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| **DEAKIN UNIVERSITY HUMAN RESEARCH ETHICS COMMITTEE****PROJECT DESCRIPTION/PROTOCOL** |  |

**Instructions for preparing the project description/protocol**

1. The purpose of the Project Description is to provide the scientific and academic background and context of a research project.
2. A Project Description is a **mandatory** component of a submission using the Human Research Ethics Application (HREA).
3. The section headings in this Project Description template represent a structure for presentation of information about a research project that meets the needs of an ethics review body.
4. Not all headings or sub-headings in this template are relevant for each research project. Where a question is not relevant please enter NA into the response box. Please do not delete the question.
5. Researchers may use visual aids embedded in the project description/protocol to assist in describing their project where appropriate (e.g. images, videos etc.).
6. Submissions of clinical trial proposals may use alternative protocol templates, such as the [SPIRIT statement](http://www.spirit-statement.org/).
7. Researchers may choose to submit an existing document (such as a protocol or project description that has already been developed) instead of developing a new document.
8. If researchers choose to submit an existing document instead of using one of the templates provided, they may need to provide indications to the ethics review body of where in the submitted document the content corresponding to the relevant fields in the template are located.
9. There is no need to duplicate information in the HREA into the Project Description or vice versa.
10. Language that is understandable to non-technical reviewers should be used.

**COVID-19**

Please indicate whether:

✓

☐ Your project is not designed to align with current COVID-19 restrictions and

1. will be delayed until all restrictions are lifted and
2. will be modified with approval prior to commencement if unforeseen flow-on effects from the virus raise new ethical issues OR

☐ Your project will be conducted once approval is granted and you have described in this application how the project’s design aligns with:

1. The current COVID-19 restrictions,
2. Deakin’s [COVIDSafe Management Plan](https://deakin365.sharepoint.com/sites/CampusReactivation/SitePages/Our-COVID-Safe-Management-Plan.aspx) and
3. Any applicable [COVIDSafe Activity Plan](https://deakin365.sharepoint.com/sites/CampusReactivation/SitePages/COVIDSafe-Activity-Plan.aspx) in place for high COVID risk activities.

☐ Where your project may include COVID-19 related risks, please acknowledge that you have taken into consideration the information provided on the [FAQs - Human Research Ethics](https://deakin365.sharepoint.com/sites/CampusReactivation/SitePages/Research-FAQs.aspx) site.

Please indicate whether your research will include direct or indirect questions related to the participants’ lived experience of COVID-19? Yes ☐ No ☐

✓

1. If Yes, tick to confirm you have:

☐ Included an appropriately tailored version of the following statement in your Plain Language Statement:

**Low risk projects only**

*“While it is not expected that participating in the research will cause you to feel distress, we recognise the challenging circumstances the COVID-19 pandemic has caused for many community members. As such, we would like to highlight that if you, or those close to you are experiencing distress, or are in need of additional support, you are encouraged to contact [insert appropriate contact details for your participants e.g. Beyond Blue, Lifeline, Suicide Call Back Service, Headspace, Kids Helpline etc.].”*

**Higher than low risk projects**

*“In addition to the risks outlined in this document, we recognise the challenging circumstances the COVID-19 pandemic has caused for many community members. As such, we would like to highlight that if you, or those close to you are experiencing distress, or are in need of additional support, you are encouraged to contact [insert appropriate contact details for your participants e.g. Beyond Blue, Lifeline, Suicide Call Back Service, Headspace, Kids Helpline etc.].”*

☐ As applicable, included the same statement at the conclusion of your survey/ questionnaire/ research instruments.

1. And that you will:

☐ Immediately review all research data for any disclosures of heightened distress, suicidal ideation or attempts or self-harm. Researchers who are unable to review their data immediately or who need clarification on what kind of time frame constitutes an acceptably prompt review, should contact research-ethics@deakin.edu.au to discuss their options.

☐ Report disclosures as described above on the [FAQ website](https://deakin365.sharepoint.com/sites/CampusReactivation/SitePages/Research-FAQs.aspx#when-conducting-research%2c-what-emerging-risks-should-i-be-aware-of-that-have-arisen-because-of-covid-19-or-its-related-restricti).

**1. Project details:**

1.1 Please provide the project title -Effect of pre-sleep α-lactalbumin supplementation in a trained population with sleep difficulties

1.2 Please provide an acronym for the project (if appropriate)N/A

1.3 Please provide the project description/protocol version numberN/A

**2. Project Team Roles & Responsibilities:**

2.1 Please provide the names, affiliations, positions and responsibilities of individuals involved in the project beyond those outlined in the HREA (e.g. technical or support staff).

Name: Ms Monica Kelly

Affiliation: Colleague - Centre for Sport Research, Deakin University

Position: PhD candidate at Deakin University

Responsibility: External researcher to provide participants with supplement to ensure double-blinding

**3. Resources:**

3.1 Please provide details of the resources necessary for the project to be conducted, and the funding or support being sought or secured.

For this project, sleep monitors (DREEM headbands and Actical activity monitors), and protein supplements (α-lactalbumin and collagen) are required, which will be supplied by seed funding supplied by the Centre for Sport Research. Additionally, the cost of provided meals throughout the intervention trials will be provided by funding available to the principal investigator (Dr Dominique Condo). All questionnaires, diaries and dietary software will incur no further cost for the project, as these subscriptions are provided by the University. The cost for biochemical analysis (plasma amino acids and melatonin) and cognitive-based software will be covered by funding attained from the Centre for Sport Research. Biochemical analysis will be completed in-house at Deakin University, with consumables such as venepuncture needles, vacutainers, and syringes to be purchased for this study.

A supervised PhD student will perform biochemical and data analysis, which will incur no further cost to the project.

**4. Background:**

Please provide:

4.1 A lay summary of the literature review (approximately 1 A4 page)

The sleep of an athlete can affect cognition, mood, sports performance, muscle recovery, and adaptation to training (Walsh et al., 2021). Athletes are thought to require more sleep than the general population, with speculations that elite athletes undergoing heavy training periods may require upwards of 10-12 hours of sleep per night (Scott, 2002). However, the typical Australian athlete does not meet the general recommendations of 7-9 hours (Lastella et al., 2015), with data indicating that up to 65% of athletes experience poor sleep (Doherty et al., 2021). As athletes experiencing poor sleep may be subject to worse health and performance outcomes, this is a population in need of sleep intervention.

Novel research suggests that diet influences the sleep of athletes (Falkenberg et al., 2021), with one such dietary factor being the amino acid tryptophan (TRP). Tryptophan is the dietary precursor to serotonin and melatonin synthesis, both of which are factors involved in the sleep/wake cycle (Silber and Schmitt, 2010). To convert into these compounds, tryptophan must be transported across the blood-brain barrier by a specific transport system that also transports other large neutral amino acids (LNAA) into the brain (Silber and Schmitt, 2010). Therefore, tryptophan must compete against other LNAAs for transportation into the brain and thus, the ratio of TRP:LNAA in blood plasma affects the ability of tryptophan to cross the blood-brain barrier. Increases in the TRP:LNAA ratio promotes tryptophan uptake in the brain which ultimately increases serotonin and melatonin synthesis (Silber and Schmitt, 2010). As tryptophan is low in most protein sources, the intake of protein decreases the TRP:LNAA ratio (Wurtman et al., 2003), which is seen to reduce sleep efficiency and increase wake after sleep onset in elite athletes (Falkenberg et al., 2021). However, evening intake of protein (tryptophan-rich sources) was associated with a reduction in sleep onset latency within this athlete group (Falkenberg et al., 2021).

There have been no studies investigating the impact of pure tryptophan supplementation on athletes, however, literature within the general population displays that tryptophan elicits a sedative effect, is more effective in those with sleep complaints, and doses at or above one gram provide the most benefit to sleep (Silber and Schmitt, 2010). In the athlete population, three studies have investigated the effect of a whey protein fraction high in tryptophan, α-lactalbumin (4.8 g TRP per 100 g protein), on sleep outcomes (Miles et al., 2021, Oikawa et al., 2019, MacInnis et al., 2020). Two studies saw no improvement in sleep or performance outcomes (MacInnis et al., 2020, Oikawa et al., 2019), which may relate to these athletes already experiencing good quality sleep (>87% sleep efficiency and <3 minute sleep latency). One study of female athletes reported increases of non-rapid eye movement stage 2 sleep following simulated competition, which may have moderated to some extent, the next day improvements of intermittent running performance (Miles et al., 2021).

Following on from these studies, there remains questions relating to the effectiveness of α-lactalbumin within athletic populations. As tryptophan supplementation is most effective in those with sleep complaints, an athletic population experiencing poor sleep may be more receptive to α-lactalbumin intake, which is yet to be explored. Further, the structure of sleep cycles (sleep architecture) following α-lactalbumin remains to be known in male athletes. Alongside sleep, the peak timing of the TRP:LNAA following α-lactalbumin ingestion has not been studied, as well as the impact of this ratio on direct melatonin measures.

The proposed study aims to answer these questions in a laboratory-based, randomised double-blinded crossover trial

4.2 A rationale/justification (i.e. how the research will fill any gaps, contribute to the field of research or contribute to existing or improved practice)

As sleep can affect the performance, mood and cognition of an athlete, athletes with sleep difficulties may be subject to worse health and sporting outcomes. Athletes with sleep difficulties may be more sensitive to the hypnotic effects of tryptophan than those athletes previously studied. The effect of α-lactalbumin on the plasma TRP:LNAA ratio of athletes across multiple timepoints is yet to be explored, which will allow for optimal timing of supplementation to be investigated following this study. Additionally, the sleep architecture of male athletes in response to α-lactalbumin remains unknown, which may showcase beneficial effects to an athlete’s recovery.

This study aims to contribute to sports nutrititon guidelines for optimising the sleep of athletes.

4.3 The research questions/aims/objectives/hypothesis

**Research Question:** Does pre-sleep α-lactalbumin intake improve the sleep, mood and cognitive performance of a trained population experiencing sleep difficulties?

The **aims** of this this study are to investigate the effect of evening supplementation of 40 g α-lactalbumin (1.9 g TRP) compared to placebo within a male- and female-trained population with sleep difficulties on:

(a) total sleep time (hrs), sleep onset latency (min), sleep efficiency (%), sleep stage duration (%, hrs), REM latency (min) and wake after sleep onset (min) as measured through portable electroencephalography (EEG).

(b) the response and peak timing of the TRP:LNAA ratio in blood plasma

(c) nocturnal plasma melatonin. This will help to better understand the direct influence α-lactalbumin has on the sleep/wake cycle.

(d) evening and morning mood as determined by the Brunel Mood Scale

(e) evening and morning sleepiness as determined by the Karolinska Sleepiness Scale

(f) post-wake cognitive performance assessed via the Stroop and psychomotor vigilance test

The **hypotheses** for this study are that evening supplementation of 40 g α-lactalbumin (1.9 g TRP) compared to placebo within a male- and female-trained population with sleep difficulties will:

(a) reduce sleep onset latency and wake after sleep onset

(b) increase the plasma TRP:LNAA ratio, with peak blood concentration reached within two hours post-consumption

(c) Increase nocturnal plasma melatonin levels

(d) reduce evening and morning depressive mood scores

(e) increase evening sleepiness and reduce morning sleepiness

(f) improve post-wake cognitive test performance

4.4 The expected outcomes

Outcomes from this research will help guide the pre-sleep nutrition of trained populations to improve their sleep, which can better performance, wellbeing and overal health.

**5. Project** **Design:**

Please provide details of:

**The research project setting**

5.1 This may include physical sites, online forums and alternatives

This research project will occur at the Deakin University Burwood campus, located in Burwood, Victoria, Australia. Participants will stay overnight within the nursing laboratories of the Burwood campus (School of Nursing and Midwifery Simulation Rooms), with testing to occur in this setting also. Note that this setting has been used previously for a sleep restriction study.

**6. Methodology:**

6.1 The methodological approach

A double-blinded counterbalanced cross-over design has been selected. The double-blinded approach means both the researchers and participants will not know which condition is being received. Through a crossover design, each participant will undertake two intervention periods, one in which they consume the experimental supplement (α-lactalbumin), and one where they will consume the collagen placebo (control condition). The counterbalanced design ensures that even amounts of participants complete the two conditions in one order, or the reserve order. As is the nature of a crossover trial, each participant will serve as their own control. The two arms of the study will involve:

- Protein dietary standardisation of 1.2 g/kg of body weight for each intervention period, to limit the impact of protein on the TRP:LNAA ratio.

- 3 × nights of 40 g α-lactalbumin supplementation (containing 1.9 g TRP)

- 3 × nights of 40 g collagen supplementation (placebo – containing 0 g TRP)

- minimum 5 × nights of washout period. The washout period is implemented to reduce any carry over effect of the experimental condition into the following intervention period.

6.2 The rationale for choices of method/s (tied to project aims/objectives)

Double blinding ensures that both the research team and participants are unaware of the treatment groups throughout the intervention periods, minimising the risk of bias. As the chosen population has sleep difficulties, a crossover design allows for the participants to act as their own controls, thus limiting confounding influences of differing sleep habits and magnitudes of sleep difficulties. Further, as participants are training throughout the project, a crossover design reduces confounding influences of training status and habitual exercise. Assigning participants to groups in a randomised counterbalanced manner ensures that the sequencing of experimental conditions does not affect results.

**7. The participants including:**

7.1 A description and the number of participants

Eighteen trained participants with sleep difficulties aged ≥18-40 years will participate in the study

7.2 The inclusion and exclusion criteria

***Inclusion:*** Participants must be ≥18-40 years old, and currently be completing structured moderate-vigorous exercise at least three times per week for a minimum total of five hours per week to meet “trained athlete” guidelines (De Pauw et al., 2013). Further, participants will be screened using the Athlete Sleep Screening Questionnaire (Appendix 1) and Pittsburgh Sleep Quality Index (Appendix 2), and those with a sleep difficulty score ≥5, global PSQI score >5 and a sleep onset latency >15 minutes will be eligible. These scores coincide with ≥mild sleep difficulty and poor-quality sleep, which are the targeted participants for this study.

Female participants are required to be naturally menstruating or taking an oral contraceptive pill. As female hormone differences across the menstrual cycle can influence sleep (Baker and Lee, 2018), participation will occur during predictable phases where the influence of hormones is low (Knowles et al., 2019).

***Exclusion:*** smoking, excessive alcohol consumption (>17 standard drinks per week), dairy allergy, high caffeine use (e.g., >5 mg∙kg-1∙d-1), antidepressant or sleep medication use, current or recently finished night shift work, recent transmeridian travel, fluctuating bedtimes, and pregnancy. These exclusions criteria relate to confounding influences that may affect sleep or the plasma TRP:LNAA ratio.

7.3 The sample size and statistical or power issues

Eighteen participants are to be recruited as per the statistical power analysis outlined in 11.4. This statistical power analysis was completed in consultation with a Deakin biostatistician, and was performed using Power Analysis and Sample Size Software (Version 16, UT, USA).

7.4 Your participant recruitment strategies and timeframes (as required in addition to that outlined in the HREA)

Mr Barnard will be the primary researcher involved with recruitment. Participants will be recruited through flyers (Appendix 11) placed at Deakin University, local gym settings and sporting clubs, with online platforms (e.g., social media) also used to reach a wider audience. With permission from university unit chairs within the School of Exercise and Nutrition Sciences (see attached Organisation Consent Form), recruitment talks will be completed during lectures and classes within relevant units. Recruitment posts will be uploaded on CloudDeakin pages with permission from unit chairs. All interested individuals will be provided with a plain language statement (Appendix 12) and are to return a consent form to either Mr Barnard or Dr Condo. No data collection will take place before written consent is received by the research team. Recruitment will commence in January 2021 (COVID permitting) and will continue until a sample size of 18 individuals are recruited.

Before the first participant is recruited, this study will be registered within the ANZCTR.

7.5 Your approach/es to provision of information to participants and/or consent (as required in addition to that outlined in the HREA)

Any interested individuals will be provided with a digital and hard copy of the plain language statement and consent form to keep. The plain language statement outlines the study design, contact details and states that an individual’s decision to be involved in the study will not jeopardise their relationship with Deakin university and the research team. The consent form is to be signed and returned before any data collection is commenced.

7.6 If necessary, the type of consent provided to different participant groups, when and where, and any arrangements to confirm that consent

Written consent. All participants will be provided with a plain language statement and are to sign a consent form before participating in the trial.

7.7 If necessary, details of who will be confirming or re-negotiating consent with participants and the process/es that will be undertaken

Participants will be able to withdraw their consent at any time during the study which will not jeopardise their relationship with Deakin University. Participants must complete the ‘withdrawal of consent form’ (attached to the Plain Language Statement) and return it to a member of the research team.

**8. Research Activities:**

What you are going to do? Please include:

8.1 The participant commitment

***COVID-19 Safety Measures***

All participants will be required to obey Victorian COVID-19 safe practices prior to entering the Deakin University Burwood campus. A COVID-19 questionnaire (Appendix 13) is to be completed prior to visiting the university for familiarisation or testing. These screening questions will include details around symptoms, contact with a confirmed or suspected COVID-19 case, contact with anyone from overseas, or any interstate or international travel within the previous 14 days. If the participant responded with yes to any of these questions, they will not be allowed to enter the campus and are to isolate and get tested as per the COVID-19 protocols outlined by state health authorities. All persons (participants and researchers) will be required to check in using QR codes to confirm their presence within the Deakin University Burwood campus. Whilst on campus, both participants and researchers are to practice good hand hygiene, cover their nose and mouth with a flexed elbow when coughing or sneezing, maintain social distancing of 1.5 metres, and comply with current mask policies. All surfaces and equipment will be thoroughly cleaned and disinfected after use, and between the use of each participant. If required, all communication that can be conducted virtually will do so via online platforms (e.g., Zoom, email). An attendance register will be kept for all participants and research staff for each day they are on campus

***Methods***

This study will be split into six phases: screening, baseline, familiarisation, and two intervention phases separated by a washout period (Figure 1). Briefly, participants will be required to report to Deakin University (Burwood campus) on a minimum of three separate occasions, which will involve a total of seven nights residing within the Deakin University laboratories (School of Nursing and Midwifery Simulation Rooms). The screening phase will involve determination of study eligibility. Familiarisation will be completed on-campus, which introduces participants to future tasks and requires them to stay overnight to become familiar with the sleeping environment. Baseline measures will determine habitual sleeping, eating, and training behaviours, helping for standardisation of these factors. For instance, habitual sleep patterns over these five days will guide participant ‘prescribed’ sleep/wake times throughout the study, and energy intake throughout the study will be matched to usual habitual intake. The intervention period will then require participants to stay overnight at Deakin University for three consecutive nights, followed by a washout period of a minimum of five nights, before the second intervention period is undertaken. A graphic overview of the study measures, timeline and details are provided in Figure 1.



**Figure 1**. *Graphic overview of the study*

***Screening Session***

To determine participant eligibility. Participants will be screened via the Athlete Sleep Screening Questionnaire (Appendix 1) and Pittsburgh Sleep Quality Index (Appendix 2), with female participants also required to complete a menstrual cycle questionnaire (Appendix 4). This can be completed via an online consult (i.e., Zoom), or in-person. Participant chronotype is also to be assessed at the screening phase (via Morning-Eveningness questionnaire (Appendix 3)). Also, after recruitment, females will complete a menstrual cycle diary for the two months preceding the study (Appendix 5), to allow for appropriate scheduling of female participants within certain phases of the menstrual cycle (i.e., early follicular phase) (Knowles et al., 2019). As fluctuations of female hormones across the menstrual cycle can influence sleep (Baker and Lee, 2018), females are to complete testing when the influence of female hormones are low (Knowles et al., 2019). This diary is also to be completed throughout the study and for one-cycle post to confirm cycle status at the time of testing.

***Familiarisation Session***

To be completed 1-week before study commencement. Participants are to become familiarised with the battery of cognitive tests, which are to be performed on a tablet using the Joggle Research application. Participants will also spend the night of the familiarisation session wearing the portable EEG (DREEM headband) equipment within the Deakin University School of Nursing and Midwifery Simulation Rooms. An overnight familiarisation session is required to limit the ‘first night effect’ when sleeping in a new environment (Agnew Jr et al., 1966). Participants will have their own individual room, with a communal living area with kitchen facilities available to convene with other participants, which four can be accommodated for at any one time. Participant height, mass, and age will also be collected at the familiarisation trial by a Level 1 ISAK-accredited technician (Mr Barnard or Dr Condo). A validation paper has been provided for the DREEM headband as Appendix 14, where DREEM’s automatic sleep staging data provides 84% accuracy compared to polysomnography.

***Baseline Measures***

Following the familiarisation trial, participant sleep, diet and training will be assessed for a five-day period (including one weekend day). Sleep will be assessed via an actigraph (Actical Z; Phillips Respironics, OR, USA), with the individual’s average habitual bed and wake times across these five-days to form their individually ‘prescribed’ bedtime and wake time for the duration of the study. Physical activity output will also be assessed using this same actigraph for the baseline measurement period. The actigraph device is to be worn by participants for the entirety of the study, to provide as a control for physical activity output and sleep outcomes across the study. An actigraph is a non-invasive small watch-like device, typically worn on the non-dominant wrist. Further, habitual food intake will be assessed through a five-day food record completed via Easy Diet Diary (Xyris Software, Brisbane, QLD), with participants providing photos and details of food quantities consumed within the app. Dietary intake will then be quantified through Foodworks (Version 9, Xyris Software, Brisbane, QLD) software by an accredited sports dietitian, which will inform the dietary standardisation (i.e., energy intake, macronutrient requirements). Training will be monitored through a five-day training diary (Appendix 10), enabling researchers to quantify typical training loads and sessions, which will detail the sessions that participants will complete throughout the intervention periods. Researchers will not be designing the training program during the intervention periods, instead, participants will be completing typical habitual training sessions outlined in this five-day period. After the baseline period has been completed, participants will be ready to commence the intervention phase.

***Randomisation***

Participants will be randomly assigned to the control or intervention condition through simple randomisation techniques in a counterbalanced manner by an external researcher. To blind both participants and the research team, the placebo and experimental drinks will be matched for taste and provided to the participants by an external researcher (as listed in section 2.1).

***Dietary Standardisation***

Participants will be provided with dietary controlled meals matching their habitual energy intake throughout the intervention periods. To limit the effect of dietary protein on the TRP:LNAA ratio, protein will be standardised to 1.2 g∙kg-1 of body weight per day during the intervention period, as this is at the lowest end of recommendations for athletes. (Thomas et al., 2016) Throughout the experimental trials, the 40 g supplement (α-lactalbumin or collagen) will be in addition to the 1.2 g∙kg-1 of body weight protein standardisation. Three main meals plus a morning and afternoon snack will be provided to the participants. The dinner meal will be lower in protein than the other main meals as to not impact the effectiveness of the experimental supplement. (Wurtman et al., 2003) Outside of provided foods, water, and approved low-protein snacks and drinks, participants will not have access to external foods.

***Experimental Sessions***

For the first three nights, participants will reside at the Deakin University laboratory (School of Nursing and Midwifery Simulation Rooms). On the first night, participants will arrive to the laboratory at 17:00. A researcher will fit participants with an indwelling cannula within the first hour of arrival (17:00-18:00). A standardised low protein dinner will then be provided, being at least four-hours before individual bedtimes. Two-hours before prescribed bedtime, participants will receive a pre-mixed protein drink, containing either 40 g α-lactalbumin (BiPRO Alpha 9000; Agropur Inc, WI, USA) or 40 g collagen protein (Collagen Regenerate; Body Science, QLD, USA). To ensure consistency across the timeline, participants are to finish the drink within ten minutes. Blood samples will be drawn immediately prior to consumption, then again at 30-, 60-, 90- and 120-minutes post-consumption (Figure 2). Subjective sleepiness will be self-rated using the nine-point Karolinska Sleep Scale (KSS) by the participants every half an hour from supplement consumption, until bedtime. Additionally, the 24-question Brunel Mood Scale (BRUMS), and the 8-item Short Recovery and Stress Scale (SRSS) will be completed by participants 90 minutes post-supplement ingestion. The cannula will be removed following the final blood sample at the prescribed bedtime, with participants then to wear the portable EEG headband (Dreem; DREEM 3, PA, France)

Participants will have individually prescribed bed and wake times (i.e., average habitual bedtime) throughout the study. Thirty minutes after waking, participants will perform the KSS, SRSS and BRUMS questionnaire. A battery of cognitive tasks to assess vigilance and reaction time will then be completed 45 minutes after waking using the Joggle Research application on a tablet device. Participants will train habitually throughout the study, however, participants are to complete the same training session (or no training) at the same time on the corresponding day of the crossover. These sessions are to be reported in a training dairy, outlining exercise duration, type and rating of perceived exertion (RPE). All procedures will be replicated for the following two days; however, cannula insertion and blood sampling will only be completed on the first night of each intervention period. An example timeline of the intervention periods is presented in Figure 2.



**Figure 2.** *Intervention period graphic timeline*. Example of a participant prescribed bedtime at 22:30 and waketime of 07:30. KSS= Karolinska Sleepiness Scale, BRUMS= Brunel Mood Scale, SRSS= Short Recovery Stress Scale, EEG= Electroencephalography, PRO= Protein. ^= completed on intervention days 2, 3, 4. #= completed every half an hour from supplement consumption to bedtime. $= completed only on night 1 of each intervention period, \*Sleep/wake time are based on average habitual times as recorded during baseline – individually standardised.

***Washout***

After completion of the cognitive tests on the morning of day 4, participants are to return to their usual residence for a minimum 5-day washout period where no testing or supplementation will occur. For five days leading into the next intervention period, participants will be required to maintain their prescribed bed and wake time, complete the sleep diary and wear an actigraphy device. Further, participants will consume their habitual diet for the entire washout period.

Following the washout period, participants will arrive back to the Deakin University laboratory at 17:00 where methods will be replicated for the next three nights. An indwelling cannula will again be inserted for the first night only, with participants crossing over and receiving the other experimental supplement for the second intervention period.

***Female participant testing requirements***

As natural variations of the menstrual cycle can affect sleep (Manber and Bootzin, 1997, Baker and Lee, 2018), naturally menstruating females will complete both intervention periods during the early follicular phase, as this is when female sex hormone levels (i.e., oestradiol and progesterone) are low and stable (Knowles et al., 2019, Reed and Carr, 2015). This phase will be determined via menses, and through menstrual cycle diaries. Also, as naturally menstruating females will have varied cycle lengths (Mihm et al., 2011), the washout phase will be individually adjusted to ensure these females complete the next intervention period during the early follicular phase. Further, females using a monophasic contraceptive pill will complete the intervention phases during days 3-21 of their mapped cycle, with no testing to be completed during the inactive pill phase (days 22-28) and the first few days of active pill recommencement.

8.2 The project duration

This project is planned to be completed within two years, with recruitment to start in January 2022. This timeline is inclusive of all aspects of the research project.

8.3 Any participant follow-up

There is no planned follow up following cessation of the study.

Participants can state their preference on the consent form (Appendix 12) if they wish to receive individual results and a summary of overall research after the study has been completed. Individuals that have selected this option, will be provided with individual booklets outlining their personal data on sleep outcomes, mood, cognition and biochemical results via a nominated email address. Participants will be provided with a copy of the research report after publication if this option is selected on the consent form.

**9. Data Collection/Gathering:**

9.1 What information are you going to collect/gather? (as required in addition to that outlined in the HREA)

Please note that prior to the recruitment of the first participant, this clinical trial is to be registered within the ANZCTR.

***Personal information***

- Name

- Email address (to be sent electronic PLS, and if selected, individual results post-study)

***Baseline measures***

- Participant physical and clinical characteristics (age, height, mass, chronotype, sleep difficulty score)

- Habitual sleep, diet and training patterns prior to the intervention

***Outcome measures***

- Sleep outcomes (including total sleep time, sleep onset latency, sleep efficiency, wake after sleep onset, sleep architecture, subjective sleep quality, and sleepiness)

- Mood score (as per the BRUMS scale in the evening and morning)

- Stress and Recovery (as per the SRSS completed in the evening and morning)

- Cognitive performance (as performed within the Joggle Research app, including calculated scores of the motor praxis task, line orientation task, digital symbol substitution task, and the psychomotor vigilance test)

- Biochemical measures (plasma amino acids and plasma melatonin)

***Data Collection***

- Blood samples across five timepoints on two separate occasions to be collected

- Menstrual cycle information (questionnaire, diary – to inform eligibility and timing of participation within the trial)

9.2 Data collection/gathering techniques: How will you collect/gather the information?

***Baseline measures***

Anthropometry – Height will be measured using a stadiometer and mass by standard scales. A level 1 ISAK-accredited technician will perform these measures.

Participant characteristics – age, medication use, alcohol and caffeine consumption will be collected at baseline using a simple questionnaire. Participant chronotype will be collected through the Morning-Eveningness Questionnaire (Appendix 4).

Sleep difficulty – Participants will be screened using the Athlete Sleep Screening Questionnaire to determine sleep difficulty score, and sleep onset latency data (Appendix 1). The Pittsburgh Sleep Quality Index (Appendix 2) will also be completed, which explores factors relating to quality of sleep.

Diet – Participants will complete 5-day food records using the Easy Diet Diary smartphone app, with participants to also upload photos of their meals to improve accuracy for later quantification. An accredited sports dietitian will then quantify these food record through Foodworks software.

Training – Participants will record all training sessions throughout the study (exercise duration (mins), type and rating of perceived exertion (1-10 scale)), with a 5-day training diary collected at baseline (Appendix 10). Physical activity output will be derived from the actigraph device.

Female menstrual cycle – Females will be required to be either naturally menstruating or taking an oral contraceptive, with a menstrual cycle questionnaire to be completed to determine this (Appendix 2). To determine menstrual phase, female participant’s menstrual cycle will be recorded in a diary for two months prior to the study, throughout the study, and one-month post in a diary (Appendix 3).

***Outcome measures***

Sleep data – collected via the DREEM headband (portable EEG) for the intervention period, and actigraphy (Actical monitor) device for the entire study period. The Actical monitor measures sleep/wake behaviours through accelerometry detected movement. Measures to be extracted from each objective sleep tool include total sleep time (hrs), sleep onset latency (mins), sleep efficiency (%), wake after sleep onset (mins). Sleep architecture data (sleep stage duration (min, %) and REM latency (min) will be extracted only from the DREEM device.

Additional subjective data will be collected using the consensus sleep diary – core (Appendix 9), which provides data related to sleep/wake times, sleep latency (mins), awakenings (n), and sleep quality (1-5 scale, i.e., 1 = very poor, 5 = very good). Subjective sleepiness data is to be collected via the 9-point Karolinska Sleepiness Scale (i.e., 1 = extremely alert, 9 = very sleepy, great effort to keep awake, fighting sleep) (Appendix 6).

Mood – Mood scores will be collected via the Brunel Mood Scale (Appendix 7), which divides mood into six dimensions including anger, confusion, depression, fatigue, tension, and vigour.

Stress and Recovery – Scores will be assessed via the Short Recovery Stress Scale (Appendix 8), which through 8-items, assesses recovery and stress across physical, mental, emotional, and overall domains in real-time.

Cognition – Numerous cognitive domains will be assessed via the tablet-based application Joggle Research. The battery of tests will be completed on a tablet device, and includes the motor praxis task, line orientation task, digital symbol substitution task, and the psychomotor vigilance test. These simple touch screen-based tasks will measure performance across multiple cognitive domains, including reaction time, spatial orientation, visual tracking, and vigilance.

Biochemical measures – Blood samples will be collected by a researcher with phlebotomy training across five-timepoints on the first day of each intervention period. Plasma amino acid analysis will then be completed at Deakin University through liquid chromatography triple-quadrupole mass-spectrometry. Plasma melatonin will be assessed through enzyme-linked immunosorbent assay according to manufacturer’s instructions.

9.3 Impact of and response to participant withdrawal

Participants will be informed about their right to withdraw from the study at any time, which will not jeopardise their relationship with the research team or Deakin University.

Data collected from a participant selecting to withdraw from the study will only be destroyed if this is requested (optional tick box on the withdrawal of consent form), otherwise all collected data will be retained for research integrity purposes.

Forms will be returned to a member of the research team, who will review the withdrawal form and store this within a locked filing cabinet.

If the number of participants that withdraw themselves from the study impacts the sample size required (n=18) then recruitment will continue in order to reach this sample size.

**10. Data Management:**

10.1 How will you store, provide access to, disclose, use/re-use, transfer, destroy or archive the information that you collect/gather? (as required in addition to that outlined in the HREA)

Digital and hard copies of data will be collected throughout this research. Digital data will be stored using the software sharing program ‘Syncplicity’, which is a secure program that is regularly backed up. Only the research team listed will have access to this data. Physical copies of data will be stored in a private lockable filing cabinet in an area of the Deakin University campus that requires security clearance to access.

Each participant will be allocated a unique study ID code. These ID codes will be linked to the participant names and stored within a spreadsheet. This spreadsheet linking participants to an ID code will be stored on this same network drive, which will only be accessible to the research team.

During the study, blood samples will be stored in -80 degree Celsius freezers in the level 2 analytical laboratories of building J at the Burwood campus. These laboratories are limited to swipe access only.

Collected data will be archived and stored for a period of 15 years following publication of results from this study. Following this 15-year period, data will be destroyed. All sensitive data contained as a physical copy will be shredded, any electronic data will be permanently deleted, and blood samples will be destroyed.

Include a data management plan in accordance with National Statement [3.1.45 and 3.1.56.](https://nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__438)

**11. Data Analysis:**

11.1 How will you measure, manipulate and/or analyse the information that you collect/gather?

Initially, data cleaning will be performed to identify missing and corrupt data. Data will be analysed using generalised linear mixed models within StataIC 16 (StataCorp LLC, TX, USA). The effect of dietary intervention (i.e., placebo or α-lactalbumin) and period (i.e., sequence receiving condition) and their interaction, will be fitted as fixed effects to determine whether there was a difference in the effect of dietary intervention over period on dependant sleep variables (e.g., sleep efficiency), mood, amino acid analysis and plasma melatonin. As training load will be a potential confounder to the nutrition-sleep relationship, this will be included as a covariate within the linear mixed model. Participant identification number will be used as a random factor to account for repeated measures in each model.

11.2 Please describe your matching and sampling strategies

There will be no requirement to create matched groups due to the repeated measures study design. Each participant will act as their own control.

11.3 Please outline how you will account for potential bias, confounding factors and missing information

*Bias*

- Double-blinded study

- Placebo and α-lactalbumin to be matched for taste. An external researcher is to provide participants with the supplement to ensure the research team remains blinded to the condition.

*-* Confounding factors such as training load may be added as a covariate during statistical analysis.

- Exercise – Physical activity output (Actical Z) and training load (training diary) will be measured throughout the trial.

- Chronotype – The Morningness-Eveningness Questionnaire will be completed to analyse any potential influence of chronotype

- Caffeine – Caffeine is not to be consumed after 12 pm each day of the intervention periods

- Diet – As protein, energy and carbohydrates can influence sleep, standardised meals will be provided to participants. A low protein dinner will be provided to minimise any potential impact on supplementation

- Sleep/wake times – Each participant will have an individually prescribed bed/wake time, which is the average habitual sleep/wake times of a participant throughout the five days of actigraphy measurements at baseline.

- As participants will be on-site, a member of the researcher will supervise and ensure that participants are not sleeping outside of prescribed hours.

- Electronic devices are not to be used in the evening due to confounding impacts on sleep.

- Light will be dimmed one hour before bedtime to limit the impact of evening light on sleep

Missing values will not be substituted in any circumstance (missing or otherwise). There are no planned data cleaning processes, with raw values only to be analysed

11.4 Please include your statistical power calculation

A two-sided t-test achieves **92%** power to infer that the mean difference is not 0.0 when the total sample size of a 2x2 cross-over design is **18**, the actual mean difference is **-0.600**, the square root of the within **mean square error** is **0.5**, and the significance level is **0.05**.

**12. Data Linkage:**

12.1 What linkages are planned or anticipated?

N/A

**13. Outcome measures:**

13.1 Please describe your outcome measures

Primary outcome measures include:

- total sleep time (hrs), sleep onset latency (min), sleep efficiency (%), sleep stage duration (%, hrs), REM latency (min) and wake after sleep onset (min) using portable EEG.

- amino acid analysis (i.e., plasma TRP:LNAA ratio)

Secondary outcome measures include:

- mood, cognition, subjective sleepiness, subjective sleep quality, stress and recovery, and plasma melatonin

**14. For research involving an investigational drug or device as part of a clinical trial:**

14.1 What is/are the drug(s) and/or device(s):

Αlpha-lactalbumin:

* Approved name – BiPRO Alpha 9000
* Trade name (if any) - N/A
* Manufacturer - Agropur
* Supplier of drug/device (e.g. manufacturer/pharmacy) - Agropur
* Approved therapeutic indication, dosage/duration in Australia – N/A
* Believed mode of action – Increased tryptophan availability 🡪 increases melatonin synthesis
(tryptophan active ingredient)
* Dosage regimen – 40 g
(tryptophan 1.9 g)
* Mode of excretion – N/A
* Known adverse events – None
* Known contra-indications or warnings – milk protein allergy and lactose intolerance
* If arrangements have been made for a Pharmacy Department to receive or dispense the drugs involved in this project, explain how the drugs will be received and dispensed for the purposes of the research project – N/A

**Placebo**

Collagen:

* Approved name – Collagen Regenerate
* Trade name (if any) - N/A
* Manufacturer – Body Science
* Supplier of drug/device (e.g. manufacturer/pharmacy) – Body Science
* Approved therapeutic indication, dosage/duration in Australia – N/A
* Believed mode of action – Placebo
* Dosage regimen – 40 g
* Mode of excretion – N/A
* Known adverse events – N/A
* Known contra-indications or warnings – N/A
* If arrangements have been made for a Pharmacy Department to receive or dispense the drugs involved in this project, explain how the drugs will be received and dispensed for the purposes of the research project – N/A

**15. Results, Outcomes and Future Plans:**

15.1 Please outline your plans for return of results of research to participants – include an ethically defensible plan in accordance with National Statement [3.1.65](https://nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__438) or [3.2.15](https://nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__725) or [3.3.36-3.3.61](https://nhmrc.gov.au/about-us/publications/national-statement-ethical-conduct-human-research-2007-updated-2018#toc__826), as appropriate.

Participants that have requested to receive their individual results on the consent form will be provided with a summary report of results at the cessation of the study.

Any results, which may require further clinical investigation will be documented, and with consent, a letter will be provided to the participant to be given to their general practitioner. The research staff will not use the results to diagnose any medical conditions.

15.2 Please describe your plans for dissemination and publication of project outcomes

Study outcomes will be disseminated to participants through individual booklets (if selected yes on the consent form), which provide personal data on anthropometry, dietary intake, sleep measures, blood analysis, and cognition and mood scores taken throughout the study. The final publication of the study will also be provided to participants selecting yes on the consent form. Further, results from this study will be disseminated at relevant conferences through presentations, and through publication in a peer-reviewed journal. For dissemination, all data will remain de-identified and participants will remain anonymous.

15.3 Please list other potential uses of the data at the end of the project

Data will not be used for any other purposes than previously described.

15.4 Please detail the project closure processes

Upon completion of all data collection and data analysis, results will be submitted to peer-reviewed journals for publication. Following publication, all data will continue to be stored per methods outlined in Section 10.

15.5 Please outline your plans for sharing and/or future use of data and/or follow-up research

The data and research findings from this study will be used to inform future research within the same field. This could include investigations of further blood markers related to α-lactalbumin supplementation within trained populations. Any follow-up research will be conducted as a separate project. Extended consent is being sought to use collected data in future research projects that are extension of, or closely related to, the original project or in the same general area of research.

15.6 Please describe any anticipated secondary use of data

There is no plan to use data collected within this project for any secondary use.

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