Research project submitted to the local ethics committee « CPP South-East VI »

Identification of new biomarkers of banana and tomato intake

IDRCB Number	Acronym
2016-A00153-48	**Project BioBanaTom**

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SUMMARY

Promotor	INRA
Main investigator	Pr Ruddy Richard
Co-Investigators	Amandine Prulière, Françoise Laporte, Nicolas Farigon
Title	**Identification of new biomarkers of banana and tomato intake**
Rational	In epidemiological studies, dietary intakes are assessed by questionnaires, an appraoch with inherent limitations associated with self-reporting : memory bias, difficulty to estimate portion size, unsufficient coverage of the dietary diversity… The use of biomarkers of food intake should markedly improve the accuracy of dietary assessment, However, only few validated biomarkers are available. A large European project (FoodBAll) was funded by the JPI HDHL to identify a large range of new biomarkers of intakes, using untargeted metabolomics on urine ana plasma samples collected in a series of controlled nutritional intervention studies.
Objective	The BioBanaTom study aims, in the framework of the FoodBAll project, at identifying new biomarkers of intake for banana and tomato, two important foods of the European diet.
Evaluation criteria	*Main Criteria*: Identification using metabolomics of plasma and urine metabolites specifically associated to banana and tomato intake, for validation as biomarkers. *Secondary criteria* : Obtention of reference metabolomics profiles which may be related to metabolic impact of an acute intake of tomato or banana.
Methodology	Cross-over randomized, open, controlled
Number of subjects	12
Undesirable effects	No indesirable effect expected beyond those link to blood sampling (small local hematoma, slight local inflammation)
Recruitment modalities	Volunteer file of UEN declared to the CNIL, mailing CHRU and INRA Clermont-Ferrand-Theix, local medias. The selection of the volunteers will be performed during medical inclusion visits.
Main selection criteria	Inclusion criteria Men and women, healthy (normal medical and biological exam), non-smoking, aged 18 to 40, BMI>18,5 and <30 kg/m^2, with no aversion for banana or tomato. Exclusion criteria Smokers, diagnosed with infectious or chronic disease, under medication (except contraception pill), having followed an antibiotic treament in the last 3 months, consuming dietary supplements several times a week, pregnant and lactating women, vegetarian as the protocol implies consumption of standardized meal containing meat.
Protocol description	Written consent will be obtained after complete information of the subjects about the objectives, and the nature and possible risks of the study (informed consent form in duplicate, one copy given to the volunteer). The protocol will be explained in details, with objectives and contraints, according to the information form, by the scientist in charge. A clinical exam and a check-up of all selection criteria will be performed by the main investigator of another medical doctor. At the inclusion visit, subjects will have a medical exam including questionnaire

	regarding past medical history, family background, medical treatments and standard clinical exam (weight, height, blood pressure) and a blood sampling. A food frequency questionnaire will also be completed. The conformity to the selection criteria will be checked during this medical exam. The selected subjects will be definitely included after validation of the selection criteria by the investigator. The volunteers will consume in 3 distinct experimental periods either a control drink (oral nutritional complement Fresubin, 250 mL), or 240g banana (and 150 ml Fresubin), or 300g tomato (with 12 g sunflower oil and 150 mL Fresubin). Every volunteer will consume the 3 test foods, in a randomized order. After test food intake, urine will be collected over 24h in 7 fractions. Blood samplings will be performed before the food consumption (t0) and in post-prandial period (4 samplings at 1h, 2h, 4h et 6h), as well as on the following day in fasting state (t24h). the volunteers will follow a tomato and banana-free diet during the study days, as well as a more restrictive diet, without plant-based food, on the sampling days and on the day before. A 24h recall will be filled for the dietary intakes on the day before the ssampling days (D2). Samples will be analyzed using an untargeted metabolomics approach (LC-MS, GC-MS et RMN) with the aim to identify new biomarkers of tomato and banana intake. Tha total volume of sampled blood will be 154 ml per subject.
Duration	Duration of the study for every volunteer : 8 weeks Total duration of the study: 6 months for the subject selection and realization of all experimental periods for all the volunteers Total duration of the project : 3 years
Indemnities	350€ per subject
Duration of exclusion	The exclusion duration for the participation of the volunteer to another study is fixed at 1 month.
Location of the study realization	Centre de Recherche en Nutrition Humaine d'Auvergne Unité d'Exploration Nutritionnelle 58 rue Montalembert, BP321, 63009 Clermont-Ferrand Cedex Agrément pour la Recherche Biomédicale n°2012 - 476
Funding	This study is part of an international research project: Project FoodBAll, JPI HDHL 2015-2017.

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LIST OF ABBREVIATIONS

ANSM : Agence Nationale de Sécurité du Médicament
CPP : Comité de Protection des Personnes
CRNH : Centre de Recherche en Nutrition Humaine d'Auvergne
INRA: Institut National de Recherche Agronomique
JPI HDHL: Joint Programing Initiative Healthy Diet for Healthy Life
FoodBAll : Food Biomarker Alliance project
LC-MS = Liquid Chromatography- Mass spectrometry
GC-MS = Gas chromatography-Mass spectrometry
RMN or NMR : Nuclear Magnetic Resonance

1 – GENERAL INFORMATION

1-1 STUDY TITTLE

Identification of new biomarkers of banana and tomato intake

1-2 STUDY IDENTIFYING NUMBER AND DATE

Study identifying number	Type
JPI HDHL 2015-2017	European Number
2016-A00153-48	IDRCB
	CPP
BioBanaTom	Acronym

1-3 NAME AND ADDRESS OF THE SPONSOR

Institut National de la Recherche Agronomique
Represented by Jean Dallongeville, Head of Human Nutrition Department
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1-5 PARTNERS

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Nurses :	**Hélène Parrot, Dominique Dumas**
Technician	**Véronique Pidou**
Dieticians :	**Aurélie Caille, Noëlle Lyon**
Cooker :	**Guy Manlhiot**

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Centre de Recherche en Nutrition Humaine d'Auvergne
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1-6 ETHIC COMMITTEE

CPP Sud-Est VI
Direction générale - CHU de Clermont-Ferrand
58, rue Montalembert - 63000 Clermont-Ferrand

1-7 LABORATORY SITE

The centre is located in the Human Nutrition Research Centre (Centre de Recherche en Nutrition Humaine d'Auvergne) Clermont-Ferrand.
The study will take place in the Unité d'Exploration Nutritionnelle (UEN) – Centre de Recherche en Nutrition Humaine d'Auvergne, 58 rue Montalembert, 63009 Clermont Ferrand France (Autorisation de lieu n°2012 - 476).

2 – INTRODUCTION AND RATIONALE

Determining dietary exposure is a challenging endeavor in nutritional research. The most common tools used in dietary assessment are based on self reporting methodology such as 24-hour recall questionnaires, food frequency questionnaires and weighted dietary records. However, the information obtained through these tools may not be entirely reliable since they depend on the individual's memory and honesty which compromises the objectivity of the dietary assessment. Through the identification of dietary biomarkers in biological fluids, nutritional researchers have found a more objective way to determine dietary exposure[1]. A dietary biomarker may be defined as a specific measurement in a biological sample that accurately reflects the intake of a specific food or food constituent. By knowing how foods are metabolized the possibility of identifying unique biomarkers for each food or food groups is possible.

There are many factors that influence biomarkers of intake discovery; individual's characteristics such as genetic variants, environmental factors, food matrix and the lifespan of the compound may change the metabolites identified in biofluids. Another key factor for discovery of biomarkers of intake is the nature of the biological sample, where accessibility plays an important role. Urine and plasma are easy access biofluids and therefore, they are the main biological sources used to assess metabolites of intake in different studies[2].

The rise of metabolomics along with different platforms such as liquid chromatography mass spectrometers (LC-MS), have allowed the assessment of thousands of metabolites simultaneously in biological samples and the recognition of patterns that may constitute a fingerprint of the intake of different foods. Recent studies demonstrated the great potential of metabolomics to discover new biomarkers of intake in intervention and cohort studies [3,4]
The diversity of compounds found in food metabolomics represents a major challenge and so in an international effort to improve dietary biomarkers identification and validation, the Food Biomarkers Alliance (FOODBALL) has been created. In this project, 22 institutions from 11 different countries will collaborate in three main tasks: 1) Discovery of new dietary biomarker using a metabolomic approach, 2) systemic validation of existing and newly discovered biomarker to achieve a good coverage of food intake in different European populations and 3) exploring biological effects using biomarkers of intake (http://foodmetabolome.org/). With the latter, the necessity of building a chemical library that allows the use of standards for further identification arises. Along with FOODBALL, The Food Compound Exchange (FoodComEx) aims to improve availability of analytical standards of biological compounds to achieve a better and easier biomarker identification (http://foodcomex.org/).

As part of INRA collaboration to FOODBALL and FoodComEx, the present project attempts to identify biomarkers of banana and tomato intake, through the exploration of the serum and urine metabolome of subjects who consumed these foods in a controlled intervention. The same study design will be applied in six different centers within Europe to assess biomarkers of the intake of 12 public health related foods.

Tomatoes were introduced to European countries in the 16th century. The origin of tomatoes may be tracked back to the Aztec civilization in Mexico. There are fifteen leading countries in the production of tomato China, US, India, Turkey, Egypt, Italy, Spain, Brazil, Iran, Mexico, Greece, Russian Federation, Ukraine, Chile and Uzbekistan which supports the fact that tomatoes are between the five most eaten vegetables in the world [5]. Tomatoes contain a large variety of micronutrients (β-carotene as pro-vitamin A, vitamin C, folate, and potassium) and other chemical compounds, including lycopene and polyphenols that are considered beneficial for health [6]. Most of the published work on tomato is mainly focused on the association between the intake of lycopene and flavonoids, such as naringenin and hesperetin, and a lower risk for chronic diseases and cancer [7], however

lycopene and flavonoids are not robust exposure biomarkers of tomato intake for various reasons (low concentrations, interindividual variation in intestinal absorption and metabolism). Tomato contains many other phytochemicals that could be revealed as exposure biomarkers or bioactive compounds contributing to the health effects of tomato.

On the other hand, bananas (*musa spp*) are among the world's leading food crops as a source of energy, mainly in tropical regions. The use of pulp and peel of the banana as medicine remedies has been documented in traditional medicine in different continents such as Africa and America. The dietary recommendations for banana intake are based mainly in their content of pro-Vitamin A compounds such as trans-α and trans-β carotenes and in their energy contribution[8]. Like tomatoes, there are no validated exposure biomarkers on the literature.

Determining biomarkers of intake of banana, tomato and different other public health related foods will allow a more precise dietary assessment in nutritional epidemiology, opening the possibility of better investigating the health effects of foods and dietary patterns. Biomarkers will also be very useful to monitor compliance in controlled intervention studies.

3 – OBJECTIVES

The overall aim of the project is to identify potential novel biomarkers of intake of tomato and banana in urine and blood of volunteers after acute consumption of these foods.

3-1 PRIMARY OBJECTIVE OF THE CLINICAL STUDY

The primary objective, within the FOODBALL project, of the present study is to identify biomarkers of food intake after an acute dietary intervention with banana, tomato and a control drink on 12 healthy subjects, by comparing the metabolomic profiles analyzed for urine and serum samples collected over 24h.

3-2 SECONDARY OBJECTIVES OF THE CLINICAL STUDY

The secondary objectives of the BioBanaTom project are:

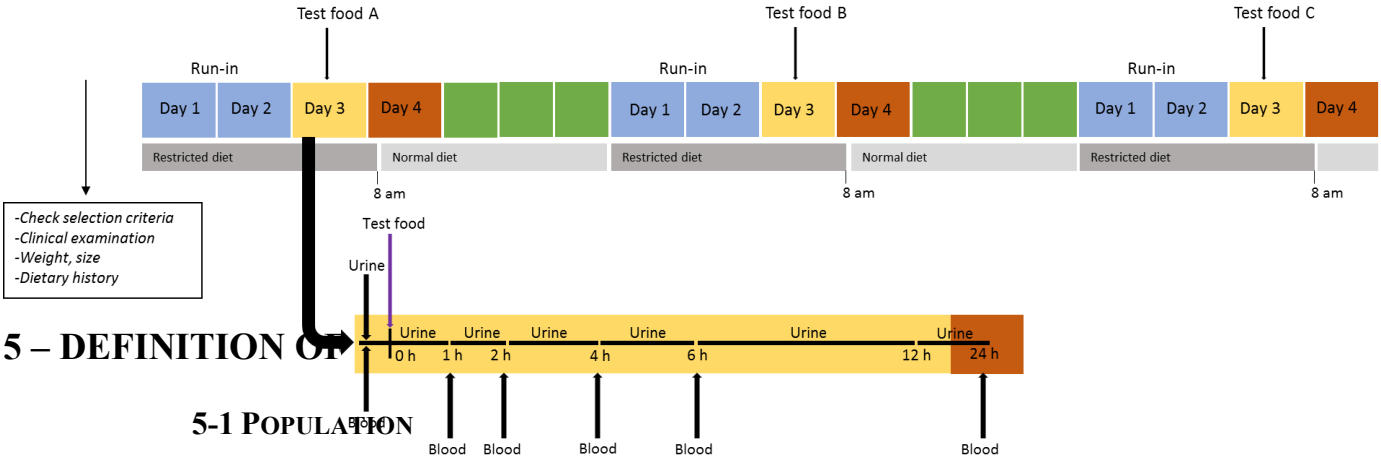
1. Collect reference urine and serum samples and associated metabolomic profiles after acute intake of banana and tomato to be shared in the FoodComEx library for analytical purposes.
2. Explore the modifications in metabolomics profiles that may be associated with metabolic effects of acute consumption of tomato and banana.

4 – PROTOCOL DESCRIPTION

The present project is a randomized, controlled, crossover study with 12 subjects. A cross-over design has been selected as each subject can serve as his/her own control thereby minimizing variations. Limitations attributed to cross-over designs are possible order or carry-over effects. These effects will however be minimized due to a randomized intervention order. The study design does not allow blinding as intervention is the ingestion of different foods.
In this study, we will assess the metabolomic profiles of human biofluids after consumption of two different foods: banana (240g peeled fruit) and tomato (300g fresh fruit) in order to identify novel biomarkers for each food. As a control diet, a high energy high protein drink, Fresubin ® 2kcal fiber (Fresinius kabi) will be used. As shown in the general study plan, the intervention comprises three dietary intervention periods of 4 days each for each food of interest and the control drink. Each

intervention period will be separated by a washout period of minimum 3 days (and maximum 10 days). Volunteers will be randomly assigned to a different food at each time point.
Volunteers will be asked to maintain their normal physical activity and follow the dietary recommendations between intervention periods; they will receive an information form containing the description and objectives of the project, and detailed instructions. A volunteer's booklet will also include simple information about nutritional metabolomics and a calendar marked with their visits to our facilities.

General study plan



5 – DEFINITION OF

5-1 POPULATION

In total 12 subjects (with the aim of 10 subjects completing the study) will be recruited for the present project of which 50% (n=6) will be men and 50% will be women (n=6) between 18 and 40 years of age. Subjects will have a BMI above 18.5 and under 30 kg/m^2, will be nonsmokers and with no disease diagnosis.

5-2 SAMPLE SIZE CALCULATION

As untargeted metabolomics is a data driven approach, which consists in detecting thousands of metabolites in biological samples with various analytical and inter-individual variability, it is not relevant to calculate a unique statistical power to determine the number of subjects. Nevertheless, previous studies showed that metabolomics allows identification of food intake biomarkers in controlled intervention studies with 4 to 20 volunteers[3,9]

5-3 SELECTION CRITERIA

5-3-1 Inclusion criteria

- Healthy males and females
- Aged 18 - 40 years
- BMI >18.5 and < 30 kg/m^2
- Willing/able to consume all test foods (tomato, banana, Fresubin drink) and the standardized meals (rice and chicken)

5-3-2 Exclusion criteria

- Smokers
- Diagnosed health condition (chronic or infectious disease)
- Taking nutritional supplements (e.g. vitamins, minerals) several times a week
- Taking medication (oral contraceptive pill is allowed).
- Pregnant, lactating

- Antibiotics treatment within 3 months prior to intervention
- Vegetarians, as standardized meals will contain meat
- Not willing to follow nutritional restrictions, including drinking alcohol during study days
- Not willing/able to give informed consent or to sign informed consent.
- Not affiliated to National Health Insurance
- Being in exclusion on the National Volunteers Data file or refusing to be registered on the National Volunteers Data file
- Currently participating or who having got 4500€ in this year to have participated in another clinical trial.
- Subjects deprived of their liberty by judicial or administrative decision.

5-4 SUBJECT WITHDRAWAL CRITERIA

Subjects may discontinue their participation in the study at any time.
The clinical Investigator may remove a subject, if, in his opinion, it is in the best interests of the subject.
A subject may be withdrawn from the study for any of the following reasons:

- Withdrawal of consent - any subject may withdraw from the study at any time
- Significant deviation from the protocol
- Lost of follow up
- Death
- Incidental illness
- Occurrence of a serious or considered serious adverse event or significant alteration in clinical and/or laboratory parameters. In these cases, appropriate actions will be taken. The sponsor will be notified immediately.

When the investigator had no news of the participant, he must have made every effort to contact him, to establish the reason for the discontinuation of treatment, and to suggest the participant to come to an end-of-study visit. If all these attempts to contact the participant failed, the investigator could then declare the participant “lost to follow-up”. The investigator should document all these attempts in the corresponding medical file. If a volunteer discontinues the study before the end of recruitment period, a new volunteer will be included with the same treatment. The investigator will record the reason(s) and the exact time of premature withdrawal of the study in the case report form. If more than one reason will be given, the investigator will indicate the main reason. The investigator will notify the sponsor of study withdrawals immediately using the page provided for this purpose in case report form by faxing to the study monitor.

In the case of withdrawal from the study due to a serious or non-serious adverse event, the investigator will make every effort to collect the information relating to the outcome of the event. If necessary, the information will be collected afterwards. This information will be recorded in that part of the case report form which concern adverse events. If the investigator cannot collect the information from a visit, he will collect it from the doctor ensuring the follow-up of the participant.

5-5 EXCLUSION PERIOD AND PARTICIPATION TO THE OTHER CLINICAL TRIAL

The subject is not allowed to participate in another clinical trial for the duration of this protocol. In addition, the exclusion period defined in this study is 1 month, timeframe during which the subject cannot participate in another clinical research protocol.

5-6 COMPENSATION FOR VOLUNTEERS

The subjects will receive 350 € of compensation for the entire participation.
In the case of a premature withdrawal, a calculation of the compensation will be made in proportion to protocol completion, as follows: 50€/kinetics.
Withdraw after the baseline visit: 0 €

Very exceptionally, the investigators may exclude an obviously non-compliant subject. In that case, the volunteer will receive no compensation.

5-7 SUBJECT RECRUITMENT

Subjects may be recruited in the community through advertisements in local newspapers, CRNH web site, emailing at INRA and CHRU, flyers at public places, and from existing UEN volunteer database registered at the CNIL.

6 – DESIGN OF TRIAL TO BE CONDUCTED

6-1 STUDY PROCEDURES

All the participants will give freely their written informed consent before their selection in the study. The investigator or co-investigator will collect written consent from each participant before, or at the latest on, the day of the first study visit. Prior to this, the investigator or his delegate will inform each participant of the objectives, benefits, risks and requirements imposed by the study, as well as the nature of the study products.

The participant will receive an information and consent form in clear, simple language. He will be allowed ample time to inquire about details of the study and to decide whether or not to participate in the study.

Subjects will come to the Investigational Site for screening to determine whether they are eligible to participate in the study. Information on demographics, anthropometrics, vital signs, relevant medical history and concomitant medication and nutritional supplements (including vitamins) will be collected. A blood sample will be collected to measure: full blood count, platelets, CRP (C Reactive Protein), glycemia, ionogramme (Na^+, K^+, Cl^-), hepatic, lipid and renal profile. A total volume of 10ml will be collected to perform this biological test. All inclusion and exclusion criteria will be checked by the investigator or co-investigator. Subjects will also complete a food frequency questionnaire (Appendix 2).

Selected subjects will be definitively included after validation of their biological test by the investigators. Subjects will be personally informed by the medical doctor in case of non inclusion if their biological test presents anomalies. Results will be delivered to them confidentially. Their medical doctor will be informed with a letter from the investigator. The results of biological tests will be kept in the envelope dedicated to each volunteer for this study and only people involved in the research will have access to documents (Nurses, co-investigators, dieticians, Clinical Research Assistant) in accordance with Good Clinical Practices.

The volunteers recruited for this project will be asked to eat three different foods, banana, tomatoes and a control drink (Fresubin ® 2kcal fiber) in order to identify novel biomarkers for each test food. Subjects will be randomly assigned to each test food at different time points of the study. As shown in the general study plan, there will be three dietary interventions:

- **Intervention 1:** Volunteers will consume the **control beverage Fresubin ® 2kcal fiber (250 mL)**.

- **Intervention 2:** Volunteers will consume **240g of peeled banana plus Fresubin ® 2kcal fiber (150 mL)**.
- **Intervention 3:** Volunteers will consume **300g of raw tomato with sunflower oil (12g) and Fresubin ® 2kcal fiber (150 mL)**.

Fresubin is a beverage composed mainly of milk protein, vegetable oils (rapeseed oil, sunflower oil) carbohydrates, vitamins minerals and trace elements (Detailed composition in Appendix 3). For the purpose of this study volunteers will drink the neutral flavored beverage.

Each intervention period will be separated by a washout period of at least 3 days (maximum 10 days). Volunteers will be asked to maintain their normal physical activity and diet between intervention periods; they will receive an information form containing the description and objectives of the project. Volunteers will be randomly assigned to a different food at each intervention period.

Following the study design shown in the general study diagram, the **run-in phase (Day1 and Day2)** participants will follow a restricted diet (described in Appendix 4) and will avoid the intake of banana or tomato in any form. On **Day 2** volunteers will also avoid the intake of banana and tomato in any form and will exclude phytochemical-rich foods such as tea, coffee, wine and other plant foods to avoid noise in the metabolomic profile (recommendations described in Appendix 5). For dinner in this day, volunteers will prepare a standardized diner which composition will be given by the investigators (rice and chicken meal, composition in Appendix 6). After dinner subjects will begin the overnight fasting period. All foods consumed on day 2 will be reported in the 24-hour recall questionnaire applied by a nutritionist the day of the dietary intervention.

In the morning of Day 3, subjects will arrive fasted at Investigational Site at around 7.30 am. As stated previously volunteers will be assigned randomly to one of the three interventions, Fresubin ® 2kcal fiber, 240g of banana plus control drink, or 300g of tomato plus control drink. Throughout the intervention, subjects will have free access to water, maximum 250ml of water per hour until 6 hours after intervention (1.5L in total).
A trained phlebotomist will place a catheter on the subject's arm before the intake of the test foods to collect the baseline sample. Then four other samples will be collected postprandially after **1h, 2h, 4h, and 6h**.

A total of **7 urine samples** will be collected. The first void of urine will be collected by the subjects at home upon the morning of Day 3 and the rest of the samples after the intake of the test foods as follows**: 0-1h, 1h-2h, 2h-4h, 4h-6h.** The urine samples corresponding to **6h-12h** and **12-24h** interval will be collected by volunteers at home until the morning of **Day 4**. Urine samples will be kept at 4ºC between collections; the use of preservatives will not be needed.

After the 6h collection of blood, the peripheral catheter will be removed and subjects will have lunch composed of rice and chicken meal, then subjects will be allowed to go home. Before leaving the Investigation center, participants will be instructed to prepare a standarized dinner based on chicken and rice. Volunteers will not be allowed to eat or drink anything except water and the standardized dinner.
On the morning of **Day 4**, subjects will arrive fasted at the investigation center at around 7.30 am in order to collect the **24h** blood sample and will deliver the **6h-12h** and **12-24h** urine collection. Afterwards participants will receive a breakfast (free composition) on site and be allowed to follow their normal diet until the run in phase of the following intervention period initiates. This procedure will be repeated for each intervention (1,2,3) separated by a minimum 3 day period (=7 days between visits).

Table 1. Summary of trial procedures

	Who	***Where***
Day 1: Run in		
Restricted diet: no banana or tomato intake	*Volunteer*	*At home*
Day 2: Run in		
Restricted diet: no intake of tomato, banana, tea, coffee, wine, fruit juices, plant foods. Dinner based on chicken and rice,	*Volunteer*	*At home*
After dinner, beginning of fasting period (8-12 hr)	*Volunteer*	*At home*
Day 3: Intervention day		
Urine collection 1 (first void of the morning)	*Volunteer*	*At home*
Placement of peripheral catheter	*Phlebotomist*	*UEN*
Blood sampling 1 at baseline (0h)	*Phlebotomist*	*UEN*
Intake of test food (banana, tomato or control drink)	*Volunteer*	*UEN*
Blood sampling 2 (1h)	*Phlebotomist*	*UEN*
Blood sampling 3 (2h)	*Phlebotomist*	*UEN*
Blood sampling 4 (4h)	*Phlebotomist*	*UEN*
Blood sampling 5 (6h)	*Phlebotomist*	*UEN*
Urine collection 2 (0-1h)	*Volunteer*	*UEN*
Urine collection 3 (1-2h)	*Volunteer*	*UEN*
Urine collection 4 (2-4h)	*Volunteer*	*UEN*
Urine collection 5 (4-6h)	*Volunteer*	*UEN*
Urine collection 6 (6-12h)	*Volunteer*	*At home*
Urine collection 7 (12-24h)	*Volunteer*	*At home*
At T6h, Lunch intake (rice and chicken meal)	*Volunteer*	*UEN*
Restricted diet: Dinner based on chicken and rice. No intake of tomato, banana, tea, coffee, wine, fruit juices, plant foods intake	*Volunteer*	*At home*
After the standardized dinner, beginning of the fasting period	*Volunteer*	*At home*
Day 4: Day after intervention		
Blood sample collection 6 (24h)	*Phlebotomist*	*UEN*
Urine Collection (first void of urine)	*Volunteer*	*At home*
Breakfast	*Volunteer*	*UEN*

6-2 SCHEME OF STUDY AND FLOW CHART

Table 2: Measurement calendar

Visit													
Day of visit	Screening visit	*Day 1* Run in	*Day 2* Run in	*Day3* Acute intervention	*Day 4*	*Day 1* Run in	*Day 2* Run in	*Day3* Acute intervention	*Day 4*	*Day 1* Run in	*Day 2* Run in	*Day3* Acute intervention	*Day 4*
Signature of subject information form and consent form	X												
Checking of eligibility criteria	X												
Clinical examination	X												
Biological test	X												
Restricted diet		X	X	X		X	X	X		X	X	X	
Biological collection													
Blood				X	X			X	X			X	X
Urine				X	X			X	X			X	X
Body assessment													
Height, weight, waist, hip circumference, body mass index.	X												
Dietary assessment													
24-hour recall questionnaire				X				X				X	
Food frequency questionnaire	X												
		Test Food A				***Test Food B***				***Test Food C***			

6-3 BIOLOGICAL SAMPLES

6-3-1 Blood samples

We will collect 6 blood samples (8 ml), one at baseline and 5 others after the acute intervention (t= 0hr, 1hr, 2hr, 4hr, 6hr, 24hr) at each of the 3 experimental interventions (Banana, Tomato, Control drink Fresubin). These samples will be collect at the Unité d'Exploration Nutritionnelle and will be stored at the Human Nutrition Unit, INRA Centre of Theix. Volunteers will arrive at the Investigation Center in an 8-12 hour fasting state and after two days of a restricted diet. All blood samples will be processed immediately after they are drawn allowing clotting at room temperature for 30 minutes. After clotting serum will be collected in aliquots and then stored at -80ºC until analysis. The whole volume of blood collected during the experimental periods is 8mL x 6 time points x3 experimental periods = 144 mL. In addition, 10 mL blood will have been collected at the screening visit. The sample collected at the screening visit will be analysis the same day of the collect by the Biochemistry Unit of Clermont Ferrand Hospital.
In total, the volume of blood collected for the whole BioBanaTom study is 154 mL.

6-3-2 Urine sampling

Participants will be instructed on collecting urine according a standardized protocol and they will be provided with containers. Subjects will be asked to collect all urine samples in marked containers at the following schedule: first void of urine before intervention, 0-1h, 1-2h, 2-4h, 4-6 h, 6-12h and 12-24h (Day 4). Urine samples will be kept at 4ºC until processing.
After complete collection, urine samples volumes will be recorded, urine will be centrifuged at 1800xg for 10 minutes at 4ºC and separated in aliquots and stored at -80ºC at the Unité de Nutrition Humaine, INRA Centre of Theix. until analysis.

Table 3: Analyses performed

ANALYSES	
Partner	**Measurements**
UNH, INRA Theix, France	Serum LC-MS metabolomics Urine LC-MS metabolomics
Max Rubner-Institut (MRI) Karlsruhe, Germany	Serum GC-MS and NMR metabolomics Urine GC-MS and NMR metabolomics

6-4 DURATION OF SUBJECT PARTICIPATION AND DURATION OF ALL TRIAL PERIODS AND FOLLOW UP IF ANY

The duration of the subject's participation is of 18 days distributed as follows:

- 3x run-in phase of 2 days (Day1 and Day2) = 6 days
- 3x intervention periods (Day3) = 3 days
- 3x collection of 24 h blood samples (Day 4) = 3 days
- 2x 3 days washout periods between interventions= 6 days

The duration of the study may be extended to 5 weeks if the wash-out periods are extended (up to 10 days).

The participation of the subjects enters within the framework of articles L1121-1 and following ones of the Public health code.

7 – TRIAL DESIGN

7-1 STUDY PARAMETERS/ENDPOINTS

7-1-1 Primary study parameter

The primary parameter in this study is the assessment of metabolites present in blood serum and urine before the dietary intervention (t=0h) and post-prandially at different time points over 24h. Metabolites will be analyzed using a non-targeted metabolomics approach (GC-MS, LC-MS and NMR). The endpoint is the identification of biomarkers of acute intake of the foods of interest through the comparison of metabolomes after either single dose of tomato, banana or control drink.

7-1-2 Secondary study parameter

A secondary endpoint is to obtain pools of urine and serum samples collected after acute intake of tomato or banana that can be shared with the international scientific community through the FoodComEx library, to be used as analytical standard or for the identification of specific metabolites of banana or tomato components.

Other secondary parameters in this study are the possible variations in endogenous metabolites detected by the metabolomics profiles that may reflect a metabolic effect of acute consumption of the tested foods. The secondary endpoint is the identification of new hypotheses on possible metabolic effects of banana or tomato that may be investigated further in others studies.

7-2 DESCRIPTION OF THE RESEARCH METHODOLOGY

7-2-1 Anthropometry

In order to minimize variation, all anthropometric measurements will be taken by the same person at the screening visit. These measurements will be conducted on the subject while they are wearing light, indoor clothing and while they are still fasting. Shoes and belts will be removed and pockets emptied.

Height measurement

The subject is placed standing at a stadiometer while wearing socks or bare feet. Hats, hair slides or highly placed hair-dos must be removed or undone. The subject is asked to stand with their back to the stadiometer's backboard ensuring their feet, buttocks and occiput (back of skull) touch the board. The measuring arm is brought down to touch the top of the subject's head, pushing hair down if necessary. This is done in duplicate, obtaining measurements to the nearest 0.1cm which will be averaged.

Weighting method

The scale is turned on (if previously off) and allowed to equilibrate. The subject removes their socks and stands on the scale. They are instructed to hold still for about 5 seconds until the scale has obtained its reading. Body weight is taken to the nearest 0.1kg. Antibacterial cleansing wipes are to be used on the scales in between uses.

Method for measuring waist circumference

For both measurements a non-stretch tape should be used. The subject should be standing with the abdomen relaxed, arms at the sides, feet together and breathing normally. If agreed by the subject, the measurement will be made directly on the skin. It should not be taken over heavy clothing.
The tape is placed around the waist, which is defined as the mid-point between the lowest rib and the supra-iliac crest. The measurement should be taken at the end of a normal expiration ensuring the tape is not compressing the skin. If the waist cannot be easily found, the investigator should ask the subject to bend over to the side - the fold is where the waist is. The measurement is read while standing at the subject's left side and it should be recorded to the nearest 0.1cm. This should be done in duplicate.

Method for measuring hip circumference

The subject should wear non-restrictive briefs or thin bottoms. They should stand erect with hands held slightly away from the sides. The investigator should squat to the side of the subject and pass the measuring tape around the greater trochanter, without compressing the skin. The measurement is recorded to the nearest millimeter.

7-2-2 Health status and dietary assessment

Dietary and nutritional status evaluation

In order to assess the dietary habits of the volunteers two instruments will be applied, 24-hour recall questionnaire and a food frequency questionnaire. The 24-hour recall will be applied in the day on intervention (Day 3) to ensure compliance to the restricted diet on day 2. During the 24r-hour recall questionnaire volunteers will be asked about the amount and type of foods and beverages they consumed the day before.

The food frequency questionnaire (FFQ) is a self-administered questionnaire where the dietary habits of individuals for the past year are assessed using a food list and beverage items categorized in 22 categories. Usual frequency of consumption will be assessed for the 22 categories of foods. The objective is to get an overall estimation of the dietary pattern of the volunteer to identify dietary patterns far outside the standard dietary patterns observed in the French population.

7-2-3. Metabolomics analysis

Urine and Blood samples will be analyzed using a UPLC-QTOF-MS (Waters Corporation, Manchester, United Kingdom) platform for the detection of metabolites, equipped with an electrospray source and a lock mass sprayer to ensure accuracy. Later on, a small number of urine samples will be analyzed using a high resolution Thermo Scientific LTQ Orbitrap Velos hybrid mass spectrometer to obtain accurate masses and molecular formulas of potential biomarkers of intake. Several online chemical databases will be queried using the accurate formula or mass to search for possible hits of chemical structures and compounds.

GC-MS and NMR analyses of the samples will also be conducted in a collaborative research unit of the FoodBAll project, at the Max-Rübner Institute in Karlsruhe.

8 – TRIAL TREATMENT, DOSAGE FORM, AND DOSAGE REGIMEN OF THE INVESTIGATIONAL PRODUCTS

8-1 INVESTIGATIONAL PRODUCT/TREATMENT

Bananas and tomatoes will be bought in a local grocery. A unique dose of 240g banana and 300g tomatoes will be given to the volunteers.

The control drink will be **neutral flavored Fresubin ® 2kcal fiber**. Volunteers will drink 250 mL on the Intervention 1 (volunteers only drink Fresubin ® 2kcal fiber as a control) and 150 ml of Fresubin plus the test food of interest on Intervention 2 (Fresubin ® 2kcal fiber plus 240g of banana) and Intervention 3 (Fresubin ® 2kcal fiber plus 300g of tomatoes).

Nutrimental composition of Fresubin ® 2kcal fiber is as follows:

Nutritional Composition		**Per 100ml**	**Per 200ml**
Energy	kcal	200	400
Protein	g	10	20
Nitrogen	g	1.6	3.2
Carbohydrate	g	22.5	45
Of which sugars	g	3.2-5.8*	6.4-11.6

Of which lactose	g	≤0.3	≤0.6
Fat	g	7.8	15.6
Of which saturated fatty acids	g	0.6	1.2
Of which polyunsaturated fatty acids	g	1.4	2.8
Of which monounsaturated fatty acids	g	5.8	11.6
Fibre	g	0, 1.5-1.6[1]	0, 3.2[1]
Water	ml	69-70*	138-140*
Osmolarity	mosmol/l	495-650	
Osmolality	mosmol/kg H2O	720-950	

Information obtained from: http://www.fresenius-kabi.co.uk/4727.htm

9 – STATISTICAL ANALYSIS

Data will be collected in ad hoc developed databases and analyzed according to the classical workflow used for metabolomics data. Data will be cleaned in a standardized way. Univariate (ANOVA with Benjamini Hochberg correction) and multivariate analyses (Principal Component Analysis, Partial-Least-Square-Discriminant Analyses, and Hierarchical Clustering analysis) will be applied to pinpoint the detected metabolites which significantly vary along time after the intake of banana or tomato.

The statistical analysis will be centralized and performed by the INRA investigators, in close collaboration with the statistician of the Metabolomics platform (Mélanie Pétéra, PFEM: Plateforme d'Exploration du Métabolisme).

10 - BALANCE BENEFITS/RISKS

The risks involved in participating in this experiment are minimal. A venous punction is comparable to a normal blood drawn and the only risk is that of a small local hematoma.
The inconveniences which can result of blood collection are possible infectious or inflammatory problems. These complications are exceptional and all the necessary measures of asepsis are taken to avoid them.
The food products that will be provided are commercially available products and for this reason do not bring any health risks.

11 – ETHICAL CONSIDERATIONS

11-1 INDEPENDENT ETHIC COMMITTEE

The protocol and the Subject informed form and consent will be submitted to The Independent Ethics Committee (CPP Sud-Est VI) and written approval from the Chair of the Ethics Committee is required before the initiation of the study.
The notification of the approval will be forwarded to the French authority ANSM. A request for authorization will be sent by the sponsor to ANSM before the start of the study.

11-2 REGULATION STATEMENT

The study will be performed in full accordance with the ethical principles stated in the Declaration of Helsinki 1964, as revised in 2008 (59th WMA General Assembly, Seoul, October 2008). The

participation of the subjects will be performed in accordance with the Code de la Santé Publique (National Health Codes), Law n°2004-806 of august 9th, 2004.

The clinical study will be conducted in compliance with the protocol, International Conference on Harmonisation (ICH), guidelines for Good Clinical Practice (GCP, as mentioned in the National Health Codes, article L.1121-3) and in the decree of the November 24th, 2006.

11-3 INSURANCE

The sponsor, INRA has an insurance *(*N° 4623359604) which is in accordance with the legal requirements. This insurance provides cover for damage to research subjects through injury or death caused by the study product and/or study procedures.

It is to note that the non compliance with the legal requirements of the research (absence of CPP opinion, absence of ANSM's authorization, non consent of the person, the continuation of a suspended or speechless research) is an exclusion clause of the guarantee.

11-4 DOCUMENTATIONS OF THE RESEARCH

Before starting the research, the investigator will supply to the sponsor a copy of his personal curriculum vitæ dated and signed and containing his number of registration in the Medical association, as well as all the investigators.
The version of the protocol accepted before submission with its appendices will be jointly signed by the investigator and the scientific in charge of the study.
For every new version of the protocol, (amendments and\or requests of the authorities), a new number and the date will be attributed and the same signatures will be collected.

The investigators agrees that the study is conducted in accordance with the Public Health law N° 2004-806 - 9 August 2004 on biomedical research, the implementing decree N° 2006-477 from 26/04/2006 amending chapter I of title II of book I of the first part of the code of Public Health relating to biomedical research and the applicable orders.

11-5 INFORMED CONSENT

All the participants will give freely their written informed consent before their selection in the study. Prior to this, the investigator or his delegate had inform each participant of the objectives, benefits, risks and requirements imposed by the study, as well as the nature of the study products.

The participant will provided with an information and consent form in clear, simple language. Two original information and consent forms will be completed, signed and dated personally by the participant and by the investigator or co-investigator for collecting the informed consent. The participant will be given one signed original information and consent form, the second original will be kept by the investigator and archived during, at least, 15 years.

11-6 ASSESSMENT OF SAFETY

11-6-1 Definitions

-ADVERSE EVENT (AE)

AE is any untoward medical occurrence in a patient or clinical investigation subject administered a study product and which does not necessarily have a causal relationship with the study product (derived from the ICH-GCP definition).

-SERIOUS ADVERSE EVENT (SEA)

SAE is any untoward medical occurrence that at any dose:

- results in death,

- is life-threatening (at the time of the event),
- requires inpatient hospitalisation or prolongation of existing hospitalisation,
- results in persistent or significant disability or incapacity,

or

- is a congenital anomaly or birth defect

-SUSPECTED UNEXPECTED SERIOUS ADVERSE REACTION (SUSAR)

A SUSAR is both:

- an SAE that is judged to be at least possibly related to the study product by the either investigator or sponsor, and
- unexpected (not listed in the Product Information Brochure)

11-6-2 (Serious) Adverse events handling

-(S)AE RECORDING

Any (S)AE as reported spontaneously by the subject or observed by the investigator or staff, is recorded on the AE form during the course of the study. The investigator must ensure that all required information is captured on this form, including the nature, onset, duration, and severity of the event, the relationship to the study product, and the action taken.

Only untoward medical events occurring after first study product intake are recorded as (S)AE (Events occurring during the Baseline period and/or before first study product intake are recorded as pre-existing condition on the Relevant Medical History and Pre-Existing Conditions page in the CRF).
When a subject undergoes a medical intervention or hospitalisation in absence of an Adverse Event (such as treatment for a pre-existing condition or hospitalisation for elective surgery or diagnosis), this intervention/hospitalisation must be reported on the AE page and not as SAE. These procedures will be handled like AEs, and timelines for reporting are the same as for reporting AEs. Complications or prolongations of hospitalisation that result from such procedures must be reported as (S)AEs, according to the applicable reporting timelines and procedures.

The severity of any (S)AE is scored as follows:

- Mild: transient or mild discomfort; no medical intervention/therapy required
- Moderate: mild to moderate limitation in activity; some assistance may be needed; and/or minimal medical intervention/therapy required
- Severe: marked limitation in activity; some assistance usually required; and/or significant medical intervention/therapy/hospitalisation required

The relationship of the (S)AE to the study product is assessed as being not related / unlikely related / possibly related / probably related / definitely related.

-SAE REPORTING BY THE INVESTIGATOR

The investigator must completed SAE report. The initial report must be sent to the delegated person by the sponsor within 48 hours (2 working days) after first notice, the follow-up report as soon as relevant new information is available.

-SAE REVIEW AND REPORTING BY THE SPONSOR

In case the SAE is a potential SUSAR, the Medical Monitor must decide whether unblinding is required.
All SAEs are reported to the accredited Ethics Committee that approved the protocol. SAEs are reported annually as line listings or according to the requirements of the Ethics Committee, SUSARs

are reported within 7 days (fatal and life threatening events) or 15 days (other events).

-FOLLOOW UP OF (S)AES

(S)AEs are followed-up by the investigator 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 practitioner, a medical specialist, or another health care provider.

11-6-3 New relevant and safety information

The Sponsor will inform the investigator and the reviewing accredited Ethics Committee if anything occurs that may negatively affect the burden or risks of participation as foreseen in the research proposal. The study may be suspended pending further review by the accredited Ethics Committee, provided that suspension does not jeopardise the subjects' health. The investigator will take care that all subjects are kept informed.

11-7 DATE HANDLING

CRFs are records of data on each subject as defined by the study protocol. Entries on CRFs shall be made complete, legible and correct using a ball-point pen. Any mistakes shall be corrected by drawing a line over the old entry and by initialling and dating next to the correction. The investigator should file all data per subject. An explanation for the omission of any required data should appear on the appropriate CRF page or other data collection forms.
The last page of each visit shall be signed and dated by the investigator to indicate the overall responsibility.
Original completed CRFs will be collected by the sponsor for data entry and further data management and statistical applications. A copy of each CRF will be archived by the investigator. At each visit the clinical monitor will review all completed CRFs (since last visit) for completeness including signature, and will compare selected data with the subject's records.

In accordance with GCP and with the national data protection laws, all information concerning the subjects in the study must be treated as strictly confidential by all persons involved in the study including the clinical, medical and statistical monitor.
Such consent shall be deemed to have been given for disclosure to any person for whom the investigator is responsible at his centre, but only so far as required for the purposes of the study, and, in the case of disclosures to staff, only if such staff are bound by obligations of confidentiality no less strict than those set out herein.

The Commission Nationale de l'Informatique et des Libertés (CNIL) is responsible for ensuring that information technology remains at the service of citizens, and does not jeopardize human identity or breach human rights, privacy or individual or public liberties. The Commission fulfils its duties in pursuance of the law of January 6, 1978 as amended on August 6, 2004.
In accordance with this law, healthy volunteers are informed that their data are processed in an automated way and that they can access this file directly through the investigator or through the physician of their choice.

INRA, sponsor of the study, signed a commitment to comply with the "Reference Method" of the CNIL for the processing of personal data carried out in biomedical research (MR-001) conducted by INRA. The investigative team of the project (UEN-CRNH) is committed to the methodology of reference MR001.

11-8 REGISTRATION ON THE NATIONAL REGISTER OF VOLUNTEERS

The healthy volunteers will be recorded on the national database of volunteers who participate to clinical researches, VRB (Volunteers for Biomedical Research) in accordance with the French regulations.

11-9 AMENDMENTS TO THE PROTOCOL

There will be no alterations or changes to the protocol without agreement of all investigators and sponsor.
If such an agreement, the planned changes will constitute an amendment that will be attached to the protocol.
Any amendment must be notified to the ethic committee if the planned changes affect the ethical or medical-scientific study (evaluation criteria, addition of a new center ….). Minor modifications do not require a review of the ethic committee.

11-10 MONITORING

The investigator must permit study-related monitoring visits, audits, review by the Ethics Committee and regulatory inspections, and allow direct access to source data and source documents provided that subject confidentiality is protected.

Monitoring includes on-site visits to assure that the investigation is conducted according to the protocol and in order to comply with applicable regulations and deadlines. On-site review of Case Report Forms (CRFs) includes the review of forms for completeness, clarity, and consistency with source documents available for each subject.

11-11 BIOLOGICAL COLLECTION

The collection of biological samples described in this protocol (blood and urine samples) is reported to the competent authority (Article L1123-12). It can be stored at the end of the research for the study of certain biological parameters of interest in the same purpose (metabolomic analysis) initially planned in the protocol All collected samples will be stored during 15 years after the end of the clinical study.

As mentioned in the protocol and subject information sheet, the collected biological samples can be stored at the end of the research, without change of purpose.

In case of a change of purpose, the Ministry of Research, the CPP and the Regional Hospitalization (if the facility is a health organization) are notified. Moreover, subjects will receive a new information which will permit them to express their opposition if they desire.

At the end of the study, urinary and blood samples collected will be stored at the Human Nutrition Unit, INRA Centre of Theix.

11-12 DATA RETENTION

The following documents will be archived in the CRNH of Auvergne to the end of the period of practical use (15 years).
These documents are:

- Protocol and appendix, any amendments,
- Information and consent forms signed (originals)
- Individual data (authenticated copies of raw data)
- Follow-up documents
- Statistical analysis

- Final report of the study

At the end of the period of practical use, all documents to be archived, will be under the responsibility of the INRA for 15 years after the end of the study according to institutional practices.
No destruction can be performed without the consent of the sponsor. At the end of the 15 years, the sponsor will be consulted for destruction. All data, all documents and reports may be subject to audit or inspection.

11-13 PUBLICATION POLICY

Investigator will have full and unrestricted access to the database with all anonymized data. Anonymised data and outcomes of this study will be shared within partners of the FOODBALL consortium (Appendix 7).

The outcomes of this study will be published in field relevant peer-reviewed journals thereby presenting the results to the scientific community. Other forms of communication include presentations at national and international meetings as well as media releases. Outcomes may be included in databases for dietary biomarkers created within the project FOODBALL.

Rules for publications have been defined in the FOODBALL consortium agreement.

12 – REFERENCES

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13 - APPENDIX

Appendix 1 : Signature page
Appendix 2 : Food Frequency Questionnaire
Appendix 3 : Nutritional Composition of Fresubin ® 2kcal fiber
Appendix 4 : Restricted diet for Day 1
Appendix 5 : Restricted diet for Day 2
Appendix 6: Composition of the standardized dinner for Days 2 and 3
Appendix 7: FOODBALL consortium

APPENDIX 1

PROTOCOL SIGNATURE PAGE

I, the undersigned, acknowledge that I agree :

To participate in the study described in the present document of the ……., according to the Clinical Good Practices, in accordance with the current regulatory texts and as required by the protocol approved by the ethics committee CPP Sud-Est VI and the ANSM and approved by the promotor.

Pr Ruddy Richard principal investigator

..... / ..... /..... .....
Jour mois année

Dr Amandine Prulière co-investigator

..... / ..... /..... .....
Jour mois année

Dr Françoise Laporte co-investigator

..... / ..... /..... .....
Jour mois année

Dr Nicolas Farigon co-investigator

..... / ..... /..... .....
Jour mois année

Appendix 2 : Dietary questionnaire

Exemple of questions translated from original in French for the volunteers:

Breakfast
At what time, foods usually consumed with quantities ?
Standing or sitting?
Same every day or changing?

Do you eat snacks in the day?
At what time? At what frequency?
What food do you eat as snacks?

What do you drink?
Water: less than 0.5l per day or 0.5 to 1l per day, or 1 to 2l/day, or more?
Wine: no or 2-3 glasses per day, or 4-7 glasses per day, or more?
Beer or cider: no or 1 can or more?
Fruit juice: no or 1 glass per day or 2-3 glasses per day, more (how many)?
Sweetened beverages: no or 1 glass per day or 2-3 glasses per day, more (how many)?

What do you drink?
Raw vegetables: every meal or once a day or once a week
Cooked vegetables: every meal or once a day or once a week
Meat: every meal or once a day or less (how many?) or never
Fish: never, once a week, more (how many?)
Eggs: never, once a week, more (how many?)

Appendix 3 : Nutritional Composition of Fresubin ® 2kcal fiber

Fresubin ® 2kcal fibre is a high energy nutritional supplement developed by Fresenius Kabi. It is composed mainly by protein milk, vegetables iols (rapeseed oil, sunflower oil), carbohydrates, vitamins, minerals and fibre. It is a lactose and gluten free product. Data available *at: http://www.fresenius-kabi.co.uk/4727.htm*

Energy and Macronutrient Composition

Nutritional Composition		**Per 100ml**	**Per 200ml**
Energy	kcal	200	400
Protein	g	10	20
Nitrogen	g	1.6	3.2
Carbohydrate	g	21.8	43.6
Of which sugars	g	3.3-6*	6.6-12*
Of which lactose	g	≤0.3	≤0.6
Fat	g	7.8	15.6
Of which saturated fatty acids	g	0.6	1.2
Of which polyunsaturated fatty acids	g	1.4	2.8
Of which monounsaturated fatty acids	g	5.8	11.6
Fibre	g	1.5	3
Water	ml	68	136
Osmolarity	mosmol/l	590-615	590-615

Micronutrient composition

Minerals and Trace Elements		**Per 100ml**	**Per 200ml**
Sodium	mg (mmol)	60 (2.6)	120 (5.2)
Potassium	mg (mmol)	160 (4.1)	320 (8.2)
Chloride	mg (mmol)	80 (2.3)	160 (4.6)
Calcium	mg (mmol)	205 (5.1)	410 (10.2)
Phosphorus	mg (mmol)	120 (3.8)	240 (7.6)
Magnesium	mg (mmol)	16 (0.66)	32 (1.32)
Iron	mg	2.5	5
Zinc	mg	1.6	3.2
Copper	µg	375	750
Manganese	mg	0.5	1
Iodide	µg	37.5	75
Chromium	µg	12.5	25
Molybdenum	µg	18.8	37.6
Fluoride	mg	0.25	0.5
Selenium	µg	13.5	27

Vitamine		**Per 100ml**	**Per 200ml**
Vitamin A	µg	150	300
Beta-carotene	µg	375	750
Vitamin D	µg	2.5	5
Vitamin E	mg	3.75	75
Vitamin K	µg	21	42
Vitamin B1	mg	0.3	0.6
Vitamin B2	mg	0.4	0.8
Nicotinamide	mg	3.75	7.5
Vitamin B6	mg	0.43	0.86
Vitamin B12	µg	0.75	1.5
Pantothenic Acid	mg	1.5	3
Biotin	µg	9.4	18.8
Folic Acid	µg	62.5	125
Vitamin C	mg	18.8	37.6

Appendix 4 : Restricted diet for Day 1

No intake of tomato in any form : raw tomato or cooked tomato, tomato products (sauce, paste, ketchup) and dishes containing tomato (pizzas, soup, ratatouille, chili con carne...).

No intake of banana in any form.

No alcohol.

Appendix 5 : Restricted diet for Day 2

No intake of tomato in any form : raw tomato or cooked tomato, tomato products (sauce, paste, ketchup) and dishes containing tomato (pizza, soup, ratatouille, chili con carne...).

No intake of banana in any form.

No intake of plant-based beverages : coffee, tea, herbal teas, chocolate, fruit juices, wine, beer.

No intake of fruits and vegetables, legumes, salad, breakfast cereals, wholegrain products.

No intake of soft drinks.
No alcohol

Animal products (meat, fish, eggs, etc…), dairy products (butter, cream, yogurt, cheese), pasta, white rice, potatoes, white bread are allowed.
Water and milk are the only beverages allowed.

Appendix 6: Composition of the standardized dinner for Days 2 and 3

- The dinner will be prepared at home by the volunteers, using food bought in local grocery.
- The dinner will be composed of chicken breast simply pan-fried with butter or margarine, boiled white rice with salt, butter or margarine, and white bread.
- The volunteers can eat as much as they want.

Appendix 7: FOODBALL consortium

Wageningen UR**, The Netherlands
University College Dublin*, Ireland
University Oslo; Norway
University of Copenhagen*; Denmark
National Institute of Public Health and the Environment (RIVM), The Netherlands
Ghent University, Belgium
University of Alberta, Canada
Technical University Denmark, National Food Institute, Denmark
Institut National de la Recherche Agronomique (INRA)*, France
Technical University München*, Germany
Research Center on Food and Nutrition (CRA-NUT), Italy
Fondazione Edmund Mach, Italy
University of Bologna, Italy
University Medical Center Groningen, The Netherlands
Maastricht University, The Netherlands
University of Barcelona, Spain
Max-Ruber-Institut*, Germany
ILSI Europe
Agroscope*, Switzerland
Helmholz Centre München, Germany
TNO, The Netherlands
Le Centre Hospitalier Universitaire Vaudois (CHUV)*, Switzerland
ZonMw, The Netherlands
Vitas AS, Norway
University of Auckland, New Zealand
University of Eastern Finland, Finland
International Agency for Research on Cancer (IARC)

**Partners within Work Package 1 (WP1) – Acute Study*
***coordinator*