavoured water to a final mass of 100 g
- 3) High dose pomace extract - 10 g extract will be accurately weighed before dissolving in flavoured water to a final mass of 100g

### 3.8 Intervention procedure

Participants may maintain normal dietary habits during the 5-day intervention period but will be instructed to refrain from drinking any alcohol. The format for phase 1 is intended to reflect the phase 2 study and is described below.

Week 1: 5 participants will start on the lowest dose of tomato pomace extract being tested (1.0 g). Participants will arrive at the research facility on the morning of each of the 5 treatment days after fasting from 10pm the night before. During the fasting period, the participant may drink as much water as he needs. At the start of each treatment day, the research nurse will record pulse rate and blood pressure and complete a gastro-intestinal well-being questionnaire (Annex 6). The participant will then ingest the tomato pomace extract. 3-h post ingestion of the extract, the research nurse will once again record pulse rate and blood pressure and complete another gastro-intestinal well-being questionnaire after which the participant may eat and drink as normal. The participant will then be free to leave the research facility with instructions to return the following morning to repeat the procedure. 24-h after the final treatment on day 5, another 10 mL fasted blood sample will be obtained for full blood count and biochemical assessment as described in the clinical screening section of this protocol (section 3.6).

Week 2: If no problems occur after 5-days ingestion at the lowest dose tested (see section 3.9 for criteria) then the next 5 participants will ingest the next incremental dose (2.5 g). The same procedure described for week 1 above, will be followed.

Week 3: If no problems occur in week 2 (section 3.9), then the last 5 participants will ingest the highest dose of extract (10 g). The same procedure described for week 1 above will be followed.

### 3.9 Stop criteria for dose escalation

Blood tests will be assessed (by a medical doctor) for abnormal variations at the end of the intervention period by comparison with the test results obtained at clinical screening. Similarly the CI will evaluate the results from the gastro-intestinal well-being questionnaires. Table 1 is the STOP criteria in the event that abnormal variations in blood test results and/or gastro-intestinal problems occur.

Table 1 Stop criteria for phase 1

Dose extract (g)	Previous dose outcome	Abnormal at current dosing	Next step	Abnormal at re-test	Next step
1.0	N/A	$\leq 2$ <sup>(a)</sup>	Escalate to next dose	n/a	n/a
		$\geq 3$	Replace and re-test	0-1 <sup>(b)</sup>	Escalate to next dose
				$\geq 2$	STOP

2.5	All normal	$\leq 2$	Escalate to next dose	n/a	n/a
		$\geq 3$	Replace and re-test	0-1	Escalate to next dose
		$\geq 2$			STOP
	1-2 abnormal <sup>(a)</sup>	$\leq 2$	Escalate to next dose	n/a	n/a
		$\geq 3$	STOP	n/a	n/a
		$\geq 3$ abnormal <sup>(b)</sup>	$\geq 2$	STOP	n/a
10	Any	0	Proceed to phase 2	n/a	n/a
		1-2	Replace and re-test	0	Proceed to phase 2
				1-2	STOP
		$\geq 3$	STOP	n/a	n/a

n/a - Not applicable; STOP - the dose-escalation study should be terminated.

If evaluation of the data indicates that the pomace extract is well tolerated at the highest dose tested, we will move onto the phase two efficacy trial.

Dr Ivan Palomo (CI) will assume responsibility for the dose escalation procedures and the decision to move into the phase two efficacy trial.

### 3.10 Participant randomization

Participants in this ascending dose human tolerance study will ingest 1, 2.5 or 10 g tomato pomace incorporated into a drink, over a 5 day period. Each treatment will be assigned a letter (A-C). Once all 15 participants have been recruited they will be block randomized to treatment by the computer programme randomization.com. The participants will be blinded to the treatment dose.

### 3.11 Participant payment

Each participant will receive an inconvenience allowance of 15,000 Chilean Peso upon completion of the study or pro-rata for non-completion.

## **4. PHASE 2 – The Effects of ingesting a tomato pomace extract on platelet aggregation**

Upon successful completion of the preliminary ascending human dose tolerance study we will conduct a parallel design human intervention trial to investigate the effects of ingesting a tomato pomace extract on platelet aggregation.

### 4.1 Study hypothesis

Daily ingestion of a tomato pomace extract for 5 days will significantly reduce platelet aggregation in humans compared with a placebo control and in a dose dependent manner.

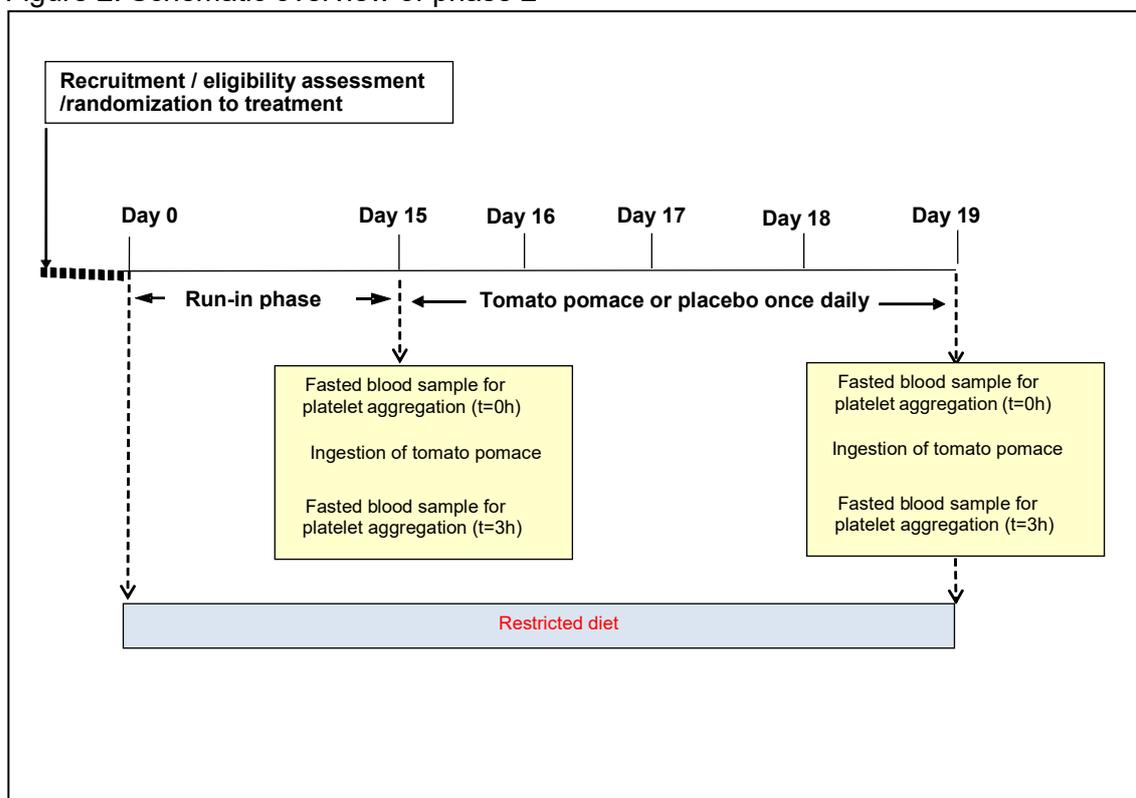
#### 4.2 Study Objectives

- 1) To determine the acute effect of ingesting two different doses of a tomato pomace extract on platelet aggregation
- 2) To determine the longer term effect of ingesting two different doses of a tomato pomace extract on platelet aggregation

#### 4.3 Study design

A single blind, parallel design (3-groups), placebo controlled trial will be conducted to assess the acute and longer term effects of ingesting a tomato pomace extract on platelet aggregation. Participants will be randomized to receive either: a) 1.0 g tomato pomace extract delivered in flavoured water b) 2.5 g tomato pomace extract delivered in flavoured water or c) Flavoured water (placebo control). A total of 99 people are required to complete (n=33/group). Each group will ingest either a treatment or placebo once daily, for 5-days. For 14-days preceding the baseline assessment day and for the 5-day period of intervention, participants will completely exclude from the diet some foods/beverages that are known to affect platelet function (tomato and all tomato based products, alcohol, cocoa and cocoa products and green tea). To assess the acute effects of the intervention on platelet aggregation, a blood sample will be obtained before ingestion (D15; 0-h) and post ingestion (D15; 3-h) of the treatment. To assess the longer term effects, further blood samples will be taken at the end of the intervention period (D19; 0 and 3-h). Figure 2 is a schematic overview of phase 2.

Figure 2. Schematic overview of phase 2



#### 4.4 Inclusion criteria

- Apparently healthy men aged between 18 and 26 years
- BMI >19.5 and <26.0

- Platelet aggregation response corresponding to  $\geq 65\%$

#### 4.5 Exclusion criteria

- Known tomato allergy
- Chronic medical conditions requiring active treatment (e.g. cardiovascular disease, diabetes, asthma)
- Gastro-intestinal disease/disorders
- Smokers
- Medically prescribed medication known to affect platelet function
- Self-prescribed medication known to affect platelet function (e.g. aspirin and non-steroidal anti-inflammatory drugs) unless participant is willing to give up.
- Bleeding disorders (e.g. haemophilia)
- Dietary supplements judged to affect study outcome
- Parallel participation in another research project which involves dietary intervention
- Blood donation within 16 weeks prior to the study
- Depressed or elevated blood pressure measurements ( $<90/50$  or  $95/50$  if symptomatic or  $\geq 160/100$  mmHg)
- Any person related to or living with any member of the study team

#### 4.6 Recruitment strategy

The study population will comprise apparently healthy men aged 18 – 26 years. A total of 99 participants are required to complete this trial. However, recruitment for this study will only cease once we are confident that the required number of participants will complete the study.

Recruitment of participants to this trial will be achieved through poster and E-mail advertisements (annex 7) placed across the University of Talca, inviting anyone who is interested in receiving information about the study to contact named researchers. Those expressing an interest will be provided with a participant information sheet (annex 8). If insufficient numbers of people are recruited from the University of Talca we may place advertisements in the local media.

After reading the information sheet, those interested in taking part will be invited to the research facility for an informal meeting with a member of the research team who will explain the study fully, focusing on the participant's involvement. Participants will be encouraged to ask questions at this stage prior to making any commitment. At the end of the meeting, participants will be given ample opportunity to consider whether they wish to take part in the study. If, following the consideration period they wish to participate in the study, they will be asked to contact a member of the research team to arrange an appointment at the research facility for the pre-study clinical screening.

#### 4.7 Clinical screening

Clinical screening for this trial will be conducted as described in section 3.6. The only addition will be the assessment of platelet aggregation. This will be assessed by the research team and those participants not meeting the platelet aggregation response range defined in section 4.4 will be excluded from the trial.

#### 4.8 Participant randomization

Participants in this parallel design study will ingest 1.0 or 2.5 g tomato pomace extract incorporated into a drink or a placebo control drink, over a 5 day period. Each treatment will be assigned a letter (A-C). Each time a participant is successfully recruited onto the study, he will be assigned the next allocated treatment. Randomization to treatment will be assigned by the computer programme randomization.com. The participants will be blinded to the treatments.

#### 4.9 Preparation of tomato pomace drinks

The tomato pomace that will be used in phase 2 is that described in section 3.7. Immediately prior to ingestion, 'drinks' containing the appropriate mass of tomato pomace extract will be prepared at the research facility as follows:

- Low dose pomace extract – 1.0 g powder will be accurately weighed before dissolving in flavoured water to a final mass of 100 g
- High dose pomace extract – 2.5 g powder will be accurately weighed before dissolving in flavoured water to a final mass of 100 g
- Placebo control - 100 g flavoured water

Flavoured water is necessary to ensure that the participants are blinded to the test product consumed.

#### 4.10 Intervention procedure

The test arm will comprise a 5 day intervention period which will be preceded by a run-in phase of 14 days. For standardisation purposes all trial procedures between days 15 – 19 will be conducted in the morning time following an overnight fast. A test arm is described below.

Days 1-14 – During the 14-day 'run-in' period, participants will be instructed to avoid foods and beverages known to significantly contribute to anti-platelet activity. A 14-day period is thought to be sufficient as this comprises the average lifespan of platelets (~ 10 days) and allows for equilibration to dietary change. The foods and beverages to be avoided include tomato and all tomato based products, alcohol, cocoa and cocoa based products and green tea. In the event that a participant self-prescribes medication for minor illness during this time period, he will be instructed to contact a member of the research team who will assess the impact of the medication on platelet function and decide whether or not the run-in period should be re-scheduled.

Day 15 – Participants will arrive at the research facility on the morning of day 15 having fasted from 10 pm the night before. Prior to conducting any clinical procedures the participant will be asked some basic health related questions (Annex 9) to establish whether or not any changes in medical conditions/medications etc. have occurred since their last visit and that they are well enough to proceed with the study day assessment. A baseline (t=0h) fasted blood sample (15 mL) will be collected by venepuncture. Once collected, participants will ingest the allocated treatment drink. A further blood sample will be obtained 3-h post ingestion (15 mL) to assess the acute effects of tomato pomace ingestion on platelet aggregation. At the end of the assessment period participants will be free to eat and drink ad libitum.

Days 16 – 18 - Participants will return to the research facility each morning having fasted from 10 pm the night before. The allocated treatment will be prepared fresh each morning by a member of the research team and ingested by the participant whilst in the research facility. On departure, the participants will be instructed to continue avoiding the foods and beverages known to significantly contribute to platelet activity.

Day 19 – Participants will return to the research facility on the morning of day 19 having fasted from 10 pm the night before. Prior to conducting any clinical procedures the participant will again be asked some basic health related questions (Annex 9) for the reasons already described. A blood sample (15 mL) will be collected by venepuncture. The participant will once again ingest the allocated

treatment drink and a further fasted blood sample (15 mL) collected 3-h later. This completes participation in the trial.

In the unlikely event that a participant is unable to attend the pre-scheduled assessment days (e.g. minor illness) they will be given the opportunity to repeat the treatment at a later date. The total blood volume collected from each participant is 85 ml (incl. screening sample).

#### *4.11 Treatment compliance*

Each participant will ingest the allocated treatment at the research facility.

#### *4.12 Participant payments*

Each participant will receive an inconvenience allowance of 15,000 Chilean Peso upon completion of the study or pro-rata for non-completion. The inconvenience payment has been calculated as follows: 2500 Chilean Peso per study blood sample (x4 samples=10,000) and 5,000 Chilean Peso for compliance with dietary restrictions.

#### *4.13 Assessment of platelet function*

Whole blood will be collected by venepuncture directly into 3.2% sodium citrate tubes (9:1 v/v) after discarding the first 2.0mL of the draw. Tubes will be centrifuged at 240 x g for 10 mins to obtain platelet-rich plasma (PRP). After aliquoting a portion of the PRP for platelet count (hematologic counter) the tubes will be further centrifuged at 650 x g for 10 mins to obtain platelet-poor plasma (PPP). The PRP will be adjusted to  $200 \times 10^9$  platelets/ L with the PPP.

Aliquots of the adjusted PRP (480  $\mu$ L) will be pre-incubated (37<sup>0</sup>C; 3 min) before the addition of the platelet agonists ADP (final conc. 2 and 4  $\mu$ mol/L) and collagen (final conc. 0.5 and 1  $\mu$ g/mL). Platelet aggregation will be measured by light transmission according to Born and Cross using a Lumi-aggregometer. The result of platelet aggregation (% maximal amplitude) will be determined using the software AGGRO/LINK.

#### *4.14 Power calculations & statistical analysis*

The primary outcome measure for this study is platelet aggregation. The sample size has been powered on data from a previous study reported by O'Kennedy et al [13], which is a study not too dissimilar to this one. In this trial, the effect size in platelet response between treatment (18 g tomato extract syrup) and control after stimulation of platelets with collagen was 17.4% with a standard deviation of 19.5%. Assuming a similar maximum difference between the control and the 2.5 g dose of tomato pomace extract, and half this difference for the 1.0 g dose of tomato pomace extract then to gain sufficient evidence to reject the null hypothesis of no differences between the 3 groups at the 5% significance level with 90% power would require 33 participants per group to complete the trial. The data will be analysed using one-way between subjects analysis of variance or its non-parametric equivalent (Kruskal–Wallis test by ranks). Post hoc comparisons between groups will use Tukey's honest significant difference (HSD) multiple comparison procedure.

## **5. Ethical considerations (Phases 1 and 2)**

### *5.1 Reporting of adverse event/reactions and serious adverse events*

**Adverse events** are defined as any undesirable experience occurring to a subject during the study, whether or not considered related to the tomato pomace extract. An **adverse reaction** is defined as all noxious and unintended responses to the tomato pomace extract related to any of the doses tested. The phrase responses to the

tomato pomace extract means that a causal relationship between the product and an adverse event is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All adverse events and reactions reported by the subject or observed by the CI or his staff will be recorded (Annex 10). A serious adverse event (SAE) is defined as any untoward medical occurrence or effect that at any dose results in death, is life threatening (at the time of the event), requires hospitalization or results in persistent or significant disability or incapacity. SAE's must be reported by the CI immediately to the University of Talca ethics committee and the study sponsor (CEAP). In this instance the trial will be immediately suspended or terminated.

#### *5.2 Liability claim*

CEAP accepts responsibility for carrying out the tests, and as such will only consider claims of participants for any damage suffered as a result of a guilty performance of CEAP. Claims arising out of participant negligence shall not be considered. Please note that CEAP will not fund any legal costs arising from an action unless awarded by a court.

#### *5.3 Informed consent*

All participants will give written informed consent before they can participate in the trial. Consent will be considered informed on the basis of written documentation (participant information sheets; annexes 2 and 8) and verbal communication describing the purpose of the trial, relevant trial procedures and foreseeable risks and benefits. Informed consent will be obtained before any study specific procedures are conducted. It will be made clear to each participant that he is free to withdraw his consent at any time and without reason.

#### *5.4 Participant fasting*

This study requires participants to be fasted on assessment days. To reduce the length of the fasting period study measurements will be conducted in the morning. Participants will be able to drink ad libitum during the fasting period. At clinical screening, a blood sample will be collected for blood glucose analysis to exclude undiagnosed diabetic participants.

#### *5.5 Food safety*

Tomato pomace extracts will be properly stored at dry conditions and controlled room temperature prior to participant ingestion. Moreover, project staff will prepare drink samples according to the Chilean Food Health Regulations of the Ministry of Health to assuring food safety and hygiene standards.

#### *5.6 Blood sampling*

In this trial blood samples will be collected by venepuncture. It is possible that this may cause a local hematoma or bruise to occur. The procedure will be conducted by an experienced research nurse and this will minimize the risk.

#### *5.7 Amendments to study protocol*

Amendments are changes made to the research after a favorable opinion has been given by the University of Talca ethics committee. Any amendments to the study protocol will be notified to the University of Talca ethics committee for approval prior to implementation.

#### *5.8 Confidentiality*

Participants will be assigned a unique code which will be used to anonymize all biological samples and data arising from this trial. Anonymized information/data will be kept in locked cabinets at the CEAP research facility and processed and stored on a computer system. Anonymized study results will be transferred to the Institute of

Food Research (UK) for statistical analysis. Only the researchers named in the approved documentation will be able to link the code with the participant.

All personal information will be kept confidential and known only to the Chief Investigator and study personnel at the University of Talca. Personal information will be kept separately to coded information in locked cabinets at the CEAP research facility for a period of 5 years.

## 6. References

1. Law, M.R. and J.K. Morris, *By how much does fruit and vegetable consumption reduce the risk of ischaemic heart disease?* European journal of clinical nutrition, 1998. **52**(8): p. 549-56.
2. Michelson, A.D., *Platelets*. 3rd ed. 2013, London ; Waltham, MA: Academic Press. xlv, 1353 p.
3. Ruggeri, Z.M., *Platelets in atherothrombosis*. Nature medicine, 2002. **8**(11): p. 1227-34.
4. Davi, G. and C. Patrono, *Platelet activation and atherothrombosis*. N Engl J Med, 2007. **357**(24): p. 2482-94.
5. Hennekens, C.H., *Aspirin in the treatment and prevention of cardiovascular disease*. Annu Rev Public Health, 1997. **18**: p. 37-49.
6. Rao, A.V., *Lycopene, tomatoes, and the prevention of coronary heart disease*. Exp Biol Med (Maywood), 2002. **227**(10): p. 908-13.
7. Willcox, J.K., G.L. Catignani, and S. Lazarus, *Tomatoes and cardiovascular health*. Critical reviews in food science and nutrition, 2003. **43**(1): p. 1-18.
8. Sesso, H.D., et al., *Dietary lycopene, tomato-based food products and cardiovascular disease in women*. J Nutr, 2003. **133**(7): p. 2336-41.
9. Burton-Freeman, B. and H.D. Sesso, *Whole food versus supplement: comparing the clinical evidence of tomato intake and lycopene supplementation on cardiovascular risk factors*. Adv Nutr, 2014. **5**(5): p. 457-85.
10. Cuevas-Ramos, D., et al., *Effect of tomato consumption on high-density lipoprotein cholesterol level: a randomized, single-blinded, controlled clinical trial*. Diabetes Metab Syndr Obes, 2013. **6**: p. 263-73.
11. Silaste, M.L., et al., *Tomato juice decreases LDL cholesterol levels and increases LDL resistance to oxidation*. Br J Nutr, 2007. **98**(6): p. 1251-8.
12. Valderas-Martinez, P., et al., *Tomato Sauce Enriched with Olive Oil Exerts Greater Effects on Cardiovascular Disease Risk Factors than Raw Tomato and Tomato Sauce: A Randomized Trial*. Nutrients, 2016. **8**(3).
13. O'Kennedy, N., et al., *Effects of tomato extract on platelet function: a double-blinded crossover study in healthy humans*. Am J Clin Nutr, 2006. **84**(3): p. 561-9.
14. O'Kennedy, N., et al., *Effects of antiplatelet components of tomato extract on platelet function in vitro and ex vivo: a time-course cannulation study in healthy humans*. Am J Clin Nutr, 2006. **84**(3): p. 570-9.
15. Fuentes, E., et al., *Effect of tomato industrial processing on phenolic profile and antiplatelet activity*. Molecules, 2013. **18**(9): p. 11526-36.
16. Fuentes, E., et al., *Protective Mechanisms of S. lycopersicum Aqueous Fraction (Nucleosides and Flavonoids) on Platelet Activation and Thrombus Formation: In Vitro, Ex Vivo and In Vivo Studies*. Evid Based Complement Alternat Med, 2013. **2013**: p. 609714.

17. Rodriguez-Azua, R., et al., *Effect of tomato industrial processing (different hybrids, paste, and pomace) on inhibition of platelet function in vitro, ex vivo, and in vivo*. J Med Food, 2014. **17**(4): p. 505-11.