PLAIN LANGUAGE STATEMENT AND CONSENT FORM

TO: Participant,

Plain Language Statement

Date:

Full Project Title: Dose response of alpha lactalbumin supplementation on serum TRP:LNAA ratio.

Principal Researcher: Dr Dominique Condo

Student Researcher: Ms. Luana A Mascarenhas

Associate Researcher(s): Dr. Spencer Roberts, Dr. David Lee Hamilton, Dr. Kate Pumpa, Dr. Kathleen Miles.

The Plain Language Statement and Consent Form contains 11 pages. Please make sure you have all the pages.

1. Your Consent

You are invited to take part in this research project.
This Plain Language Statement contains detailed information about the research project. Its purpose is to explain to you as openly and clearly as possible all the procedures involved in this project so that you can make an informed decision whether you are going to participate.

Please read all sections of this Plain Language Statement carefully. Please feel free to ask questions about any information contained within this document. You may also wish to discuss the project with a relative or friend or your local health worker.

Once you understand what the project is about and if you agree to take part in it, you will be asked to sign the Consent Form. By signing the Consent Form, you indicate that you understand the information and that you give your consent to participate in the research project.

You will be given a copy of the Plain Language Statement and Consent Form to keep as a record.

2. Purpose and Background

Researchers are investigating the effect of α-lactalbumin (ALAC) intake (a protein high in tryptophan) on the serum Tryptophan levels and the serum TRP:LNAA ratio to identify its optimal recommended dosage to elevate these levels in the blood. Tryptophan is an amino acid that is studied to have benefits on sleep, mood, and cognitive performance. The proposed mechanism, through which these beneficial changes are seen, is by increasing the tryptophan availability to the brain, which can be measured through blood plasma concentrations. Tryptophan usually competes with other LNAAs (Large Neutral Amino Acids) to increase its availability to the brain as they are transported by the same carrier molecules. Previous studies have reported that α-lactalbumin intake has sufficiently increased serum tryptophan availability, however, they contain varied doses and lack a comparison over the dose dependent effect of the supplement on the ratio. Through this study the researchers aim to understand this better and to identify the optimal α-lactalbumin (ALAC) supplement dosage and timing.

3. Eligibility

To be eligible for this study, the following inclusion criteria is required to:

- Training to complete at least 150 to 300 min moderate-intensity activity or 75–150 min of vigorous-intensity activity a week, plus muscle-strengthening activities 2 or more days a week.
- Aged between 18-35yrs.
- Be free of any clinically diagnosed sleep disorders (insomnia or any others)
- Not be consuming any sleep medication or sleeping aids to improve your sleep quality such as melatonin, chamomile, or other herbs.

Exclusion criteria include:

- excessive wine or beer consumption (>17 standard drinks per week),
- dairy allergy,
- high caffeine use (e.g., >5 mg·kg-1·d-1),
- antidepressant or mood stabilizers use,
- current or recently finished night shift work,
- recent travel across time-zones,
- pregnancy.

4. Procedures:

We would like to invite you to participate in a research study in which we will examine the effect of α-lactalbumin protein on amino acids within the blood.

Screening

Firstly, we will require you to complete a brief screening questionnaire online to assess your eligibility for this study. If you meet the inclusion criteria and are selected successfully, you will be contacted by our research team for a detailed explanation of the study protocol and scheduling of your visits to the Deakin University, Burwood Campus Laboratory to participate in the study. You will also be instructed and guided around filling out a 3-day diet recall using a smartphone app Easy Diet Diary to record your regular meal intake, 3 days prior to the first visit to the Deakin University lab.

Pre-requisites:

- Before arrival for your first scheduled visit to the Deakin Laboratory, you need to complete a 3-day dietary recall using the Easy Diet Diary (EDD) smartphone application.
- Additionally for each visit, you need to be fasting with a gap of >2.5hrs from your last meal, upon arrival at the Deakin University Campus.
- You will be provided with an instruction booklet of activities permitted over the next 3 hours at the laboratory. You will be discouraged from using screens including mobile phones, tablets, TV, laptops, and other blue light emitting devices.
- For this study, you will be required to visit the Deakin University laboratory on four different occasions to complete all four doses of the supplement, each separated by a minimum gap of 3 days in between (explained below).

Wash-out (minimum three nights)

The wash-out phase is designed to limit any carry-over effect of the supplements going into the next experimental protein dose, ensuring that the experimental protein dosages are adequately separated. During the wash-out period, you will be required to continue your regular eating and training practices. This is to ensure that your patterns stay consistent, and do not affect the subsequent supplement dose outcomes. During this wash-out period, no supplement will be provided, and you may eat as per usual.

Intervention

- On each intervention day, you will be required to report to the university at **17:00 hrs with a fasting period 2.5 hrs before arrival (14:30 hrs)**.
- Upon arriving, a researcher will take your basic height and weight measures and hand you with a short questionnaire on chronotype.

- Next, a trained researcher will also insert a cannula into your forearm vein for blood samples to be taken.
- You will then be randomly allocated a single dose of the α-lactalbumin (BiPRO Alpha 9000; Agropur Inc, Appleton, WI), in a crossover design (you will complete all 4 doses on 4 different occasions). Both you and the research team will not be aware which dose you are assigned, so that the trials are not biased.
- Two simple questionnaires will be handed to you for completion at three time-points during each session (Before supplement consumption, Immediately after supplement consumption (T=0 mins), and 2 hrs after supplement consumption (T=210 mins).
- Small amounts of blood will be drawn (5 mL) and saliva swab samples will be taken at various time points during the 3hr intervention period.
- Post completion of the study protocol, you will be screened for drowsiness using a short questionnaire, viz. Karolinska Sleepiness Scale (KSS), to assess your alertness levels and to rule out any drowsiness. This is precautionary and will be carried out to ensure your readiness to drive post the intervention.
- All participants will be handed a boxed dinner meal before they leave. On completion of all 4 intervention doses, participants will receive reimbursement via a $100 gift card.

A graphic overview of the study design is provided below in Figure 1.

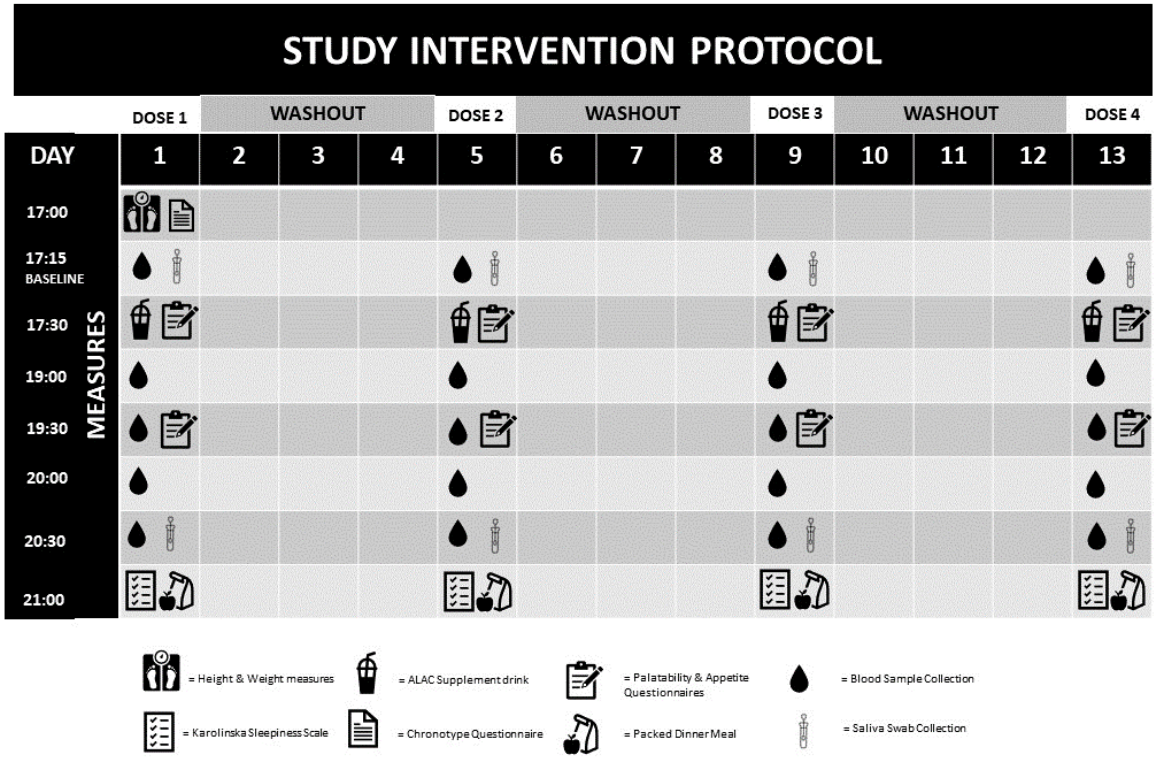

Figure 1 -*Graphical description of the study protocol. Example of a participant prescribed all 4 doses of alpha - lactalbumin supplement in random order and the measures taken at different time intervals.*

Study Measures:

- Plasma tryptophan, large neutral amino acids through blood sample collected.
- Serum melatonin levels through saliva samples.
- Palatability, appetite, and preference of the ALAC protein supplement via questionnaires

Participant Commitment:

- Online brief screening questionnaire
- 3-day diet recall via Easy Diet Diary smartphone app
- Chronotype questionnaire will be administered once during the first visit to the laboratory.
- 4 intervention sessions at Deakin University Burwood campus for each dose of ALAC supplement (separated by a minimum 3-night wash-out period)
- A cannula will be inserted on the day of each intervention (4 occasions total), with five blood samples to be taken on each of these days. (0 mins, 90 mins, 120 mins, 180 mins and 210 mins)
- A saliva sample will be taken twice (time 0 mins and time 210 mins) post ALAC supplement consumption on each of the 4 occasions.
- Palatability and Appetite Questionnaires during each of the four doses of ALAC supplement consumption.

5. Collection of Blood and Saliva Samples

By consenting to take part in this study, you also consent to the collection and use of blood samples as specified below. Blood samples will be taken via cannulation of a vein in your forearm at five timepoints on four different intervention days of the study. These blood samples will be stored in the laboratory freezer for later analysis of amino acids within blood plasma. Saliva samples will be collected at two points during each intervention day via an oral saliva swab and the extracted and stored in the laboratory freezer for later analysis of plasma melatonin levels.

6. Possible Benefits

Previously, α-lactalbumin supplementation has been investigated in several studies around improving mood and sleep outcomes positively. Within these studies, a wide range of dose between 5 to 60 g α-lactalbumin have been implemented amongst participants to study varied outcomes. Due to the investigative nature of the α-lactalbumin, it is yet to be approved by the TGA. The α-lactalbumin provided is commercially available in the USA and is approved by the Food and Drug Administration for use as a food ingredient.

By participating in this research, you will help to determine the optimal dosage of α-lactalbumin in an active population, thus guiding future research on the use of this optimal dosage for possible benefits on sleep and mood outcomes.

7. Possible Risks

There are few foreseeable risks throughout this study albeit minimal, including risks associated with blood sampling. There is a risk of bruising at the blood collection site, along with a small risk of infection. Researchers carrying out the blood sampling will be certified for this procedure, thereby reducing the risk of bruising. Infection risk is minimised by using sterile equipment for blood collection. The amount of blood taken throughout this study is minimal (5 mL samples). There is also a risk involved with possible drowsiness post consumption of the supplement which will be screened for using a validated tool i.e., Karolinska Sleepiness Scale before you leave the laboratory. In the unlikely event that you score >6.8 on the KSS scale (indicating drowsiness which could impair alertness while driving), you will be requested to either stay back on Deakin University campus where temporary sleeping arrangements will be made for the night, or you will be provided with alternative transport arrangements to get home safely. Kindly note this in advance to plan to be dropped off to Campus before the trial or be prepared to leave your vehicle on campus in case need arises.

Alpha-lactalbumin is not approved by the Therapeutic Goods Administration, however, has been supplemented previously in multiple studies at various dosages ranging from 5g to 60g. The α-lactalbumin supplement used in this study has received Generally Regarded as Safe (GRAS) approval by the US Food and Drug Administration.

8. Privacy, Confidentiality and Disclosure of Information

Any information obtained in this research project that can identify you will remain confidential and will only be used for the purpose of this research project. It will only be disclosed with your permission, except as required by law. Forms with identifying information will be stored using secure password protected software, with any physical copy of data stored within a locked filing cabinet when not in use.

A unique participant ID code will be used on all forms and data collected from you, and not with your name or any other identifying information. These data will be stored on a password protected Deakin network. Only the investigators at Deakin University will have access to this data. Any sharing of data with investigators outside of Deakin will occur only in a coded, anonymous way, with no identifiable or personal information to be shared.

The results of this study will be presented at scientific conferences, in scientific journals and research theses, with all information provided to remain anonymous. Your identity and personal information will not be disclosed. As a clinical trial, data is required to be retained for a minimum of 15 years as per research conduct policy.

9. Results of the Project

Upon completion of all intervention sessions, you will be provided with the final research report once it has been published. A member of the research team will send this information to you via email. Please indicate on the Consent Form attached below if you would like to receive this information. The research staff will not use the results to diagnose any medical conditions.

10. Participation is Voluntary

Participation in any research project is voluntary. If you do not wish to take part in this project, you are not obliged to. If you decide to take part and later change your mind, you are free to withdraw from the project at any stage.

Your decision whether to take part or not to take part, or to take part and then withdraw, will not affect your relationship with the research team, the School of Exercise and Nutrition Sciences, or Deakin University. You will also have the option to withdraw any data collected from the study should you wish to do so.

Before you make your decision, a member of the research team will be available so that you can ask any questions you have about the research project. Sign the Consent Form only after you have had a chance to ask your questions and have received satisfactory answers.

If you decide to withdraw from this project, please notify a member of the research team and sign the withdrawal of consent form before withdrawal. Please also indicate whether you wish to withdraw any previously collected information from the study.

11. Payments to Participants

You will receive a $100 gift card for your participation in the trial.

12. Further Information

If you require any further information or if you have any problems concerning this project you can contact the Principal Researcher Dr Dominique Condo, or the Student Researcher – Ms. Luana A Mascarenhas.

Dr. Condo will be available at:

Work email: dominique.condo@deakin.edu.au

Work telephone: 03 9251 7309

Ms. Mascarenhas will be available at:

Work email: l.mascarenhas@deakin.edu.au

Mobile telephone: 047849959

13. Complaints

If you have any complaints about any aspect of the project, the way it is being conducted or any questions about your rights as a research participant, then you may contact:

The Human Research Ethics Office, Deakin University, 221 Burwood Highway, Burwood Victoria 3125, Telephone: 9251 7129, research-ethics@deakin.edu.au

Please quote project number (2023-XXX).

14. Ethical Guidelines

This project will be carried out according to the *National Statement on Ethical Conduct in Human Research* (June 2007) produced by the National Health and Medical Research Council of Australia. This statement has been developed to protect the interests of people who agree to participate in human research studies.

As per good clinical practice, this research will undergo continuous monitoring in the form of annual reports. Given the small sample size and capacity of participants undergoing the study at one time, Dr Condo (CI) will monitor participants throughout the study to ensure there are no adverse effects of taking the supplement. This is unlikely given it is a food product safe for human consumption.

15. Source of Funding

This research is partly funded by industry grant from Agropur Inc, Appleton, WI.

PLAIN LANGUAGE STATEMENT AND CONSENT FORM

TO: Participants,

Consent Form

Date:

Full Project Title: Dose response of alpha lactalbumin supplementation on serum TRP:LNAA ratio.

Reference Number: (2023-XXX)

I have read and I understand the attached Plain Language Statement.

I freely agree to participate in this project according to the conditions in the Plain Language Statement.

I have been given a copy of the Plain Language Statement and Consent Form to keep.

The researcher has agreed not to reveal my identity and personal details, including where information about this project is published, or presented in any public form.

The aims, methods, anticipated benefits, and possible risks of the research study have been explained to me.

The researcher has agreed not to reveal my identity and personal details, including where information about this project is published, or presented in any public forum.

I understand that I am free to withdraw my consent at any time during the study, in which event my participation in the research study will immediately cease.

I extend my consent for the use of my data in future research projects that are extensions of, or closely related to, the original project or in the same general area of research. ☐

Do you wish to receive a final publication of this study? **(Yes / No)**

If you answered 'Yes' to the above question, please provide your email address below:

……..

Participant's Name (printed) ……

Signature: …… Date: ……

PLAIN LANGUAGE STATEMENT AND CONSENT FORM

TO: Participant,

Withdrawal of Consent Form

(To be used for participants who wish to withdraw from the project)

Date:

Full Project Title: Dose response of alpha lactalbumin supplementation on serum TRP:LNAA ratio.

Reference Number: (2023 -XXX)

I hereby wish to WITHDRAW my consent to participate in the above research project and understand that such withdrawal WILL NOT jeopardize my relationship with Deakin University.

Participant's Name (printed) ……

Signature ……Date ……

Please post or email this form to:

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