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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](mailto: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 on the sleep and performance of athletes with sleep difficulties within habitual environments

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 at Deakin University and Centre for Sport Research

Position: PhD candidate at Deakin University

Responsibility: Randomising participants to crossover conditions in a counterbalanced manner and preparing and matching supplements for taste.

**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 (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. All online questionnaires, diaries and dietary software will incur no further cost for the project, as these subscriptions are provided by the University. All equipment required for performance testing is available to the research team at Deakin University (i.e., Dynavision light board, force plates) and therefore will not require any additional funding. Participants will not be reimbursed for participation in this study.

**4. Background:**

Please provide:

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

Data from the UK indicates that up to 65% of athletes experience poor sleep quality (Doherty et al., 2021). For an athlete, adequate sleep is vital for optimal health, wellbeing, and performance, with inadequate sleep increasing the risk for illness, injury, and poor sporting performance (Watson et al., 2020, Möller-Levet et al., 2013, Kirschen et al., 2020). With Australian athletes failing to meet general guidelines and many athletes experiencing poor quality sleep (Lastella et al., 2015), investigation of novel aids to improve the sleep of this cohort are warranted.

The relationship between diet and sleep is a growing area of interest, with recent data suggesting protein influences athlete sleep (Falkenberg et al., 2021). Consumption of protein in the evening was associated with a reduction in sleep latency for these elite athletes. This was speculated to be due to frequently reported consumption of whey protein in the evening, which is high in the amino acid tryptophan (Layman et al., 2018). 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). Evening tryptophan intake elicits a sedative effect, with data indicating that supplementation is most effective for individuals struggling to fall asleep (Silber and Schmitt, 2010). Additionally, tryptophan supplementation has previously shown a “loading effect”, whereby sleep characteristics were further improved at the latter end of the week-long supplementation (Spinweber, 1986). This effect is yet to be studied in high tryptophan-based protein sources such as whey, with the effects of sub-chronic supplementation to be further investigated. A whey protein, α-lactalbumin, has the highest tryptophan content of commonly consumed protein, with α-lactalbumin supplementation displaying similar sleep inducing effects as pure tryptophan for individuals with sleep complaints (Layman et al., 2018, Silber and Schmitt, 2010).

Three studies have investigated the impact of evening alpha-lactalbumin supplementation on the sleep of athletes, producing modest findings (MacInnis et al., 2020, Miles et al., 2021, Oikawa et al., 2019). Two studies observed no significant influence on athlete sleep (MacInnis et al., 2020, Oikawa et al., 2019), whilst one Australian study observed a significant increase in N-REM stage 2 sleep duration following evening supplementation of 40 g α-lactalbumin (Miles et al., 2021). However, these studies recruited athletes without known sleep difficulties, which tryptophan-based supplementation appears most effective in populations with sleep onset insomnia. As athletes with sleep difficulties are prone to accumulating sleep debt, the impact this has on performance measures sensitive to sleep is also to be further investigated.

Tryptophan does not appear to be an ergogenic supplement in itself, however, improvements in sleep may promote enhancements in cognition and mood, improving sport performance (Williams, 2005). The impact of sleep on exercise and sports performance differs between modalities, with performances involving maximal effort over short periods of time appearing somewhat unaffected by sleep loss (Kirschen et al., 2020, Reilly and Edwards, 2007). Alternatively, skill-related fitness components such as reaction time (Jarraya et al., 2014), and accuracy (Reyner and Horne, 2013) appear sensitive to sleep loss. Sleep restriction data suggests that although athletes can perform singular, maximal efforts without impairment following sleep loss, athletes are unable to maintain performance across repeated bouts of physical activity (Fullagar et al., 2015). Data also indicates that as sleep debt accumulates, extended aerobic performance is likely impaired (Roberts et al., 2019). As successful performance in team sports demands high levels of technical skill, aerobic functioning and repeated efforts (Farley et al., 2020), athletes experiencing sleep difficulties and accumulating sleep debt may underperform. Alpha-lactalbumin supplementation may prove a viable strategy to reduce cumulative sleep debt and resultant decrements to performance in athletes with sleep difficulties.

The proposed study will investigate the impact of evening α-lactalbumin supplementation on the sleep and sports-related performance of athletes experiencing sleep difficulties, through a field-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 effects of α-lactalbumin on sleep following more than three days of supplementation remains to be known in any population group, with previous data from pure tryptophan literature indicating a “loading effect” may occur (Spinweber, 1986). Therefore, this study will observe the effects of sub-chronic (7-days) α-lactalbumin supplementation on athlete sleep and performance. Further, the ecological validity of α-lactalbumin is to be investigated in more depth, with sleep sensitive performance outcomes to be performed following sub-chronic supplementation.

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

4.3 The research questions/aims/objectives/hypothesis

**Research Question:** Does pre-sleep α-lactalbumin intake improve the sleep and sports-related performance of athletes experiencing sleep difficulties within their habitual environment?

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-athlete population with sleep difficulties in their habitual environment on:

(a) total sleep time (h), sleep onset latency (min), sleep efficiency (%), and wake after sleep onset (min) as measured through actigraphy and sleep diaries.

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

(c) performance as measured by the Yo-Yo Intermittent Recovery Test Level 1, and 30-second continuous jump test

(d) reaction time via Dynavision light board.

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

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-athlete population with sleep difficulties in their habitual environment will:

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

(b) increase evening sleepiness and reduce morning sleepiness

(c) increase Yo-Yo Intermittent Recovery Test Level 1 distance, and reduce fatigue index across the 30-second continuous jump test

(d) reduce reaction time

(e) reduce evening and morning depressive mood scores

4.4 The expected outcomes

This research is expected to help formulate sports nutrition guidelines to help improve the sleep of athletes. Improvements in sleep can result in better sports performance, wellbeing and overal health for athletes.

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

Please provide details of:

**The research project setting**

5.1 This may include physical sites, online forums and alternatives

Participants will primarily remain within their habitual setting (i.e., home) for the duration of the study, which is where they will also be taking the prescribed dietary supplements. However, on seven occasions, participants are required to present to the Deakin University Burwood campus, located in Burwood, Victoria, to complete sports-related performance testing.

**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 reverse 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 total daily protein intake on sleep.

- 7 × nights of 40 g α-lactalbumin supplementation (containing 1.9 g TRP). The effect of sub-chronic α-lactalbumin supplementation remains to be studied, with previous tryptophan literature displaying a potential “loading effect” with one-week of supplementation (Spinweber, 1986). A one-week supplementation period has been selected to measure this potential loading effect.

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

- minimum 6 × nights of washout period. The washout period is implemented to reduce any carry over effect of the experimental condition into the following intervention period. Previously, 2 g supplementation of pure tryptophan for three nights displayed improved sleep characteristics up to four days post-supplement cessation (Schneider-Helmert, 1981). As this effect has not been explored in studies supplementing α-lactalbumin, a minimum 6-night washout will be observed.

- 6 × nights of post-study measures to be completed

- 6 × sports-related performance testing days to be completed. Two baseline sessions, testing after each intervention period, and testing after each 6-day washout 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. Additionally, continuing measures of objective sleep for 6 nights following each intervention period allows for any potential “loading effect” of supplementation to be observed.

The α-lactalbumin supplementation dosage of 40 g has been selected as this has been used in all previous athlete literature (MacInnis et al., 2020, Miles et al., 2021, Oikawa et al., 2019), with one study displaying improved sleep characteristics (Miles et al., 2021). Also, within non-athlete literature, 40 grams was calculated as the optimal amount of α-lactalbumin to be supplemented to improve sleep metrics through general linear mixed modelling (Halson et al., 2020).

Two baseline sports-related performance tests are to be recorded to limit learning effects, whereby performance is stabilised following two physical performance tests (Hopkins et al., 2001). Additionally, the sports-related testing is to occur after the 6-day washout periods to observe any “continuing effect” that α-lactalbumin supplementation may have on performance.

**7. The participants including:**

7.1 A description and the number of participants

Twenty-four athletes 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 actively competing in a team-sport and registered with a club at a minimum community level. Participants will be recruited from a range of invasion team-sports (e.g., football, rugby, soccer) (Lamas et al., 2014), with participants to be completing structured 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 and measures have been selected as these equate to ≥mild sleep difficulty and poor-quality sleep as per the ASSQ and PSQI (Samuels et al., 2016, Buysse et al., 1989), with the National Sleep Foundation classifying sleep latencies ≤15 minutes as good sleep quality (Ohayon et al., 2017).

Female participants are required to be naturally menstruating or taking an oral contraceptive pill (see Section 8.1 “Female participant testing requirements”). 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 (Britton et al., 2020)), dairy allergy, high caffeine use (e.g., >5 mg/kg/d), antidepressant or sleep medication use, current or recently finished night shift work, recent transmeridian travel, fluctuating bedtimes, and pregnancy. This exclusion criteria relates to confounding influences that may affect sleep or the plasma TRP:LNAA ratio.

7.3 The sample size and statistical or power issues

Twenty-four 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 relevant invasion team-sports organisations including but not limited to the Australian/Victorian Football League, Basketball Victoria, and Football Victoria. Researchers will contact these sporting organisations and seek permission to distribute information about the study to their athletes via email. Recruitment will also occur via social media, reaching out to potential participants that are actively competing in a team-sport, and meeting the inclusion/exclusion criteria. Additionally, participants will be recruited through flyers placed at Deakin University (Appendix 11). 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 24 individuals are recruited.

Before the first participant is recruited, this study will be registered within the Australian New Zealand Clinical Trials Registry (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 performance 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***

Graphical user interface

Description automatically generatedThis study will be divided into six main phases: screening, baseline, familiarisation, and two intervention phases separated by a washout (Figure 1). Each of these phases will be discussed in the following sections.

**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. As participant chronotype can be a confounder to sleep (Facer-Childs et al., 2018), this is 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 the intervention periods 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 Deakin University Burwood sports science facilities where they will be completing testing throughout the study. Participants will be given a briefing on the sports-related tests and will perform these in part to become familiarised with each test (i.e., Yo-Yo Intermittent Recovery Test Level 1, 30-second continuous jump test, and reaction time via Dynavision light board). 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).

***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. An actigraph device is a non-invasive watch-like device, which is to be worn by participants for the entirety of the study for recording of physical activity and sleep data. 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. This food-record software will inform dietary standardisation throughout the intervention periods, with an accredited sports dietitian providing individualised meal plans that provide 1.2 g/kg/day protein, and match habitual energy intake. Training will be monitored through a five-day training diary (Appendix 10), enabling researchers to quantify typical training loads and sessions, which can be a confounder to sleep outcomes.

Following these baseline measures, participants are to complete two baseline recordings of the performance tests, separated by a minimum of one day (i.e., Yo-Yo Intermittent Recovery Test Level 1, 30-second continuous jump test, and reaction time via Dynavision light board). The second baseline performance testing day will be one day prior to the commencement of the intervention period. Multiple physical performance tests are required to reduce learning effects, with physical performance stabilising following two testing sessions (Hopkins et al., 2001). Both baseline testing days will again be completed within the Deakin University Burwood sports-science building.

***Randomisation***

Participants will be randomly assigned to the control (placebo) or intervention condition (α-lactalbumin) 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 prepared and matched for taste by an external researcher (as listed in section 2.1), with Mr. Barnard then delivering pre-made supplements to the participants at an arranged location.

***Dietary Standardisation***

Participants will be provided with an individualised meal plan that matches their habitual energy intake throughout the intervention periods. To limit the effect of dietary protein on sleep, 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. The listed dinner meals will be lower in protein than the other main meals as to not impact the effectiveness of the experimental supplement (Wurtman et al., 2003).

To monitor compliance, participants are to take an image of the meals being consumed over the intervention period and upload them to Easy Diet Diary to ensure they are following the individualised meal plans.

***Experimental Sessions***

During the intervention periods, participants will consume either the 40 g α-lactalbumin or placebo (40 g collagen) two hours prior to average habitual bedtime for seven nights (Figure 2). Participants will receive a text-message reminder at individually standardised times each night to consume the supplement and fill in evening questionnaires. Ninety minutes post-supplement consumption (thirty minutes prior to prescribed bedtime), participants will complete the nine-point Karolinska Sleepiness Scale (KSS) as this has previously been a time of peak sleepiness following tryptophan consumption (Chauffard-Alboucq et al., 1991, Åkerstedt and Gillberg, 1990). Additionally, at this timepoint, the Brunel Mood Scale (BRUMS) will be completed to assess mood status, and the 8-item Short Recovery and Stress Scale (SRSS) to assess recovery. Participants will be sleeping in their home environment, with sleep measured objectively via actigraphy (Actical Z; Phillips Respironics, OR, USA) and subjectively through sleep diaries.

Thirty-minutes upon rising, participants are to again complete the KSS, BRUMS, and SRSS questionnaires, and a sleep diary detailing subjective sleep measures. A text message will be sent through as a reminder to complete these tasks. Throughout each day, participants will fill out a training diary, and report training duration (min), exercise type and rating of perceived exertion (RPE) for each session (or report that no training was completed). These measures allow for researchers to observe intensities of the training sessions, as well as potential exercise confounders between experimental periods (Day et al., 2004, Wang and Boros, 2021). The intervention period is represented graphically below in Figure 2.

Graphical user interface, application

Description automatically generated

**Figure 2.** *Intervention period graphic timeline*. Example of a participant prescribed bedtime at 22:30 and wake time of 07:30. KSS= Karolinska Sleepiness Scale, BRUMS= Brunel Mood Scale, SRSS= Short Recovery Stress Scale, PRO= Protein. \*Sleep/wake times are based on average habitual times as recorded during baseline – individually standardised.

***Performance Testing***

Following one week of dietary supplementation on day eight (both intervention periods), participants will undergo the same battery of performance tests as performed at both baseline sessions (i.e., Yo-Yo Intermittent Recovery Test Level 1, 30-second continuous jump test, and reaction time via Dynavision light board). These tests are to be completed at a previously agreed upon time within the Deakin sports science building, aiming to be within four hours of waking to limit confounding influences of increased circadian drive later in the day (Facer-Childs et al., 2018). Testing will commence with a 10-minute dynamic warmup, followed by the Yo-Yo Intermittent Recovery Test, 30 second continuous jump test, and reaction time testing. Recovery time will be given between activities, with an example timeline provided in Figure 3.

Graphical user interface, application, Teams

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**Figure 3.** *Intervention period graphic timeline*. Example of a participant with a prescribed wake time of 07:00. KSS= Karolinska Sleepiness Scale, BRUMS= Brunel Mood Scale, SRSS= Short Recovery Stress Scale. \*Sleep/wake times are based on average habitual times as recorded during baseline – individually standardised

***Washout***

Following the performance testing, the minimum 6-day washout period will begin. During this washout period, habitual diet will be resumed and no placebo or α-lactalbumin will be consumed. For the immediate six days following both intervention periods, participants will continue to wear the actigraphy device, and complete the sleep and training diary to observe any “continuing effect” of α-lactalbumin supplementation (Schneider-Helmert, 1981). Those athletes observing an extended washout period, need only to take these sleep measures for the immediate six days preceding the supplementation period, to observe any continuing effect after supplement cessation. Further, prescribed sleep/wake times are to be maintained for at least five days leading into the next intervention period, limit any potential confounding influence of changes to participant sleep/wake cycles.

***Post-Washout Performance Testing***

Following both intervention periods, six days of “washout” will be observed where no supplementation will occur. Immediately following these six days, sports-related performance testing will occur (as described above). This testing is to observe whether any potential continuing effect of α-lactalbumin on sleep translates to improved physical performance. Please refer to Figure 1 for a timeline of the study.

***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 (between intervention periods) 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, and sports-related performance 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)

- Mobile phone number (to receive text-message reminders throughout the intervention periods)

***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, 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)

- Sports-related performance (measured via the Yo-Yo Intermittent Recovery Test Level 1, and 30-second continuous jump test)

- Reaction time (measured via Dynavision lightboard)

***Data Collection***

- 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 3).

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 sleep quality.

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 records, and provide individualised meal plans in accordance with habitual energy intake.

Training – Participants will record all training sessions during the baseline measurement period, and throughout the entirety of the study (exercise duration (mins), type and rating of perceived exertion (1-10 scale)). A 5-day training diary will be collected at baseline (Appendix 10). This diary is to be filled out online via REDCap software. 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 4). 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 5).

***Outcome measures***

Sleep data – collected via actigraphy (Actical Z) 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 (h), sleep onset latency (min), sleep efficiency (%), and wake after sleep onset (min).

Additional subjective data will be collected using the consensus sleep diary – core (Appendix 9), which provides data related to sleep/wake times, sleep latency (min), 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). These measures are to be completed using REDCap online software.

Mood – Mood scores will be collected via the Brunel Mood Scale (Appendix 7) using REDCap software, 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) via REDCap, which through 8-items, assesses recovery and stress across physical, mental, emotional, and overall domains in real-time.

Sports-related performance – Participants will perform the Yo-Yo Intermittent Recovery Test Level 1 and the 30-second continuous jump test on six occasions, twice at baseline, following both intervention periods, and following both 6-day washout periods. These tasks are sensitive to accumulated sleep debt and simulate real-world components of invasion sports, where athletes need to withstand fatigue and perform multiple effort sequences.

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.

The calculated sample size of 24 takes into consideration a dropout rate of 10%. If the number of participants that withdraw themselves is greater than 10%, recruitment will continue until an adequate sample size is met.

**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.

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, and sports-related performance. 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 will prepare and mask each supplement’s taste to ensure the research team and participants remain blinded to the condition.

*-* Confounding factors such as training load will 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 to be avoided in the evening of the intervention periods

- Diet – As protein, energy and carbohydrates can influence sleep, diet will be standardised through individualised meal plans. A low protein dinner will be assigned within the meal plan to minimise any potential impact on supplementation effectiveness. With participants in their habitual environments, photos of each meal are to be uploaded to Easy Diet Diary app to display adherence to the individualised meal plans provided.

- 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 within their habitual environments, text messages will be sent to each participant reminding them to consume the supplement and fill in evening questionnaires, and another reminder sent to complete morning questionnaires.

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

Missing values will not be substituted in any circumstance (missing or otherwise).

11.4 Please include your statistical power calculation

Statistical power calculations are based on the primary outcome of sleep onset latency within previous literature (Miles et al., 2021). A two-sided t-test achieves **82%** power to infer that the mean difference is not 0.00 when the total sample size of a 2x2 cross-over design is **24**, the actual mean difference is **13.0**, the standard deviation of the paired differences is 18.56, 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 (h), sleep onset latency (min), sleep efficiency (%), and wake after sleep onset (min) using actigraphy.

- Sports related performance (Yo-Yo intermittent recovery test level 1, 30-second continuous jumps test, reaction time via Dynavision)

Secondary outcome measures include:

- mood, stress and recovery, subjective sleepiness, and subjective sleep quality

**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 will provide personal data on anthropometry, dietary intake, sleep measures, mood, recovery, and sports-related performance measures 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 continue to be 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 α-lactalbumin supplementation, sleep, and performance. 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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