Department of Health
Swiss Distance University of Applied Sciences
(Fernfachhochschule Schweiz - FFHS)
Nutrition Research Group

Request for participation in nutrition research

Study title: The effect of chitin and ascorbic acid on dietary iron absorption from *Tenebrio molitor* larvae in young women.

Dear Madam

Hereby, we would like to inform you about our iron bioavailability study and ask you whether you would like to take part in it. The aim of this nutrition study is to measure the absorption of iron from an insect-based meal that will be in the form of a soup. We are specifically looking for female study participants with a marginal iron status to enroll in our study.

By reading this information material, you can learn all about this study. Your participation is voluntary. The following study information sheet will help you in your decision on whether to take part or not. Before taking a final decision, you can ask any questions in a conversation with the investigators.

If you want to participate, please sign the declaration of informed consent at the end. With your signature you confirm that you have read and understood the study information sheet. If you have questions or something is unclear, please ask the investigators.

The study information sheet and declaration of informed consent consist of four parts:

If you read **Part 1**, you will get an overview of the study. In **Part 2**, we explain the entire process and background of the study in detail. **Part 3** contains the information on data protection and insurance. With your signature at the end of the document, **Part 4**, you confirm that you agree to participate. This study is initiated by Prof. Dr. Diego Moretti from Fernfachhochschule Schweiz (FFHS) as the sponsor. The local principle investigator is Dr. Nicole Stoffel. Please contact the study coordinator email address**:** **insecte@ffhs.ch** if you have any further questions or one of the following study staff:

Sponsor

Prof. Dr. Diego Moretti

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Part 1: Summary Study Information

What are we aiming for with this study?

The overall aim of this study is to measure the absorption of iron from *Tenebrio molitor larvae*, a commercially available edible insect in Switzerland, and thus estimate its potential as a source of iron in our diet. Based on earlier studies in our group, we know that dietary antinutritional compounds play a key role in insect iron absorption. Chitin is a polysaccharide present in insect biomass, which is known to bind iron and could potentially prevent its absorption during digestion. However, it is not yet fully understood if and to which extent chitin negatively affects absorption of iron from *Tenebrio molitor*. By comparing iron absorption in presence and absence of chitin (potential iron absorption inhibitor) and vitamin c (known iron absorption enhancer); we aim to investigate how iron absorption from an insect-based meal can be optimized to better understand its potential as an iron source. In **Chapter 1,** you will learn more about the scientific background of the study.

What does it mean for you to participate in this study?

Participation in this study will involve 9 appointments of approximately 20-30 minutes at the study center located at ETH Zurich: building GLC, room D35.3 and D35.3, Gloriastrasse 37/ 39 , 8092 Zurich,. We will first invite you for a screening visit that will include 1 blood withdrawal and a short questionnaire. Based on the screening visit, we will decide whether you can participate in the study. If you can participate in the study, you will consume 7 different meals (3 insect-based meals and 4 control meals without insects) on 7 separate days as breakfast over an overall period of 47 days. Meals will be consumed on day 1, 2, 16, 17, 18, 32, and 33. In addition to the screening visit, blood samples of 6 mL will be withdrawn on day 16, 32, and 47. Consult **Table 1** and **Table 2** for a visual overview of the screening and study visits (pages

7-8). In **Chapter 2,** you will find a detailed schedule of the study visits and more information on the course of the study.

What are the advantages and risks for you associated with the study?

At the end of the study, you will be informed about your iron status, and you can consult us on this. This study has minimal risks; the blood withdrawals can lead to small bleeding, a slight effusion of blood or an infection in very rare cases. The intake of stable isotopes, which we use to determine the iron bioavailability, carries no risk. See **Chapter 3** for more information on risks and benefits of participating in this study.

Which responsibilities do you have when participating in the study?

If you participate, you don't need to take special precautions. For the success of our study, we ask you to come to all appointments on time. We require you to come in a fasted state to all study visits; this means no food intake after 8 pm and no beverage intake including water after midnight the evening before. After the breakfast at our study facility, you will need to wait for 3 hours before you can eat or drink anything else.

What rights do you have when you participate in the study?

You are free to choose if you would like to participate in the study or not. If you decide to participate, you have the right to withdraw from the study at any time without needing to specify any reasons nor facing negative consequences. During the study, we will collect medical data about you, as well as biological samples (blood).

What happens with your data?

We comply with legal requirements about privacy. We will only use your data for the aims specified in this study (assessment of iron bioavailability). The obtained data will be stored safely and reported in an encoded form. Only the responsible investigators and the members of the ethical committee have access to the original data under strict confidentiality.

Content table

Part 2: Detailed Study Information

Title of the study

The effect of chitin and ascorbic acid on dietary iron absorption from *Tenebrio molitor* larvae in young women.

1. The scientific background of the study

1.1 What is the aim of the study?

The aim of the study is to measure the absorption of iron from the edible insect *Tenebrio molitor larvae* in women and subsequently estimate the potential of edible insects as a source of dietary iron. Additionally, the study aims to determine if the presence of chitin and ascorbic acid influences how much iron is absorbed from the insect-based meals.

1.2 Why is the study important?

Worldwide, more than 2 billion people are affected by anemia and iron deficiency remains a leading cause of anemia. Animal products are a key dietary source of well-absorbed iron. However, due to the high environmental burden of their production and by considering the growing world population, there is a need to develop viable sustainable and nutritional alternatives to animal food products. From earlier research, we know that *Tenebrio molitor* larvae contain a substantial amount of iron, which is moderately well absorbed. Moreover, we know that antinutritional food components contained in the insect biomass can result in a decreased iron absorption. With the current study, we specifically aim to investigate if and to which extent chitin, a polysaccharide found within the exoskeleton of insects, can have a negative effect on iron absorption. Chitin is an understudied food component present in insects but also in other foods which could potentially serve as meat alternatives, such as mushrooms. Additionally, we aim to study to which extent the addition of vitamin c (ascorbic acid) can increase iron absorption from an insect-based meal. Knowledge of iron absorption inhibitors and enhancers can help optimize and formulate insect-based meals for improved iron absorption.

To measure the absorption of iron by your body we will use stable iron isotopes. To distinguish the iron from *Tenebrio molitor* from the iron that exists naturally in your body already, the insects have been grown so that they contain the isotope “labels”, which we can later measure in your blood (see **Glossary**, page 12).

Tenebrio molitor larvae are recognized as an edible insect in the Swiss food legislation. The *Tenebrio molitor* larvae used for the study are produced by Ensectable AG (Endingen, Switzerland), whose products are also available in Coop supermarkets. This study follows the

federal human research act; furthermore, we respect all international recognized guidelines. The cantonal ethical committee of Zurich (KEK Zurich) has reviewed and approved this study.

1.3 Can I participate in this study?

You can participate in the study if you:

- Are a female between the age of 18 - 45 years old
- Have a body weight below 70 kg
- Have a normal body mass index (18.5 – 24.9 kg/m^2)
- Have low iron stores (being in the lower half of the distribution of serum ferritin at screening)
- Are able to read and communicate comfortably in English
- Have an open-minded attitude towards consuming insect-based meals

You cannot participate in the study if you:

- Have difficulties with blood withdrawals
- Have any food allergies, especially to crustacea, dust mites, sea food, gluten, milk, or eggs
- Have taken any antibiotics in the last 4 weeks prior to the study and during the study
- Have any chronic digestive, renal and/or metabolic diseases
- Are on long-term medication (except for contraceptives)
- Consume any mineral or vitamin supplementation in the last 2 weeks prior to the study and during the course of the study
- Are pregnant or intend to become pregnant on the study date or within 30 days after study end date
- Are currently breastfeeding or have been breastfeeding in the 6 weeks before study start date
- Had a blood transfusion, donated blood, or experienced significant blood loss in the last 4 months
- Participated in any clinical study within the last 30 days or plan to participate in one at the same time
- Smoke more than one cigarette per day
- Cannot comply with study protocol (e.g., not available on certain study appointments)
- Are unable to understand the information sheet and the informed consent form due to cognitive or language reasons
- Are anaemic (hemoglobin below 12 g/dL, will be measured by us during screening visit)
- Have an elevated C-reactive protein concentration above 5.0 mg/L (will be measured by us during the screening visit)

2. What to expect when participating in the study

2.1 If I take part in the study, what is expected of me?

For the success of this study, you will need to closely adhere to the study schedule (see **Chapter 2.2**) and follow all instructions by investigators. Further, we kindly request you:

- to be on time for all appointments.
- to inform us of any infection, symptoms, or any change in your general health condition.
- to report intake of any type of medication, such as non-prescription medication (painkillers, cold or allergy medication, etc.) including alternative medicine (for example herbal therapy), as well as any prescribed medication.
- to contact us immediately if you are unable to keep an appointment.

2.2 What are the detailed schedules for the screening and study visits?

Study participation is voluntary and will involve 1 screening visit of approximately 30 minutes and 8 fixed study visit appointments of approximately 30 minutes. All 9 appointments will take place at the study site of ETH Zurich: ETH Zurich: building GLC, room D35.3 and D35.3, Gloriastrasse 37/ 39 , 8092 Zurich,.

Screening Visit

If you are interested to participate in the study, we will invite you for a screening visit to establish whether you can participate in the study (consult **Table 1** for an overview). The screening visit will last approximately 30 minutes and will involve a short questionnaire. As part of the screening, we will withdraw a venous blood sample of 6 mL to determine if you have anemia or systemic inflammation (hemoglobin, ferritin, and C-reactive protein concentrations), in which case you would not be able to participate in the study. We will also measure your height and weight. Pregnancy is excluded by self-declaration. If in doubt, we will provide you a pregnancy test. You will be able to try a small portion of the insect-based meal (vegetable soup with pulverized insects), so that you can decide if you would be able to consume it during the study day.

Table 1: Schedule of screening visit

Screening visit (time)	Appointed time and date (20 to 30 minutes)
Questionnaire	x
Height and Weight measurements	x
Meal consumption	Try small insect-based meal
Blood withdrawal (6 mL)	x

Study Visits

If you fulfill all requirements to participate in our study based on the screening and you are willing to take part, we will invite you to **8 fixed appointments spread over 47 days.** You must be able to attend all appointments to be able to participate in the study.

On the **evening before all study appointments, you must stop eating and drinking beverages after 8 pm and stop drinking water after midnight**, so that on the study visit you

will be in a fasted state. To measure iron absorption it is important that you are in a fasted state on each of the study days (mornings), and that your breakfast will consist of the test meal (soup with insect powder). This also means that you will not be able to drink coffee or tea on the mornings of the study visits.

The entire duration of the study is equal to 47 days. On study day 1, 2, 16, 17, 18, 32, and 33, we will ask you to consume one of the 7 different test meals. These seven meals will be comprised of a vegetable soup with or without pulverized insects given with a bread roll. The meals will be consumed at our study site as breakfast between 7 and 9 am together with 500 mL bottled water, after which you must wait for another 3 hours before you can consume anything else. On day 16, day 32 and day 47 of the study, venous blood samples (6 mL) will be collected after an overnight fast for the determination of iron status (hemoglobin and ferritin), inflammation status, and iron absorption.

Please consult **Table 2** for a visual overview of the study timeline. In total, you will consume 7 test meals (3 insect-based and 4 meals without insects) and you will give 3 blood samples of 6 mL during the course of the study.

Table 2: Study timeline

	Study period							
Day in the study	**12.5.2025**	**13.5.2025**	**26.5.2025**	**27.5.2025**	**28.5.2025**	**10.6.2025**	**11.6.2025**	**24.6.2025**
Short questionnaire	X	X	X	X	X	X	X	X
Meal consumption	X	X	X	X	X	X	X	
Blood withdrawal			X			X		X

2.3 When does my participation in the study end?

Your participation in the study is successfully completed with the blood collection on day 47 of the study. It is possible that we might have to ask you to end the study early because of the occurrence of any of the criteria listed in **Chapter 1.3**.

2.4 What are my rights as a study participant?

You have the free choice to decide whether you would like to take part in the study after you have carefully gone over this information sheet and asked any questions you might have. Once agreed to participate, you will have the right to withdraw from the study at any point during the study and you do not need to specify the reason for your discontinuation. Please do not hesitate to contact the study team regarding any questions or concerns that you may have.

3. Risks and benefits of participating in the study

3.1 How do I benefit from the study?

If you participate, you will be informed about your iron status free of charge. We will measure your hemoglobin, ferritin, and C-reactive protein (inflammation parameter). These three parameters will be used to evaluate your iron status. If your hemoglobin levels are low, we will advise you to seek medical advice from a doctor. Please note that the study does not offer adequate iron supplementation for iron depleted women. A doctor's consultation is recommended at the end of the study. Moreover, you will be compensated for your participation in the study (see **Chapter 3.4**).

3.2 As a study participant, are there any risks?

Participation in this study poses little or no risk. The only risks in this study are related to the blood withdrawals, which will be carried out by qualified and experienced nurses. In rare cases, blood withdrawals can lead to small bruising, bleeding, and swelling at the puncture site. In very rare cases, an infection can occur at the puncture site. The total amount of blood taken (including screening) is 24 mL, which is a very low amount when compared to a standard blood donation where a person donates 350 mL of blood. Hence giving blood samples is of minimal risk.

A possible discomfort associated with your participation in this study could be the fasting requirement and the regulated intake of the test meals at specific times in the morning.

The intake of stable isotopes is of no risk and a usual method in the research of human nutrition. Stable isotopes are frequently used in research including vulnerable groups such as infants and pregnant women. The reason why we conduct a pregnancy test in case of doubt and ask you for self-declared non-pregnancy is that the iron absorption increases significantly during pregnancy. This makes the interpretation of the study results more difficult; therefore, we avoid inclusion of pregnant women in the study.

3.3 Will I be informed about the study findings?

We will inform you about the study findings and your individual iron status. In case there are any new findings that may affect your safety or participation in this study, we will inform you both orally and in writing. You have the right to inform the person in charge of the study if you do not wish to receive any information regarding the study findings. If you provide additional consent (please see "Consent form for the re-use (further use) of coded health-related data and biological materials", attached), we may use your data or samples for further, yet undefined, research purposes, as outlined in the respective consent form.

3.4 Do I receive any monetary compensation?

If you participate and complete this study, you will receive a compensation of 170 CHF. If you withdraw from the study before termination, you will receive a proportionate compensation. The compensation is meant to compensate for your time in participating to the visits foreseen in the study. A separate compensation for travel costs to the study location is not foreseen.

Part 3: Data protection, insurance, and study funding

4.1 Are my personal information kept confidential?

We will collect personal and medical data from you for this study, including your name, date of birth and address. This data, and any other information that could identify you, will be encoded, which means that we will assign you a study ID code. Only the study coordinator and Sponsor-Investigator will know who belongs to which ID code. Any other researchers will only work with the encoded data. The list of codes will be kept in a secure place by the Sponsor-Investigator.

It is possible that relevant public authorities (Cantonal Ethical Committee of Zurich - KEK Zurich) will audit the study and may require access to uncoded data. In case of any study related emergencies, a representative of the insurance company may access uncoded data, if necessary, for the evaluation of your case. In these exceptional circumstances, all individuals having access to uncoded data are obliged to respect and uphold your absolute privacy and confidentiality.

We will not publish your name in any report, publication, or on the internet. The study team is responsible for the compliance to the national legal requirements concerning your privacy.

4.2 What will you do with my samples and personal information?

Your samples and personal information are kept confidential and used to obtain the results for the present study. The Sponsor-Investigator is responsible for the safe handling of your data and samples after the end of the study. Any data collected during the study will be stored securely for a maximum of 10 years at FFHS. Additionally, if you agree in the consent form below, this will allow storage of your blood samples for future research aims, the samples will be stored for 5 years to allow retrospective analyses of new study questions. This additional consent is fully voluntary, and you can also opt to participate in the study without this additional consent. You have the right to withdraw consent for this study at any time. If you choose to withdraw before completing the study, your biological material (blood) and medical data that we have collected until then, will still be analyzed and interpreted for the study results to be accurate. The samples will then be destroyed after the analyses and not kept for retrospective analyses as described above. Any further projects using your data will be subject to a separate application and approval by the applicable ethics committee.

5.1 If I take part in the study, will I be insured?

In the unlikely event that health problems arise from this study, which are due to the fault of FFHS, they are covered by the third-party liability insurance of FFHS. Accident insurance (i.e., on your way to the study location) and general health insurance are, however, the responsibility of each individual study participant. In any case, should you have any complaints relating to the study (regarding your health or otherwise) please contact the Sponsor-Investigator or the study coordinator.

6.1 Funding of the study

The project is funded by the Swiss National Research Foundation.

How to contact the study team

For any questions or emergencies that occur during or after the study, please contact the following study email address (study staff have access to it): **insecte@ffhs.ch** or the following persons:

Study coordinator/Investigator:	Laila Hammer Nutrition Research, Department of Health, FFHS Zollstrasse 17, CH-8005 Zurich Tel.: +41 44 512 09 63 | laila.hammer@ffhs.ch
Sponsor:	Prof. Dr. Diego Moretti Nutrition Research, Department of Health, FFHS Zollstrasse 17, CH-8005 Zurich Tel.: +41 44 512 09 20 | diego.moretti@ffhs.ch
Principal Investigator:	Dr. Nicole Stoffel Dr. Nicole Stoffel ETH Zurich Laboratory of Nutrition and Metabolic Epigenetics, Schmelzbergstrasse 7, 8092 Zürich E-Mail: nstoffel@ethz.ch

Glossary

What is *Tenebrio molitor* larvae?

Tenebrio molitor larvae, often named as mealworms, is an edible insect permitted in Switzerland as a novel food since 2017. The EU has approved *Tenebrio molitor* larvae for as a novel food in 2021. Products containing *Tenebrio molitor* larvae are commercialized by a variety of Swiss and international suppliers. *Tenebrio molitor* larvae contained in the insect-based meals in the present study will be in the form of a dry powder.

What is chitin?

Chitin is an abundant and ubiquitous polysaccharide present in the insect biomass composed of β- 1,4-linked N-acetyl-D-Glucosamine monomers. Chitin is characterized as metal binding polymer and potentially inhibits iron absorption in the gastric and duodenal digestive environment.

What are stable isotopes?

Isotopes are atoms of an element that have an equal number of protons and electrons but differ in the number of neutrons. The difference in the number of neutrons results in a different atomic mass. The isotopes that we use in this study also exist naturally in small quantities. You might know that some isotopes are radioactive, though others are not. The isotopes that are used in this study are very stable and not radioactive. The intake of such stable isotopes in the very small quantities used in this study is of no risk.

What is hemoglobin, ferritin, and C-reactive protein (CRP)?

To determine your iron status, we will measure those three parameters in your blood. Hemoglobin is an iron containing component of the red blood cells and is needed for the oxygen transport in human body. A low hemoglobin level refers to anemia, this means the oxygen is not well distributed in the body. Ferritin is the storage molecule for iron. A low ferritin level indicates iron deficiency and can decrease physical and concentration performance. C-reactive Protein (CRP) is an unspecific parameter of infection and can already be elevated when you have a slight cold. A high CRP level leads to an increase in ferritin and hinders the interpretation of your iron status.

Informed consent form

Please read this form carefully and ask us any questions you might have. Your written consent is required for participation.

Study title: The effect of chitin and ascorbic acid on dietary iron absorption from *Tenebrio molitor* larvae in young women.

Study location: ETH Zurich: building GLC, room D35.3 and D35.3, Gloriastrasse 37/ 39 , 8092 Zurich,

Name of Co.Investigator: Laila Hammer

Name and surname of Participant: _____

Date of birth: _____

- ✓ I participate in this study on a voluntary basis and can withdraw from the study at any time without giving reasons and without any negative consequences. The data and samples collected up to the point of withdrawal will still be evaluated as part of the study.
- ✓ I have been informed orally and in writing about the aims and procedures of the study, the advantages, and disadvantages, as well as potential risks.
- ✓ I have read the information sheet for study participants. My questions related to participating in the study have been answered satisfactorily. I have been given a copy of the information sheet for study participants and a copy of the informed consent form.
- ✓ I will be informed of results and / or incidental findings that directly affect my health. If I do not wish to be informed, I will inform the study coordinator.
- ✓ I was given sufficient time to make a decision about participating in the study.
- ✓ With my signature, I certify that, to the best of my knowledge, I fulfill the requirements for participating in the study stated in the information sheet for study participants.
- ✓ I have been informed that any possible damage to my health, which are directly related to the study and are demonstrably the fault of FFHS, are covered by the general liability insurance of FFHS. However, beyond the above mentioned, my health and/or accident insurance (e.g., for the commute to or from the study location) will be applicable.
- ✓ I agree that the responsible investigators/Sponsor and/or the members of the Ethics Commission have access to the original data under strictly observed rules of confidentiality. I understand that my data samples will only be passed on in coded form.
- ✓ I am aware that during the study, I have to comply with the requirements and limitations described in the information sheet for study participants. In the interest of my health, the investigators can, without mutual consent, exclude me from the study.
- ✓ For non-native English speakers, I confirm that I understand English well enough to understand every aspect of this information sheet for study participants, with all its legal consequences.

Place, Date	Signature of study participant:

Confirmation by the study staff: I hereby confirm that I have explained the aims, objectives and meaning of this study to this study participant. I guarantee that applicable laws in relation to the conduct of this study will be followed by the study team. Should any new information become available during the study which could influence the participant's decision to take part in the study, I will inform them as soon as possible.

Place, Date	Name and signature of Co-investigator:

Consent form for the re-use (further use) of coded health-related data and biological materials

Fernfachhochschule Schweiz
Mitglied der SUPSI

Please read this form carefully and ask us any questions you might have. Your written consent is required for participation.

Study title: The effect of chitin and ascorbic acid on dietary iron absorption from *Tenebrio molitor* larvae in young women.

Study location: ETH Zurich: building GLC, room D35.3 and D35.3, Gloriastrasse 37/ 39 , 8092 Zurich,

Name of Co-Investigator: Laila Hammer

Name and surname of Participant: _____

Date of birth: _____

- ✓ I give consent that my personal coded data and biological materials, collected for the clinical trial or research project named above, may be further used by FFHS and/or its research collaborators in future research projects performed in Switzerland or abroad related to topics not yet defined. Research institutions abroad must comply with the same data protection standards as apply in Switzerland.
- ✓ I understand that the samples will be stored in coded from, and the key will be kept in a secure location by the Sponsor-Investigator.
- ✓ My consent is valid for an unlimited period, unless and until consent is withdrawn.
- ✓ I understand that I can withdraw my consent for further use of my personal coded data and biological materials, at any time by simply informing the investigator without any justification.
- ✓ If I withdraw my consent for further use of my personal coded data and biological materials, I give consent that the FFHS and its research collaborators may continue to use my personal coded data and biological materials, as fully anonymized data, or samples, i.e., with no identifiable data available. If this is not possible, I understand that FFHS and/or its research collaborators will stop exploiting my coded data and samples.

By signing below, I agree that my coded personal data may be used for any future scientific research. If I do not sign, my data will be used in the frame of the aforementioned study protocol only.

Place, Date	Signature of study participant:

Confirmation by study staff: I confirm that I have explained the aims, objectives and meaning of the further use of coded health-related data and biological materials agreement to the participant to the best of my knowledge.

Place, Date	Signature of Co-Investigator: