THE ViTaL-D STUDY

Investigating the Impact of Vitamin D Biofortified Tomatoes on Serum Levels of 25(OH)D.

Quadram Institute Science Health Food Innovation

John Innes Centre

UNIVERSITY OF SURREY

NHS Norfolk and Norwich University Hospitals NHS Foundation Trust

Invitation

We invite you to take part in the ViTaL-D study, a 21-day dietary intervention study (a research study where people change or add something to their diet to see how it affects their health) currently being launched by the Quadram Institute Bioscience (QIB) in collaboration with the University of Surrey, the John Innes Centre, and the Norfolk and Norwich University Hospital (NNUH).

What is the purpose of the study?

In this study we have developed a tomato that has been bred to increase their vitamin D content, we refer to this as biofortification. To do this, scientists have changed (gene edited) a specific part of the tomato plants' DNA (genes, inherited traits) so that it develops vitamin D in the fruit and leaves of the plant where usually there is very little. This gene-editing technology enables the tomato to accumulate (biofortify) provitamin D3, which converts to vitamin D3 after ultraviolet (sunlight) exposure, offering a vital dietary source especially for people following plant-based diets who typically lack vitamin D from plant foods.

This innovation addresses vitamin D insufficiency in a natural, accessible, and environmentally friendly way that supports plant-based nutrition with minimal changes to the tomato makeup, its growth, or appearance. Additionally, findings from this study may lay the groundwork to help us understand if these tomatoes could fill and/or supplement the nutritional gaps in diets that avoid animal products, contributing to better health outcomes in a growing population of vegetarians, vegans, or those who prefer whole foods over supplement tablets.

Vitamin D deficiency is a significant problem in the UK. It is estimated that 1 in 5 people in the UK can be deficient during the winter and spring. Vitamin D is a nutrient that our bodies need to stay healthy, and it's sometimes called the "sunshine vitamin" because your body can produce it when your skin is exposed to sunlight.

Vitamin D helps the body absorb calcium and phosphorus which is important for building and maintaining strong bones, teeth and muscles. It also helps support your immune system to fight off infections and illnesses. Some research also links low vitamin D levels to mood disorders such as depression.

In the UK from late spring to summer (April – September time) most people can produce the vitamin D that they need from direct sunlight on the skin when outdoors. In the autumn and winter there is not as much sunlight so your body cannot make enough vitamin D alone.

Taking vitamin D supplements and eating foods higher in vitamin D is recommended in these winter months (year-round for some people). However, most foods are very low in vitamin D, especially plant sources.

People are more likely to have vitamin D deficiency if they; have darker skin (e.g. have an African, African-Caribbean or south Asian background), are over 65, are pregnant or breastfeeding, are obese, or do not get much sun exposure (from covering their skin, or those that are confined indoors for long periods).

The aim of this study is to compare whether the soup made with the biofortified tomatoes with higher levels of vitamin D would result in higher levels of active vitamin D in the blood of those who have eaten it, compared with people that have eaten a normal tomato soup.

To test this, we will make four/three [delete as appropriate] different soups:

1) Made from the biofortified tomatoes (high pro-vitamin D, premature form of vitamin D) [delete and renumber as appropriate].
2) Made from biofortified tomatoes that have been exposed to UV light (UV light matures the vitamin D into a form that is used by your body, similar to a supplement).
3) Made with tomatoes that have not been biofortified.
4) Made with tomatoes that have not been biofortified but have an added supplement of vitamin D (we use a vitamin D supplement called lanolin which is made from a waxy substance found on sheep wool which means this is suitable for vegetarians but not vegans).

We will look at how each of these soups affect the levels of vitamin D in participants that consume the soup every day for 21 days (alongside your normal diet). If you took part, you would not know which soup you have been given (and the study team would also not know until after the trial has happened).

We will not ask you to change any element of your diet or normal supplementation/medication routine to take part in this study.

Why have I been chosen?

We hope to recruit 76 healthy participants. You have been approached because one or more of the following applies:

- You are signed up to our participant database and consented to be informed of research studies at the Quadram Institute.
- You have seen an advert/poster/news article about the study and have requested further information from us about the study.
- You have been identified by your GP to be informed of this study because you may have had low vitamin D levels in prior blood tests.

To join the study, you will need to:

- Be willing to eat a tomato soup daily for 21 days.
- Have access to a smartphone or tablet device and internet access.
- Be at least 18 years old.
- Be able to understand the study and provide informed consent.
- BMI 18.5 to 35.
- Live within 70 miles of the Quadram Institute.
- Have blood vitamin D (25-hydroxyvitamin D (25[OH]D)) levels between 25-40 nmol/L (we will collect a blood sample at the screening visit to test what your levels are so don’t worry about knowing what your levels are before).

You will not be able to join the study if you:

- Have a known allergy or intolerance to tomatoes or to any of the soup components (see ingredients list below) of the test soups.

Soup Ingredients

Water, Tomatoes, Soup Base (Vegetables: [Swede, Onion, Carrot, Leek, Peas], Glucose Syrup, Maize Starch, Vegetable Oils [Palm, Sunflower], Palm Fat, Sugar**MILK** Proteins, Parsley, Flavourings (Contain **CELERY**), Emulsifier (Mono And Diglycerides Of Fatty Acids), Ground Turmeric, Sage Extract], **MILK** Proteins, Salt, , Flavour Enhancers (Monosodium Glutamate, Disodium 5' Ribonucleotides), Yeast Extract (Contains **BARLEY**), Stabilisers (E331, E452), Acidity Regulator (E340), Emulsifiers (E471, E472e), Anti Caking Agent (E170), Maltodextrin, Colour (Beetroot, Riboflavin), and Vitamin D (Lanolin).

ALLERGY ADVICE: For allergens, see ingredients in CAPS & **bold**. This product may also contain traces of Soya

- Have a hypercalcaemia, hyperparathyroidism, kidney stones, bone diseases (osteomalacia) or sarcoidosis.
- Have anaemia, immunocompromised or viral infections that may compromise immunity. This will be assessed on a case-by-case basis by Quadram Institute Medical Advisor.
- Have any acute or chronic illnesses that affects the outcome of the study such as a gastrointestinal disorder. This will be assessed on a case-by-case basis by the Quadram Institute Medical Advisor.
- If you have just started taking or changed your dose of a vitamin D supplement in the last month or at any point during the study or if you are regularly taking more than 400 International Units (IU) of vitamin D (equivalent to a '1-A-Day' vitamin D supplement containing 10 micrograms (µg) of vitamin D).
- Are on any medication regimen that may alter vitamin D absorption they are as follow but not limited to bile acid sequestrants, hormone replacement therapy, thiazides, calcium channel blockers, isoniazid, anti-seizure medications, and antacids.
- Any dietary restriction that prevents the consumption of the study soups or follow a diet programme which requires fasting for multiple days (soups are not suitable for vegans).
- Have abnormally low or high blood pressure (i.e. ≤90/60, ≥160/100).
- Plan to become pregnant during the study duration, pregnant or breastfeeding.
- Are currently taking part in an intervention study.
- Are a registered blood donor and have donated a large quantity of blood within the last 6 weeks. Registered blood donors should abstain from blood donations for the duration of the study.
- Are unable to give written or verbal informed consent.
  Unable to provide your GP contact details (this can be just the surgery details if you do not know the name of your GP).
- Fall under the line management structure or live in the same household with any of the research team, or the QIB Human Studies Team.
- Have a physical or mental health condition that might make your participant in the study difficult or unsafe, or that could affect the study results. This will be assessed on a case-by-case basis by Quadram Institute Medical Advisor.

Do I have to take part?

No, it is entirely up to you to decide if you would like to take part. If you do decide to take part, you are free to withdraw from the study at any point, without giving a reason. To withdraw from the study, you can inform a member of the study team at any of your study visits or contact the study team via email ViTaL-D@quadram.ac.uk to let them know you have decided to withdraw from the study.

If you decide not to take part, or to withdraw from the study at a later point, this will not affect any medical care you receive or affect your participation in future studies.

If you withdraw from the study, we will stop collecting any more samples or data from you. With your consent, we will keep any samples or data that have already been collected in the study until the point you have withdrawn. We will keep your consent form and contact information after withdrawal; we will use it for safety/ audit purposes only for at least 15 years after the study.

What would I have to do if I decide to take part?

This is a dietary intervention study that lasts approximately 21 days. There are four/three [delete as appropriate] groups of participants in the study;

1) **Group 1** – eats 1 soup daily made from biofortified tomatoes. These tomatoes have been genetically changed to produce a substance (7-DHC) that can turn into vitamin D in your body. By testing these, we want to see how much this genetic change alone affects your vitamin D levels, without any extra treatment like UV light. [delete this arm as appropriate]
2) **Group 1/2** [delete as appropriate] – eats 1 soup daily made from biofortified tomatoes that have been exposed to UV light. These are the same genetically changed tomatoes as above, but they've also been exposed to UV light, which helps boost their vitamin D content. We're testing these to see if they work as a good natural source of vitamin D after the UV treatment.
3) **Group 2/3** [delete as appropriate] – eats 1 soup daily made from tomatoes that have not been biofortified. These are regular tomatoes with no changes made to them and no added supplements. We're using them as a baseline, so we can compare how the other tomatoes affect your vitamin D levels compared to just eating normal tomatoes.
4) **Group 3/4** [delete as appropriate] – eats 1 soup daily made from tomatoes that have not been biofortified but have an added supplement of D3 (we use a vitamin D supplement called lanolin which is made from a waxy substance found on sheep wool). These are regular tomatoes, but we've added a vitamin D3 supplement to them. We're testing these to see if eating them with a supplement is as effective (or better) than eating the gene-edited tomatoes that naturally produce vitamin D. The quantities of vitamin D are consistent with group 2 (UV light exposed biofortified tomatoes).

The study team will not know which group you have been assigned to until after the study visits have finished and the data has been analysed, we call this "blinding", and we use this method to try to make sure we are not bias to any group in the study when we analyse the results.

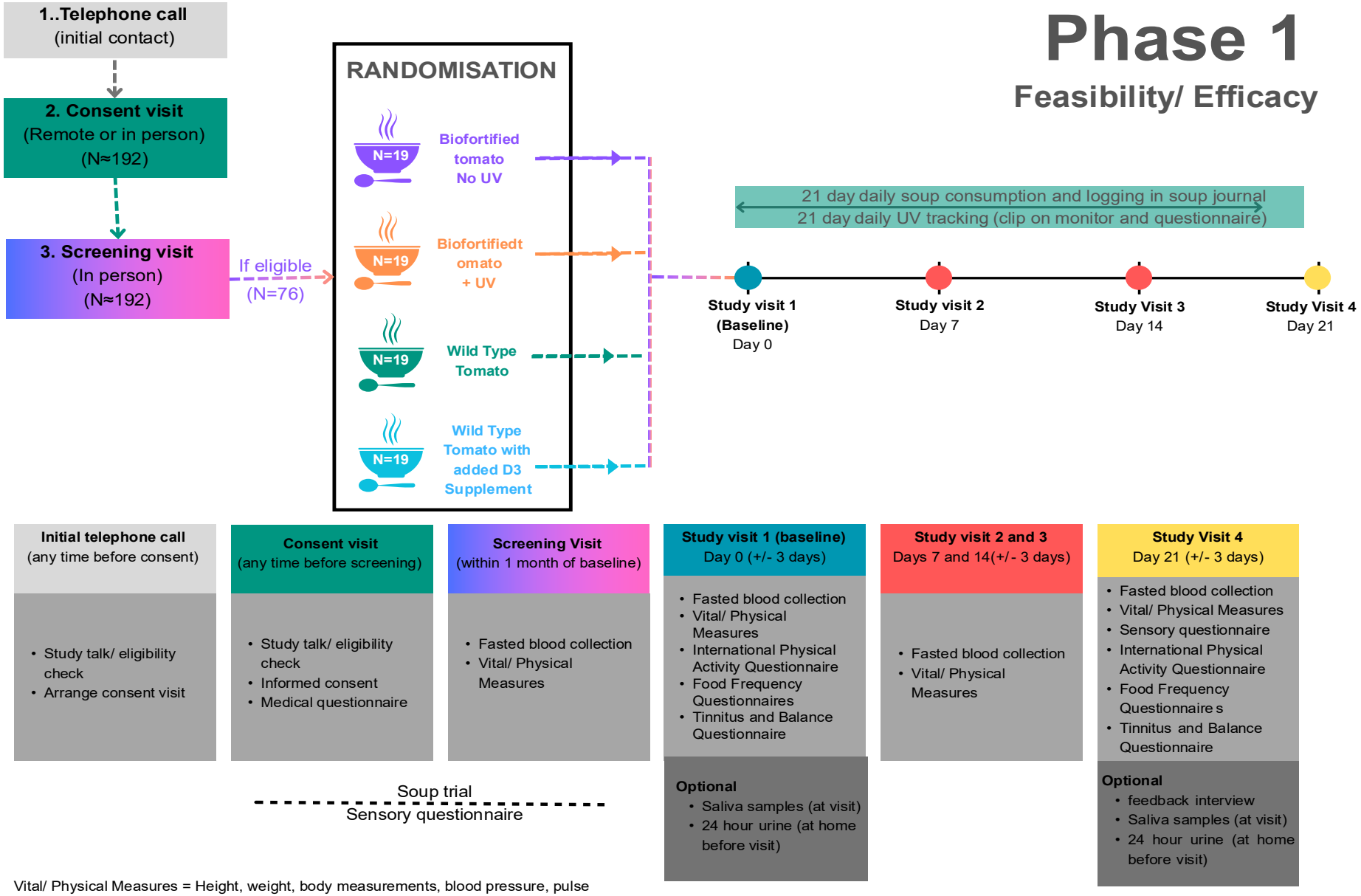
As vitamin D can be produced by skin UV exposure, it is important that we understand the level of UV exposure that you have had during the study, so we will also be asking you to wear a UV tracker every day that you are a part of the study. The UV tracker is clipped onto your clothes and is monitored using a third-party app (sun-a-wear) which you will need to download to your mobile phone – we will set you up an account using a Quadram Institute email using a unique participant number so that you do not need to enter your personal details such as your name. We will download this data from the app at the end of the study.

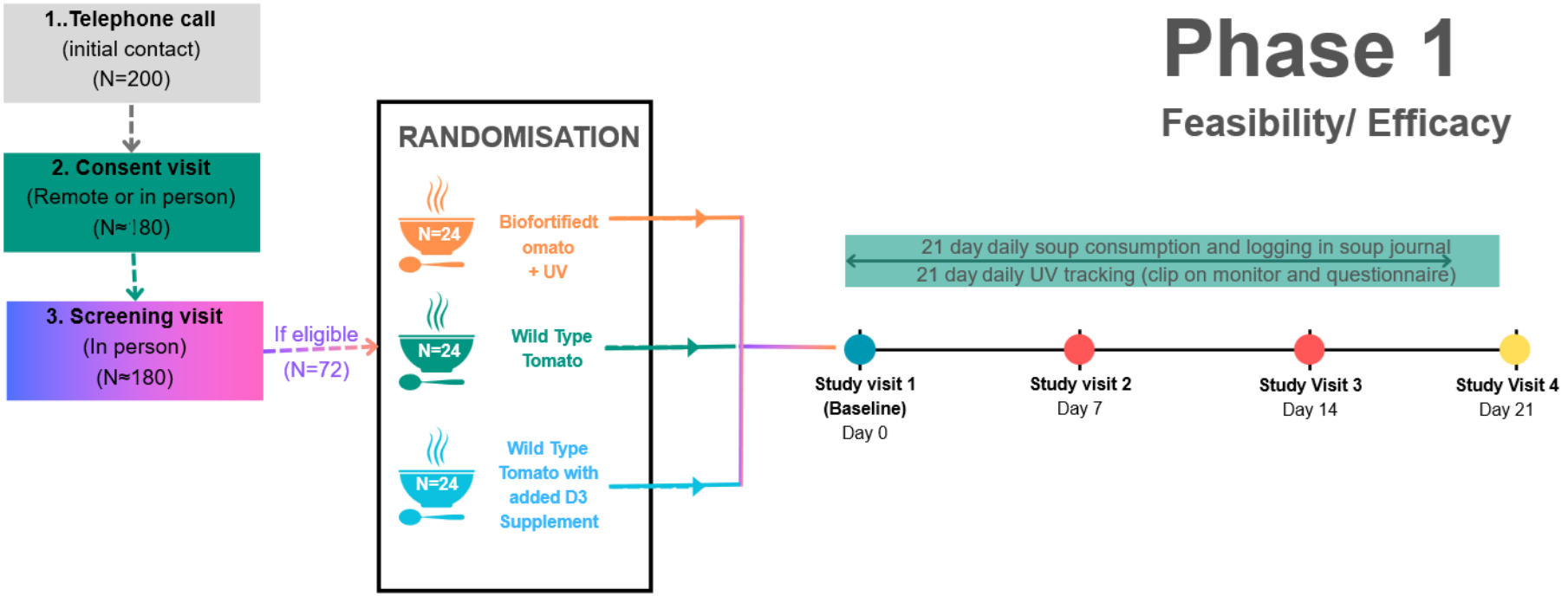
We will also ask you to complete a daily journal that will ask you if you have had any foods that are high in vitamin D, and how much of your skin was exposed to UV light when you went outside. We expect that the time commitment outside of the appointments below would be approximately 30 minutes every day for 21 days.

The table below shows an overview of each of the study appointments for the ViTaL-D study and you will find more information about each of the study visits on the next page. In total we would ask you to attend the Quadram Institute 6 times throughout the study (or 5 times if you chose to have a video consent visit). These visits would last between 30 minutes – 1.5 hours.

Prior to all study visits, you will be asked to avoid alcohol for 72-hours, as well as not take part in any intense exercise the evening before (such as playing rugby/football/netball, hiking, jogging, or activities which cause heavy sweating).

Figure 1. ViTaL-D study overview [delete 4 or 3 arm study figure as appropriate]





Initial telephone call (any time before consent)	Consent visit (any time before screening)	Screening Visit (within 1 month of baseline)	Study visit 1 (baseline) Day 0 (+/- 3 days)	Study visit 2 and 3 Days 7 and 14(+/- 3 days)	Study Visit 4 Day 21 (+/- 3 days)
• Study talk/ eligibility check • Arrange consent visit	• Study talk/ eligibility check • Informed consent • Medical questionnaire	• Fasted blood collection • Vital/ Physical Measures	• Fasted blood collection • Vital/ Physical Measures • International Physical Activity Questionnaire • Food Frequency Questionnaires • Tinnitus and Balance Questionnaire	• Fasted blood collection • Vital/ Physical Measures	• Fasted blood collection • Vital/ Physical Measures • Sensory questionnaire • International Physical Activity Questionnaire • Food Frequency Questionnaires • Tinnitus and Balance Questionnaire
			Optional • Saliva samples (at visit) • 24 hour urine (at home before visit)		**Optional** • feedback interview • Saliva samples (at visit) • 24 hour urine (at home before visit)

Soup trial
Sensory questionnaire

Vital/ Physical Measures = Height, weight, body measurements, blood pressure, pulse

The Study Facility

The NIHR (National Institute for Health and Care Research) Clinical Research Facility (CRF) is an NHS facility managed by staff from the Norfolk and Norwich University Hospital (NNUH) and located in the Quadram Institute.

NIHR Norfolk Clinical Research Facility
Quadram Institute
Rosalind Franklin Road
Norwich, NR4 7UQ

Description of appointments

The study team will send you reminders before each of your appointments.

While there is flexibility in when we hold your initial call, consent, and screening visit. For the remaining visits it is important that they are held on specific days. The first study visit must be within 1 month of your screening visit, and the remaining visits (Study visits 2, 3 and 4) are on set days (7 days apart) to maintain consistency between participants which means the information we get from the study is as reliable and meaningful as possible. We will arrange these dates with you so that they fall on convenient dates for you.

1. Telephone contact (initial contact) (15 minutes)

If you reach out to us to let us know you are interested in finding out more, we will phone you to discuss the study and answer any questions you might have. We check the eligibility criteria that we can over the phone, and if you meet those criteria, we will book you in for a consent visit at the National Institute of Health and care Research (NIHR) Norfolk Clinical Research Facility (CRF) which is based at the Quadram Institute. We will provide you with a copy of the consent form that we will discuss with you at this appointment so that you have time to think of any questions you might like to ask.

2. Consent visit (30 minutes)

This visit gives you the opportunity to meet the study team and see where the study is held. We will go through the study in detail with you and answer any questions you may have. If you decide to continue, we will ask you to complete a consent form. A copy of your consent form will be sent to your GP with a copy of this Participant Information Sheet to be inserted into your personal medical records. You will also be given a copy of the consent form to keep.

If you would rather do this visit via video chat from home (remote consent), we can arrange this too. You would need to have access to a computer/tablet with good internet connection at home for us to arrange this. Please let one of the team know if you would prefer this to an in person visit.

Once you have completed the consent form, we will also ask you to complete a medical questionnaire to check that you meet some of the requirements of the study.

3. Screening visit (30 minutes)

To check you are eligible and that it is safe for you to take part in the study, we will need to collect some physical measurements (height, weight, blood pressure, pulse, hip/waist measurements) and some blood samples from you. We will send these blood samples for analysis and let you know within 2 weeks whether you meet the criteria to join the study. We will also provide a copy of these results to your GP.

The blood samples that we need are fasted blood samples which means that you cannot eat or drink anything other than plain, unflavoured water within **12 hours** of your appointment. We know that fasting can be difficult, so to make it as easy as possible, we will arrange your appointment for the morning so that most of your fast will be overnight. We will also provide refreshments for you at your appointment for after you have had your blood sample collected.

We ask participants to fast before their blood tests because eating can affect the levels of certain substances in the blood, like vitamins and hormones. Fasting ensures that the results we get are accurate and not influenced by recent meals. This helps us get a clear picture of how their body is processing vitamin D and other nutrients related to the study. We also ask that you do not drink any alcohol within 72 hours (3 days) of your appointment or take part in any in any intense exercise the evening before (such as playing rugby/football/netball, hiking, or jogging at 6mph).

If you are eligible, we will arrange your first study visit at the Quadram Institute at a convenient time for you. This should be within ***2 months*** of your screening visit.

To make sure that you are happy to eat the tomato soup for 21 days, at the end of the screening visit, we will provide you with a soup to try between the screening visit and your first study visit. When you eat this trial soup, we would like to ask you to complete a sensory questionnaire (within 1 hour of eating the soup if possible) that will ask you things like how you found the taste and texture of the soups.

4. Study visits 1, 2, 3, and 4

During this 21-day stage of the study, we will ask you to eat your assigned soup and wear a clip-on UV monitor (that clips onto your clothes) every day for 21 days. We will ask you to record in a daily diary any soup that you eat, and information on how much skin you have had exposed when you go outside (for instance, were you wearing long sleeves, or did you have suncream on).

It's really important that you don't miss any of your daily soups or UV monitoring because these are key parts of the study. However, if for any reason you have not been able to consume your soup, or log your UV exposure, we ask that you note this in your daily diary.

During this stage in the study, we will ask you to visit the CRF at the Quadram Institute 4 more times. At each appointment we will:

- Collect a fasted blood sample from you which means that you cannot eat or drink anything other than water for 12 hours before your appointment. We will provide refreshments for you for after you have had your blood sample collected.
- Collect some physical measurements from you (height, weight, blood pressure, pulse, hip and waist measurements).
- Collect spot urine at the start of your study visits to measure how your body has absorbed and broken down the tomato soup.
- Provide you with the soups you need until your next appointment (receive 9 soups at your first visit and 8 soups for Visit 2 and Visit 3, meaning you will receive 25 soups in total).
- Provide UV tracking equipment that you need until your next appointment.

On the first and last study visits (study visit 1 and 4) we will ask you to complete a series of questionnaires that ask you about your diet and lifestyle. These questionnaires should take about 45 minutes to complete.

Within an hour of eating the last soup (where possible), we would like to ask you to complete another sensory questionnaire (the same as the one you completed at the beginning of the study) that will ask you things like how you found the taste and texture of the soups.

Optional extras

We are also asking participants if they would be willing to take part in 3 optional parts to this study. You can take part in the study without doing any of these:

a. **Saliva samples (study visits 1 and 4)** - Our trained study team will show you how to collect these at your appointment if you choose to donate these samples. For the saliva samples, it is very important that you do not smoke 1 hour before your visit, and do not chew gum or brush your teeth during the fasting period (12 hours before your appointment).

b. **24-hour urine samples (study visit 1 and 4) -** If you would be willing to provide urine samples, we will provide you with a large collection container to take home with you to collect your urine for 24 hours before to your study visit 1 and 4 appointments.

c. **Feedback interview** - On your very last visit with us (study visit 4) we would also like to ask you a few feedback questions to see how you found taking part in the study. If you decide to take part in this element, you can choose to provide your answers verbally (which we will record), or as a video recording. Audio recordings will be turned into written text by one of our team at the Quadram Institute. We will use these quotes, and audio/video recordings in publications, presentations and at outreach events to increase awareness of, and to improve the participant experience of taking part in human research at the Quadram Institute. We will share with you any quotes/ videos made from these interviews before we publish them and we will give you the opportunity to change your mind about them being made public.

Frequently Asked Questions

What are you specifically testing, and what will happen to my samples?

Blood samples

We will test your blood samples for:

1) **25-hydroxyvitamin D -** Vitamin D must go through several processes in your body before your body can use it. The first step happens in your liver where vitamin D is turned into to a chemical known as 25-hydroxyvitamin D. The 25-hydroxy vitamin D test is the best way to monitor vitamin D levels. The amount of 25-hydroxyvitamin D in your blood is a good indication of how much vitamin D your body has.
2) **Parathyroid hormone (PTH) –** Your liver and kidneys need an enzyme to turn vitamin D into the form that is useful for your body. Parathyroid hormone is responsible for increasing the activity of this enzyme.
3) **Calcium and phosphorous:** these tests will help us monitor how vitamin D and parathyroid hormone affect these minerals in your blood.
4) **Serum cholesterol and triglycerides (Study visit 1 and 4) -** These are fats in the blood. We want to see if the daily soup consumption affects cholesterol and triglyceride levels, which are important for heart health.
5) **Urea and electrolytes (Study visit 1 and 4) –** These tests help us monitor how well your kidneys are working throughout the study, ensuring that the tomato soup has not altered your electrolytes or changed the normal physiology of this vital organ.
6) **Liver Function Tests (Study visit 1 and 4):** These tests help us monitor how well your liver is working, ensuring that the tomato soup doesn't negatively affect this vital organ.
7) **Full blood count (Study visit 1 and 4) -** To check your blood for any obvious signs that you may not be able to absorb Vitamin D (for instance, if you are anaemic).

These samples will be processed at the Norfolk and Norwich University Hospital (NNUH) and the results will be added to the hospital's electronic results system where your GP can access them. These samples will have your personal information recorded on the blood tubes (fully identifiable to you) so that the results will appear on your hospital medical records and can be accessed by

your GP, but these samples will not be stored after they have been analysed or accessed outside of the NNUH laboratory team.

We will also analyse your blood samples at the Quadram Institute for the following tests:

1. **Bone health markers (P1NP and CTx)** - These tests help us see if there are any changes in bone health during the study by looking at proteins linked to bone growth and breakdown.
2. **Plasma carotenoids and lycopene levels** - Carotenoids and lycopene are nutrients found in tomatoes. We're checking how much of these nutrients get absorbed into the body from the soups and if participants are sticking to the soup diet.
3. **Serum 7-DHC, 1,25(OH)2D, 24,25(OH)2D, and C-3 epi 25(OH)D:** These tests give us more detailed information about how your body is processing vitamin D. They help us understand how effective the tomato soups are at improving your vitamin D status.

These results are not clinical tests and useful only for research purposes so will not be notified to your GP.

Saliva samples

We are collecting saliva samples to explore if daily tomato soup consumption affects two different aspects of oral breakdown and absorption of vitamin D.

1. **Vitamin D binding proteins** - These are proteins in saliva that help carry vitamin D through the body. These proteins are important to help understand how different individuals move vitamin D to the rest of the body where it's needed, and by checking saliva, we can see if the soups have any impact on how well vitamin D is transported.
2. **Salivary 7-DHC, 1,25(OH)2D, 24,25(OH)2D, and C-3 epi 25(OH)D** – enzymes (compounds which help change the chemistry) which convert vitamin D from soups into their physiologically active form are found in gums. By examining the saliva for these chemicals give us more detailed information about how your body is processing vitamin D. They help us understand how effective the tomato soups are at improving your vitamin D status.

These samples will be processed at the Quadram Institute. These results are not clinical tests and so will not be notified to your GP.

Urine samples

We'll collect two distinct urine samples.

1. **Urine Riboflavin** – a spot urine will be collected to test to measure the riboflavin (vitamin B2) how your body passes this vitamin in your urine after you have consumed tomato soup. This measures your adherence to the tomato soups.
2. **Urine 7-DHC, 1,25(OH)2D, 24,25(OH)2D, and C-3 epi 25(OH)D** – Urine collected over 24 hours will be measured to give us detailed understanding of how much vitamin D and related compounds are excreted, which helps us understand how different indviduals processes vitamin D from the soups.

These samples will be processed at the Quadram Institute. These results are not clinical tests and so will not be notified to your GP.

Any remaining sample after we have done the tests above (blood, urine, or saliva) will be stored at QIB with only your unique participant identification number (none of your personal information will

be on the sample containers) until the end of the study when we will destroy your sample, unless you give us your permission to donate your sample for use in further research. If you give us this permission, we will transfer your samples to the Norwich Research Park (NRP) Biorepository where they may be used for future ethically approved research after the ViTaL-D study has finished. For more information about the NRP Biorepository please see their website here: www.biorepository.org.uk.

Physical Measurements (height, weight, BMI, pulse, blood pressure, hip and waist measurements)

We collect these physical measurements to gain a comprehensive understanding of your baseline health status and monitor any potential changes throughout the study. These indicators, including body mass index (BMI) and blood pressure, may influence vitamin D absorption and overall response to the intervention. Additionally, the amount of pigmentation of your skin and the tendency of your skin to burn or tan are important factors on how you may respond to the tomato soups and how your body responds to vitamin D. We will scan your hands and arms with a special camera that measures the colour of your skin into a number value.

Daily Journal - UV tracker and skin exposure questionnaire

We ask participants to log their skin exposure to the sun along with wearing a UV tracker (Replior) to better understand how much sunlight they're absorbing. The amount of exposed skin affects how much vitamin D the body can produce, while the UV tracker measures the strength and duration of sunlight. By combining this information, we can more accurately assess the effects of sunlight on their vitamin D levels and separate it from the impact of the soups in the study. The UV tracker is made by company in Sweden called Replior. When you download the app, we will provide you with log in details under a pseudonym (we will use a code instead of your name) to protect your identity as far as possible, however, the app will collect location data within a 5km proximity to provide accurate UV index information. The data is transmitted securely via the app and stored in their sister company, Complior, data centres in Sweden.

A variety of questionnaires are used in this study to help researchers understand how differences in an individual such as how they like or dislike the soup; what they eat in their regular diet; and the amount of physical activity they do can impact how vitamin D levels change in an individual after they have eaten the tomato soups. Additionally, there is a questionnaire for some individuals who may experience balance disturbances or ringing in the ears.

1. ***Sensory evaluation questionnaire***

This questionnaire is to understand how you experience the soups in this trial, including how full they make you, and how you think they look and taste etc. so that we know for any future trials how to improve the soups if needed. *Notably, this is a whole tomato product which includes the seeds and skins of the tomato.*

2. ***Food Frequency Questionnaire (FFQ) and International Physical Activity Questionnaire***

These questionnaires will ask you about your normal diet and lifestyle. These questionnaires will give us important background information that can help us to really understand how these soups would affect different groups of people. These questionnaires are completed online using a third-party website, the company, Viocare, is based in the USA – we will set you up an account under your unique participant number so that you do not need to enter your personal details and we will

ask you to complete these at your appointments on our tablets so they will not be able to access details of your personal devices.

3. Food Frequency Questionnaire for Vitamin D Intake

This questionnaire will ask you about the vitamin D-rich foods in your normal diet. This is to complement the FFQ described above. This will give us important background information that can help us to understand how your background diet affects your vitamin D levels when you eat the soups. These questionnaires are completed online using our institute's REDCap (Research Electronic Data Capture) software and we will ask you to complete these at your appointments on our Tablets.

4. Tinnitus and Balance Disturbance

This questionnaire will only need to be complete if you currently have ear or head noises (eg ringing) or balance disturbances. Studies suggest that there may be a link between low vitamin D and such symptoms. Those experiencing these symptoms will be asked to complete this questionnaire to understand and quantify the level of burden they are experiencing with these symptoms. These questionnaires are completed online using our institute's REDCap (Research Electronic Data Capture) software and we will ask you to complete these at your appointments on our Tablets.

What are the risks and benefits of taking part in the study?

There are no direct benefits to you by taking part of the study. However, this is the first in man human study involving gene edited tomatoes biofortified with provitamin D. Gene editing (e.g. CRISPR) alters genes (inheritable traits) that already exist within the tomato plant and avoids insertion of foreign DNA genes like genetically modified (GMO) foods. Gene edited foods such as tomatoes, soybeans, canola, mushrooms, and fish have been consumed since 2019 in the US and 2021 in Japan. In both these countries gene-edited foods have been regarded as safe as traditionally bred foods, with no evidence of its harm to health to date.

This study has 3/4 [delete as appropriate] groups, and we do not know which you will be assigned to while you are taking part in the study. You may not receive the soups made with the gene edited tomatoes. We also do not know if the biofortified (gene edited) tomatoes will have the desired effect of raising vitamin D levels. By participating, individuals will contribute to advancing scientific understanding of how biofortified foods, like vitamin D-enhanced tomatoes, can improve vitamin D levels. This research could lead to better dietary strategies for addressing vitamin D insufficiency in the wider population.

All food products provided in this study are microbiologically tested for your safety.

There can be slight discomfort associated with taking blood from a vein. You may develop a bruise at the site of the blood sample, which should fade over a few days. If the bruise becomes increasingly more painful or red, please notify the study team as soon as possible.

Do I get paid for doing this?

While participation in the study is voluntary, we do recognise that taking part may cause you some inconvenience. As a thank you for taking part in the study, we will reimburse you to the maximum total value of £75 at the end of your participation in the study broken down as follows:

- £15 - attendance at a screening appointment
- £15 for each attendance at study visits 1 (baseline), 2, 3, and 4

Mileage to the Quadram Institute (current rate is 45p/mile) along with any parking charges in the Quadram Institute visitor car park will be reimbursed. Public transport fares will be reimbursed with a valid receipt. Participants may require transport by taxi, if they can't reach the CRF by car or public transport. In this case, a taxi will be arranged by the study team and pre-paid.

The inconvenience payment and travel expense reimbursement will be made after completion of your screening visit and after the completion of the study, at study visit 4 (week 3) or earlier if you are excluded or withdraw from study. Alternatively, upon request payments can be made after study visit 2 and study visit 4. We will need you to bring your bank details along with you to your screening appointment so we can fill in the forms to reimburse you.

What if new information becomes available or the study changes?

If there are changes to the study or new information becomes available, we will contact study participants. If these changes are significant, you may be asked to sign another consent form.

What if there is a problem, or I would like to make a complaint?

If you have any concerns or complaints about the study, you can speak to the Principal Investigator Dr Jennifer Ahn-Jarvis (jennifer.ahn-jarvis@quadram.ac.uk, 01603255052) or Co-Investigator Dr Oana Ancu (oana.ancu@quadram.ac.uk) who can address any queries. If your concerns or complaints remain unresolved, you can contact the chairperson of the QIB Human Research Governance Committee (HRGC), Dr Antonietta Hayhoe, either by telephone (01603 255030) or email (antonietta.hayhoe@quadram.ac.uk).

If you wish to complain or have any concerns about the way you have been treated whilst taking part in this study at the NNUH clinics (NIHR Norfolk CRF), there will be a local hospital complaints procedure that you can follow. The PALS (Patient Advice and Liaison Service) is a confidential service designed to support patients, relatives and carers. Their website is pals@nnuh.nhs.uk and the office has an answerphone which is available 24 hours a day: 01603 289036 or 01603 289045.

What if something happens to me while I am on the study?

QIB accepts responsibility when conducting clinical studies and as such will give consideration to claims from participants for any harm suffered by them as a result of participating in the trial, with the exception of those claims arising out of negligence by the participants. QIB has liability insurance in respect of research work involving human participants. Please note that the institute will not fund any legal costs arising from any action unless awarded by a court. As this study involves the NIHR Norfolk CRF, which is an NHS facility, indemnity is provided through the NHS.

Will my GP be informed?

Yes, it is routine practice to inform your GP of your participation in a study at the Quadram Institute. When you give your consent by signing the informed consent form you have agreed that we are allowed to contact your GP.

How will we use information about you?

We will need to use information from you for this research project.

This information will include your:

- Name
- Date of Birth
- Contact details
- NHS number (needed to generate requests laboratory test)
- Address of your General Practitioner (GP)
- General health, lifestyle and dietary information collected from questionnaires
- Physical measurements (height, weight, BMI, pulse, blood pressure, hip and waist measurements)
- Data produced from samples you provide (blood, and if you choose to also provide urine and saliva samples)
- Data collected from the UV monitor you will wear
- Clinical test data (blood test results)

People will use this information to do the research or to check your records to make sure that the research is being done properly.

People who do not need to know who you are will not be able to see your name or contact details. Your data will have a code number instead.

Quadram Institute Bioscience is the sponsor and data controller of this research and is responsible for looking after your information. We will keep all information about you safe and secure by:

- All personal and study-related data is stored on secure, encrypted databases accessible only to authorized team members. Physical documents, such as consent forms, are kept in locked, secure storage at our facilities.
- To protect your privacy, we replace your personal identifiers (such as your name) with unique codes whenever possible. This ensures that your information is not directly linked to your identity in study records.
- Access to your personal information is limited to key research team members who need it for study purposes. Strict access control ensures that only approved personnel can view or handle your data.

Please note that the Quadram Institute has CCTV cameras in use for security purposes.

International data transfers

We will share data about you outside the UK for research related purposes:

- To administer and analyse your food frequency questionnaire and international physical activity questionnaire which is done by an American company called Viocare. The data collected from the FFQ will have no personal or identifiable information. Information about your location will not be shared since questionnaires will be completed during your clinic visits. The data will be held and analysed by VioCare®. All the data collected from the questionnaires will be kept anonymous (no personal identifying information), so no data management agreements are required.
- To log and analyse your UV exposure using the UV monitor and app. The UV monitor is developed by a Swedish company called “Replior”. No UV data is stored in the mobile devices used to connect the the UV sensor. The GPS position is collected from the mobile

device when sending data to the back-end system (server, data processor, etc). Replior have stated that stated they will not be able to identify you.

We will only share the data that is needed. We will also make sure you can't be identified from the data that is shared where possible. This may not be possible under certain circumstances – for instance, if you have a rare illness, it may still be possible to identify you. If your data is shared outside the UK, it will be with the following organisations:

- The company that administers the Food Frequency and International Physical Activity Questionnaires, as well as the UV monitor company.

We will make sure your data is protected. Anyone who accesses your data outside the UK must do what we tell them so that your data has a similar level of protection as it does under UK law. We will make sure your data is safe outside the UK by doing the following:

- (some of) the countries your data will be shared with have an adequacy decision in place. This means that we know their laws offer a similar level of protection to data protection laws in the UK.
- we use specific contracts approved for use in the UK which give personal data the same level of protection it has in the UK. For further details visit the Information Commissioner's Office (ICO) website.
- we need other organisations to have appropriate security measures to protect your data which are consistent with the data security and confidentiality obligations we have. This includes having appropriate measures to protect your data against accidental loss and unauthorised access, use, changes or sharing.

Once we have finished the study, we will keep some of the data so we can check the results. We will write our reports in a way that no-one can work out that you took part in the study.

We will keep your study data for the minimum period of time required by our internal policy which is 15 years. The study data will then be fully anonymized and securely archived or destroyed.

What are your choices about how your information is used?

- you can stop being part of the study at any time, without giving a reason, but we will keep information about you that we already have
- you have the right to ask us to remove, change or delete data we hold about you for the purposes of the study. We might not always be able to do this if it means we cannot use your data to do the research. If so, we will tell you why we cannot do this
- If you agree to take part in this study, you will have the option to take part in future research using your data saved from this study. For more information about the NRP Biorepository please see their website here: www.biorepository.org.uk.

Where can you find out more about how your information is used?

You can find out more about how we use your information, including the specific mechanism used by us when transferring your personal data out of the UK.

- our leaflet [**www.hra.nhs.uk/patientdataandresearch**]
- by asking one of the research team
- *If you have any concerns about our use of your personal information, you can make a complaint to our Data Protection Advisor by email (dpa@nbi.ac.uk) or phone (01603 450067).*

- *You can also complain to the Information Commissioner's Office (ICO). The ICO can be reached through helpline number 0303 123 1113. More information can be found on the ICO website: https://www.ico.org.uk.*

Will I be told the results of the study?

At the end of the study, we will provide you with feedback about what we have found as a result of your help and what it may mean for future research (no matter which group you were assigned to). We will regularly update our website to fill you in on the exciting research that we have done using your samples.

The data resulting from the study may be published in scientific journals or presented at meetings. The data presented is anonymous. Your name will not appear anywhere in any of the results presented, shared, or published. Unfortunately, we are unable to provide individual results to anyone.

Who is funding this study?

This study is funded by an award from a government research organisation called the Biotechnology and Biological Sciences Research Council (BBSRC). The study has also received funding as part of a PhD studentship called "EDESIA: Plants, Food and Health" which has been funded by the Wellcome Trust.

Who has reviewed this study?

This research project has been reviewed by the QIB Human Research Governance Committee (HRGC), as well as an external Research Ethics Committee (REC). These are groups of independent people who review research to protect your safety, rights, well-being, and dignity. The study protocol will be formally registered on a publicly available database (Clinicaltrials.gov) to support transparency of this research.

What should I do now?

If you are interested in taking part in the study after reading this information sheet, you can express your interest by:

- Completing our expression of interest form on our website: www.quadram.ac.uk/Vital-D.
- Contacting the research team on: Vital-D@quadram.ac.uk
- Complete the expression of interest form below and return it to us using the pre-paid envelope enclosed (if you are receiving a paper copy of this information sheet).

If you are not interested in taking part in the study or you are not eligible, you need not do anything. No one will contact you further about the study.

Thank you for taking the time to read this information.

THE ViTaL-D STUDY

Investigating the Impact of Vitamin D Biofortified Tomatoes on Serum Levels of 25(OH)D.

Expression of Interest form

I am interested in taking part and/or finding out more information about this study and am happy to be contacted by the research team with the contact details provided below.

Title: Mrs / Mr / Miss / Ms / Dr / Other (please specify) _____

Full Name: _____

Address: _____

_____

_____

Telephone: _____

Mobile: _____

I am happy for a message to be left via my telephone/mobile number: **YES / NO**

Preferred time to call: _____

E-mail address: _____

Are your Vitamin D levels lower than they should be? YES / NO / Don't know

(it is not essential for you to know this before expressing an interest, we can test this at your screening appointment if you decide that you would like to take part).

Expressing an interest does not commit you to taking part in the study.

Please return this form digitally (scanning the form) by email or using the return envelope that was provided or contact the study team at [insert study phone] to provide a freepost envelope.