Title: The Effects of Ashwagandha (Withania Somnifera) Supplementation on Exercise Performance in Female Footballers

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Title

The effects of ashwagandha (*Withania somnifera*) supplementation on exercise performance in female footballers.

Summary

English: The objectives of the thesis are to determine the effects of ashwagandha (ASH) on muscle strength, muscle recovery and to assess habitual dietary intake of female footballers. Across one phase, two experimental research papers and one descriptive research paper will be produced; it will involve one double-blind, randomised and placebo-controlled trial and a comparative analysis of nutritional intake in female footballers. The thesis will involve participants supplementing ASH in the form of a capsule containing KSM-66 (600 mg with 30mg withanolides) or placebo once a day for 28 days, on a singular occasion. The objectives of the first two papers are to determine the effects of ASH on muscle strength, perception of recovery, perception of exertion, perceived wellness and perceived muscle soreness. For the paper on muscle strength, the methodology will include hand grip test, counter movement jump (CMJ), squat jump (SJ), peak power, medicine ball throw and rate of perceived exertion (RPE) via the Borg Scale. For the paper on muscle recovery, the methodology will include a questionnaire on sleep, stress, fatigue and delayed onset of muscle soreness (DOMS) test via the Hooper Index (HI), total quality recovery (TQR) questionnaire and a supplement satisfaction questionnaire. The objectives of the third paper are to evaluate the dietary intake of professional female footballers and compare the values against published nutritional recommendations. The methodology will involve self-tracking of dietary intake via the Snap-N-Send method and data will be stored and analysed with the software Nutritics. The study will be conducted with a professional club in Barcelona. The use of ASH is becoming prevalent in athletes, despite lack of information regarding dosage guidelines or lack of research on its benefits for exercise performance. The proposed study will contribute to the overall scientific knowledge of ASH and whether it may demonstrate benefits from short-term use. Additionally, the study will determine if there are benefits for female athletes that wish to increase strength and reduce perception of pain or soreness related to exercise. The ethical considerations for this study are predominantly the safety and comfort of the participant. An informed consent form will be given to the participant, containing information about data collection, personal information, safety of the supplement and their right to drop out of the

study at any point. The proposed start date is January 2024, and the study will collectively last for 36 days across a singular phase. The supplement is provided by Zenement España, a supplement company based in Barcelona.

Spanish: Los objetivos de la tesis son determinar los efectos de la ashwagandha (ASH) sobre la fuerza muscular y la recuperación muscular, así como evaluar la ingesta dietética habitual de las futbolistas. Se elaborarán dos estudios, uno de investigación experimental y un segundo de investigación descriptiva; el primero implicará un ensayo doble ciego, aleatorizado y controlado con placebo y el segundo un análisis comparativo de la ingesta nutricional en futbolistas femeninas. El primer estudio requerirá que las participantes tomen suplementos de ASH en forma de una cápsula que contenga KSM-66 (600 mg con 30mg withanólidos) o (en el grupo control) un placebo, una vez al día durante 28 días, en una sola toma. Los objetivos del primer estudio son determinar los efectos del ASH sobre la fuerza muscular, la percepción de la recuperación, la percepción del bienestar, la percepción del esfuerzo y las agujetas. Se presentará un artículo con datos sobre la fuerza muscular, en el que la metodología incluirá pruebas de agarre con la mano, salto con contramovimiento (CMJ), salto en cuclillas (SJ), potencia máxima, lanzamiento de balón medicinal y el índice de esfuerzo percibido (RPE) a través de la escala de Borg. Un segundo artículo evaluará la recuperación muscular y el bienestar, la cual se medirá mediante una prueba de valoración del sueño, el estrés, la fatiga aparición retardada de dolor muscular (DOMS) mediante el Hooper Index (HI), un cuestionario de recuperación de calidad total (TQR) y un cuestionario de satisfacción de uso del suplemento. Los objetivos del segundo estudio (y tercer artículo) son evaluar la ingesta dietética de las futbolistas profesionales y comparar los valores con las recomendaciones nutricionales publicadas. La metodología consistirá en el auto-registro de la ingesta dietética a través del método Snap-N-Send; posteriormente los datos se almacenarán y analizarán con el software Nutritics. El estudio se realizará con un club profesional de Barcelona. El uso de ASH es cada vez más frecuente en los atletas, a pesar de la falta de información sobre las pautas de dosificación o la falta de investigación sobre sus beneficios para el rendimiento en el ejercicio. El estudio propuesto contribuirá al conocimiento científico general sobre el ASH; por una parte, pretende demostrar los beneficios de su uso a corto plazo, y por otra, el estudio determinará si existen beneficios para las atletas que desean aumentar su fuerza y reducir la percepción de dolor o molestias relacionadas con el ejercicio. Las consideraciones éticas para

este estudio son predominantemente la seguridad y la comodidad del participante. Se entregará a los participantes un formulario de consentimiento informado con información sobre la recogida de datos, la anonimidad de los datos personales, la seguridad del suplemento y su derecho a abandonar el estudio en cualquier momento. La fecha de inicio propuesta es enero de 2024, y el estudio durará colectivamente 36 días en una fase singular. El suplemento lo suministra Zenement España, una empresa de suplementos con sede en Barcelona.

Background and current status

Ashwagandha (ASH) is a medicinal plant with adaptogenic properties, clinically studied to demonstrate anti-stress, anti-inflammatory, anti-tumour, immunomodulatory, analgesic, neuroprotective and antioxidative effects (Bonilla et al., 2021). Those effects have encouraged intrigue for the use of ASH in sports performance. Notably, a study by (Wankhede et al., 2015) studied the effects of ASH on muscle strength and reported significant increases after 8 weeks of supplementation alongside a resistance training programme. The study has its limitations as participants' food habits were not considered, and subjects were previously untrained and exclusively male. The experimental group demonstrated considerable physiological benefits post-intervention compared to the placebo group, but more controls are needed in future studies to affirm the effects of ASH and muscle strength. A 4-week study by (Tripathi et al., 2016) explored muscle power alongside VO2 max among males. Improvements were found in speed, distance travelled, and muscle power (measured with a hand grip dynamometer) compared to the control group. Despite favourable results, limitations include being an open-label study, exclusively male participants and no food diary of participants. A 12-week study by (Ziegenfuss et al., 2018) researched the effects of ASH on perceptive pain, body strength and a cycling time trial. Improvements were shown in upper and lower body strength, perception of recovery and muscle soreness. There are strengths to this research, such as keeping track dietary intake during the study, well-trained participants and a double-blind control. However, the limitations include an exclusively male sample and questionable cycling time trial results that do not reflect the scores of highly trained individuals. None of the participants in the above studies had any adverse reactions. Even with the aforementioned limitations, the above studies are heavily referenced as confirmation that ASH is a 'proven' beneficial supplement for exercise performance. More studies with better controls and

monitoring of confounding factors are needed to verify the efficacy of ASH in exercise performance. Additionally, new research should be performed on participants who are female, highly trained or athlete level and dietary intake should be closely monitored.

Justification of the study

The justification to study female footballers is due to the lack of research in this specific area. There are public calls within sports science to expand research on performance and explore methods of how players can improve performance and the recovery process to avoid disruption of play and training. A study by (O'Donnell et al., 2018) noted the connection between cortisol and sleep: a 354% increase in cortisol post-match was found in elite female athletes, resulting in significantly reduced sleep time, quality and quantity; these factors are crucial in not only the recovery process but to general wellbeing and performance. Some of the ASH benefits are explained by its role in cortisol regulation in the HPA axis. If ASH reduces raised cortisol in female athletes, there is evidence that it could lead to improved sleep and as a result benefit overall health and performance. The objective of the thesis is to determine whether four weeks supplementation of ASH has the ability to improve muscle strength performance and perception of wellness and muscle soreness.

There is a fast-growing interest in ASH among practitioners, athletes and active individuals. However, there is an emerging amount of anecdotal feedback from users who supplement ASH in extreme doses and for a long period of time that they experience anhedonia or emotional numbness as a side effect. This is echoed in clinical research, where the active ingredient of ASH, called withanolides, is given to rats in extreme high doses (equivalent to 7-20 commercial tablets daily) for a long duration; the side effects are likened to antidepressants such as lorazepam (Bhattacharya et al., 2000*).* This may be due to continuous buffering of cortisol in the HPA axis (Lopresti et al., 2019). Additionally, current published research on ASH and exercise is not of high quality, as voiced by Asker Jeukendrup, one of the world's leading sports nutritionists (Jeukendrup, 2023). Current studies on ASH and exercise are mostly between 8-12 weeks. Despite no adverse reactions or toxicology reported in any of the clinical studies, in the author's opinion, this time length of study runs the risk of an anhedonia response due to consistently reduced cortisol. There are no general guidelines on usage, but the sports industry is amplifying the use of ASH by accrediting products as Informed Sport

Certified (specifically KSM-66, a third-party tested, branded product of ASH that is extracted from the root and without the use of alcohol or chemical solvents). If one commercial tablet of ASH supplemented daily for four weeks shows physiological benefits, it is in the athlete's best interest to cycle ASH intermittently instead of ingesting it continuously for a long duration. If four weeks of ASH supplementation does not exhibit effects, the conversation should begin on whether it is a beneficial supplement to use in general.

The justification to monitor professional female footballers' habitual food intake is to assess whether they receive adequate nutritional intake to complement their match and training schedule. It is opportunistic research, as food diaries will already be logged during the familiarisation of the muscle strength and recovery study. However, it is beneficial to analyse current dietary habits and compare it against recently published recommendations for female footballers (Dobrowolski et al., 2020). The benefit of this analysis is to potentially provide sports nutrition practitioners with insight on areas that may require improvement or attention in regard to female footballer's dietary intake and how they can be further supported.

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Hypothesis

1. Short-term ASH has beneficial effects on muscle strength in female footballers
2. Short-term ASH has positive effects on perception of muscle recovery following intense exercise in female footballers
3. Female footballers are not consuming recommended levels of nutrients for optimal sports performance

Objectives

1. Evaluate the effect of ASH on muscle strength in female footballers
2. Evaluate the effect of ASH on the perception of recovery in female footballers
3. Evaluate habitual dietary intake female footballers

Methodology

Design

1. Muscle strength and recovery RCT: Double-blind, randomised, placebo-controlled trial.
2. Food intake analysis: Observational, descriptive and cross-sectional research design

Subjects

The study population will be female footballers who belong to a professional club. Specifically female footballers who are signed to CE Seagull in Barcelona, Catalunya.

Sample size

The sample size was generated using G*Power (Version 3.1.9.6). Using the peak power variable in a study analysing ASH and muscle soreness (Diehl, 2021), the estimate of the effect size of Cohen's d equals to 0.53 and therefore used in the sample size calculation for this proposed research. There is no further calculation to convert Cohen's d into Cohen's f to make it appropriate for ANOVA, as when there are only two groups Cohen's f is the same as Cohen's d (University of Michigan, 2021). Using ANOVA repeated measures between factors, effect size of 0.53, 80% power, 5% risk error in a two-parallel-group design and three measurements, the overall sample size is 30 participants, with 15 subjects per group. The calculation provided by G*Power can be found in the appendix.

Selection criteria

Inclusion for participants who are:

- Female
- Playing football professionally
- At a sub-elite to elite standard

- Healthy and free of disease

Exclusion for participants with:

- Active supplementation with other ergogenic aids
- Medication or contraceptives (to not interfere with existing treatment)
- Thyroid conditions (due to interaction with cortisol)
- Diabetes or certain autoimmune conditions (to not interfere with existing treatment)
- Active pregnancy (to not interfere with existing treatment)
- Allergies to nightshades such as tomato, aubergine, potatoes and peppers (as ASH is an herb in the nightshade group)
- No signing of the consent form

The withdrawal criteria will be participants who request to withdraw and do not wish to proceed, or do not complete the required study tests.

Intervention

The intervention will be 600mg of KSM-66, a branded supplement derived from the root extract of the herb ashwagandha, with a consistent rate of 5% withanolides for 28 days. It is the most clinically studied extract of the herb and is the only type of ashwagandha to receive third party testing certification (Informed Sport, Informed Ingredient, BSCG, Clean Label Project). The laboratory where the product is made, Ixoreal Biomed, has received Current Good Laboratory Practices (cGLP), quality controlled and tested against heavy metals and pesticides.

A highly important factor of KSM-66 is its sole use of extraction from the root of ashwagandha, thereby avoiding the leaves. Extracts derived from the leaf contain Withaferin A and Withanone, which are considered cytotoxic (Priyandoko et al., 2011; Khedgikar et al., 2015; Rossato Viana et al., 2022). A paper by (Siddiqui et al., 2021) states that Withanone is the active metabolite that can induce liver toxicity with overuse. In a previous case report by (Björnsson et al., 2020), liver injury of moderate severity was reported in five patients after

supplementing ashwagandha. The listed ashwagandha supplements responsible for the injuries had no information on extraction process or what part of the herb was used, no certification and had unstated levels of withanolides.

This amplifies the importance of sole root extraction, controlled levels of withanolides and third-party testing for ashwagandha supplements, such as KSM-66. The safety and tolerability of root-extracted KSM-66 is evident in scientific literature (Raut et al., 2012; Sharma et al., 2018; Salve et al., 2019). In a trial by (Verma et al., 2021b) body weight, body temperature, pulse rate, respiratory rate, systolic and diastolic blood pressure, haematological and biochemical parameters were analysed to be normal in males and females after 8 weeks of daily consumption of 600mg KSM-66.

Muscle strength will be predominantly based on the data from the hand grip test using the hand-held dynamometer, CAMRY model. The participants will squeeze the dynamometer with all their ability on each hand, and the strength values will be determined by an average of each hand's measurement.

During the 28 days, participants will have 12 training sessions and 4 official matches. It is likely the participants will experience menstruation during the study. However, recent findings suggest there is no change in strength or exercise performance from short-term fluctuations in reproductive hormones (Colenso-Semple et al., 2023).

Dosage

For 28 days, participants in the experimental group will ingest 600mg KSM-66 (30mg withanolides) once daily at midday with a meal. Participants in the control group will ingest a HPMC capsule containing 600mg of chickpea flour once daily at midday with a meal.

Potential benefits

Benefits will vary between subjects, however ASH supplementation may:

- Reduce levels of cortisol, resulting in:
    - Improved sleep
    - Improved muscle strength

- Improved cardiorespiratory fitness
- Improved perception of recovery
- Reduced difficulty of exertion during exercise

(Bonilla et al., 2021)

Potential side effects

Side effects of ASH are uncommon, however large doses (exceeding 1,250mg daily, based on a clinical trial examining tolerability) may induce:

- Direct irritation to the intestinal mucosa, resulting in:
  - Nausea
  - Vomiting
  - Gastrointestinal upset

(Raut et al., 2012, LiverTox, 2019)

Regarding the potential side effects of KSM-66 specifically, a trial by (Chandrasekhar et al., 2012) noted adverse effects in subjects after 60 days of 600mg KSM-66 supplementation. Overall, there were 6 adverse events with ASH and 5 with placebo. They included congestion, decreased appetite, cough and cold symptoms. The placebo group experienced fever, headache, tiredness and abdominal pain, making it unclear as to whether KSM-66 has notable or established side effects.

Intervention group

The intervention for the experimental group will be KSM-66 ashwagandha (600mg ashwagandha containing 30mg withanolides, once a day with meals for 28 days, ingested at midday) supplied by the company Zenement based in Barcelona. The branded product of KSM-66 is made by Ixoreal Biomed.

Control group

The control group will receive rice flour in a HPMC capsule, once a day with meals for 28 days, ingested at midday. The rice flour will be a commercial product found in supermarkets. To achieve randomisation, simple random allocation will be used to prevent bias amongst participants. The protocol will follow the randomisation guide by (Arifin, 2012) and will be

achieved in SPSS software. It will be a double-blind study as the main researcher will not know which participants supplement ASH or placebo.

Variables

Dependent**:**

Muscle strength research:

- Hand grip strength in kilograms (kg) using a CAMRY dynamometer. The variable of interest will be the mean of the right and the left-hand measurements
- Seated medicine ball throw in metres (m)
- Counter movement jump (CMJ) in watts per kilogram ($W \cdot kg^{-1)}$
- Squat jump (SJ) in centimetres (cm),
- Peak power in watts per kilogram ($W \cdot kg^{-1)}$, using the results from SJ
- Rate of Perceived Exertion (RPE) via Borg Scale

Muscle recovery research:

- Perceived ratings of sleep, stress, fatigue and Delayed Onset Muscle Soreness (DOMS) via Hooper Index (HI)
- Total Quality Recovery (TQR) questionnaire
- Supplement satisfaction questionnaire

Food diary analysis research, compared against (Dobrowolski et al., 2020):

- Energy intake (kcal)
- Energy availability (kcal/kg fat free mass/day)
- Protein (g/day)
- Carbohydrates (g/day)
- Fat (%)
- Fluids (L/day)
- Vitamin D (mg/day)
- Calcium (mg/day)
- Iron (mg/day)

All data will be collected via Snap-N-Send and stored using Nutritics dietary analysis software (Research Edition, v5.096, Dublin, Nutritics, 2019). The Snap-N-Send method was analysed in a doubly labelled water study and was shown to have enhanced validity for assessing athlete total energy intake (Costello et al., 2019). The Nutritics software was clinically studied to be a useful and valid tool for healthcare professionals to measure macronutrients and micronutrients, demonstrating that its analysis values positively correlated with plasma markers (Watkins et al., 2020)

Independent**:**

- The treatment of ASH: 600mg KSM66 (30mg withanolides) once a day for 28 days

Data collection

Study data will be collected manually and entered into Blanquerna's OneDrive system. All data sheets and files will be backed up on to Google Sheets and shared with associated researchers and tutors of the project. This allows for data to be reviewed by collaborators.

Dietary intake data will be collected via Whatsapp using Snap-N-Send. The data will then be manually entered and stored using Nutritics and access will be shared with collaborators.

Data analysis

For the muscle strength and recovery research, dependent variables will be described as the mean and standard deviation and calculated using and **ANOVA repeated measures, between factors** as there will be three time points. A descriptive analysis will be carried out for all the data collected in the data collection notebook. To assess the normality of the data distribution, the **Kolmogorov-Smirnov** test will be used.

For the food diary analysis, dependent variables will be described as the mean and standard deviation and determined using **one-sided t-test** to compare against data averages of nutritional recommendations for female footballers (Dobrowolski et al., 2020). **Pearson's r** will be used to examine correlations between the selected variables.

Ethical considerations

The ethical considerations for this study are predominantly the safety, comfort and consent of the participant:

- Epistemic injustice will be avoided in the study by the researcher listening to the participant and valuing their opinion and feelings on the research subject
- The safety and tolerability of ASH has been clinically studied in humans and mice and is deemed safe to ingest. However, participants will be informed of rare, potential side effects and their right to leave the study at any time
- Personal information (name, contact details) of the participant will not be shared with a wider network or in the research papers, but will be used by the researcher during the study to check-in on the participant. They will be contacted for their questionnaire scores during the research on perceptive muscle recovery – if the participant does not agree to this method, they may decline or drop out of the study
- There will be data collection during the study, and it is important to not violate the participants right to privacy when asking personal questions via the questionnaires. The participant may decline to answer any question they deem unsuitable
- Participants will be made aware that they will be observed for the data collection during the study and may decline this if it makes them uncomfortable
- If the researcher fails to respect personal, cultural or religious values that makes the participant feel uncomfortable, they have the right to drop out of the study
- The participants will be informed about the aims of the study and will be asked to sign an informed consent form (the form can be found in the appendix)

Scientific and social value

The study is contributing to the generation of scientific knowledge. For a supplement that is widely used, the existing knowledge on its effects on athletes and females is substantially low. In addition, there is very little existing research on short-term supplementation of ASH as the focus is currently on 8-12 weeks supplementation. This study will inform as to whether the supplement can provide performance benefits after 28 days, as the author believes this is a more realistic and safer dosage duration. This research therefore holds relevance in scientific advancements and may provide new dosage guidelines to users. Another aspect of scientific

relevance is the response to the public calls for more research on this specific area of ASH and sports performance.

There is high social relevance and value for the female athlete population, who are likely to experience strict training schedules with limited recovery time between training and matches. If significant benefits are found in recovery from ASH, there is a safe and low-cost method for athletes to adapt into their routine. Additionally, if there are benefits found in this thesis, practitioners who work directly with female athletes will be informed of up-to-date practice with ASH and can pass on information to athletes. Importantly, if the thesis shows no benefits across any measures, both athletes and practitioners will be informed of the supplement and its credibility.

The study holds high social and innovative relevance as it will collect, summarise and present existing published research that indicates a) overuse in high dosage and duration may lead to consistently lowered cortisol and b) consumption of solely root extraction of the herb is highly recommended in order to avoid potentially ingesting the cytotoxic metabolites of Withaferin A and Withanone, which are found in the leaves of the herb. Providing safety and information for users and future users of ASH is the crux of this thesis.

Scientific-technical validity

All of the instruments and questionnaires used in the proposed study are established in peer-reviewed literature and deemed both valid and reliable. Two measures will be taken for each tool. The first instance will be the baseline measure and will occur on-site at the training ground gym in the evening during a typical training session. The second measure will take place after 28 days of supplementation (half ASH, half placebo) in the same location and time of day. Each participant will be given a timeslot to enter the gym and perform their assessments before continuing with normal resistance-training session. The tests using instruments will be monitored by two strength and conditioning coaches, and the data will be collected by two sports scientists. The following evaluation instruments are as follows:

- Hand grip strength of the participants will be measured with a CAMRY dynamometer and the predominant measure to assess muscle strength. It is a commonly used tool in sports performance and demonstrates validity and reliability in relation to athlete

strength (Cronin et al., 2017). The validity for the use of a CAMRY dynamometer is documented by (Huang et al., 2022) when comparing against the Jamar dynamometer. The CAMRY dynamometer is inexpensive, easily accessible and provides valid results

- Counter movement jump (CMJ) and squat jump (SJ) jumping tests will be used to assess estimation of muscle power in the lower limbs of the participants. These tests will be performed in the on-site gym at the football club, using appropriate equipment to carry out the tests. Valid and reliable scores are obtained via CMJ and SJ (Markovic et al., 2019)
- Seated medicine ball throw will be used to assess explosive power in the upper body. Participants will sit on the floor with legs fully extended and back against the wall to throw a 4kg medicine ball as far as possible. This test has validity and reliability when assessing muscle strength and general athletic ability (Stockbrugger & Haennel, 2001)
- Peak power will be calculated using the centimetres measurement of SJ with the following formula: (60.7) × (jump height [cm]) + 45.3 × (body mass [kg]) − 2055 (Sayers et al., 1999). The reliability and validity of average peak power is acceptable (Natera et al., 2023)
- Participants will be asked to provide their rate of perceived exertion (RPE) after the previous tests are complete. This will be retrieved using the Borg Scale (Borg, 1998). It is a popular tool in published literature on sports performance, and provides a valid and reliable measure of exercise intensity and exertion from the participant (Lea et al., 2022)
- The following day, post-resistance training recovery will be assessed by the Total Quality Recovery (TQR) questionnaire, developed by (Kenta y Hassmén, 1998). This questionnaire monitors sleep quality, emotional state, stress and other subjective markers following exercise. Its validity is documented by (Osiecki et al., 2015), when compared against creatine kinase (CK) levels in football players; a marker that has been found to be a strong indicator of stress and recovery in athletes. The high CK levels were significantly associated with the lower the TQR score, making the subjective questionnaire relevant to physiological recovery. The TQR questionnaire is a valid alternative to evaluate recovery and is easy applied in this proposal

- On the following day, participants will be asked to rate their delayed onset of muscle soreness (DOMS), sleep, stress and fatigue via Hooper Index. The Hooper Index is a low-cost monitoring tool to measure wellness markers, such as perceived sleep quality, stress, fatigue and muscle soreness. It is shown to strongly correlate with exercise fatigue in professional soccer players (Rabbani et al., 2019)
- The final evaluation tool will be a supplement satisfaction questionnaire. This will ask participants to rate their satisfaction with the supplement, either ASH or placebo, on a scale between 1 and 5 (1 = very satisfied; 5 = very dissatisfied). They will additionally be asked to measure their willingness to use it in future (1 = very unwilling; 5 = very willing). This questionnaire has been used by (Kuehl et al., 2010b) in a previous study evaluating muscle pain following a course of supplementation
- The food diaries of participants will be received via Whatsapp by the Snap-N-Send method, which is a titled and photography-based mobile assessment and considered a valid and reliable method for assessing energy intake (Costello et al., 2017).
- The tool to analyse nutritional data will be Nutritics, electronic software that is commonly used to analyse food diaries. Electronic diet assessments are scientifically valid to measure macronutrients and micronutrients, and there is additional benefit from being low-cost (Raatz et al., 2015; Ferrara et al., 2019).

Equitable selection of subjects

Recruitment and selection of participants will be aided by the selected liaison of the football club, who are already familiar with the club structure, the players and the personal health information of the players. Presentations will be given during training sessions about the study. During the presentations with the desired participant groups, all the information of the study will be displayed with easily understandable and digestible information, without stating misleading or false expectations. Published research about the supplement will be included in one of the presentation slides for transparency. In the presentation it will be made clear that participants may receive either the supplement or a placebo. It will be made clear that participation is optional and not mandatory. It will be made clear there is no reward or compensation for joining the study, except for receiving a food report back with nutritional guidance to optimise their sports performance (as participants will have been tracking their

diary throughout the study) provided by the author and the collaborator. As the author and in-house collaborator are registered sports nutritionists, the reports hold authenticity. After the presentation has finished, participants can independently choose to sign up. The presentation would be done a month before the study would begin, giving subjects time to consider their participation and to sign the consent forms. In the presentation the following selection criteria will be shown:

Benefit / risk ratio

There is minimal risk posed to participants involved in this study, as there are no reported harms from supplementing KSM-66 in clinical research and no adverse effects have been reported. Additionally, it is a commercially available product that has been certified safe to supplement. As KSM-66 is made from root extraction of the ashwagandha herb, and therefore not made of the leaves, there is less toxicological risk as the metabolites Withaferin A and Withanone are removed in the process (further elaboration in the *Thesis – Intervention* section of this document) making KSM-66 safer than other products of ashwagandha. Potentially, a participant could have an undiagnosed illness or allergy to nightshades and the supplement gives side-effects. Previous literature has not reported any instances of this happening. It is unlikely as athletes typically have a medical team at their disposal, who provide health checks and make sure they are fit to play. Due to the short-term time length and reasonable dosage of the study, there is minimal risk for over-supplementation leading to consistently reduced cortisol or gastrointestinal problems. There is low to no risk pertaining to psychological, social or economic factors. The study has been designed with participant comfort in mind and the timeline, training sessions, performance and recovery tests mimic the participants' usual regime, allowing for ease of participation and reduced risk factor.

The anticipated benefits outweigh the risks. The supplement of KSM-66 has shown to improve muscle strength, recovery, cognition and sleep in published literature. These are highly important factors to the lifestyle of an athlete and provide social, psychological and physical benefits.

Privacy

All personal data and information collected or generated in the study will be stored on OneDrive Blanquerna and protected in accordance with the current legislation on personal data protection (Regulation (EU) 2016/679 and in the Organic Law 3/2018 of 5 December on Data Protection). No one, except some of the staff directly related to this study, will be able to know the identity. Data will be stored only for the duration of the study and during the process of analysing the data.

Integrity

There is no known conflict of interest in this study. There will be no plagiarism within this study and all and words will be original to the study. There will be no fabrication or invention of results, and data will be analysed and published accurately and transparently. All collaborators will be employed staff members of the football club who are collaborating in the thesis. The gym facilities where the tests will be measured and performed are on-site of the football club and where the players have weekly gym training sessions. The gym facility is a licensed establishment. The participants will be informed of the results and will have access to their individual data.

Work plan

A visual representation of the study timeline can be found in the appendix. A description of each step is as follows:

0. Approval from the ethical committee
1. Participants are informed about the aims of the study and sign the consent form. Information about age, BMI, years of practice and health history is collected
2. Participants start tracking their food diaries via an app remotely for three days over the weekend prior to the supplementation for familiarisation
3. Baseline measures for muscle strength will be taken on the following day at the club gym, coinciding with a regular gym training session. The measures will be taken prior to starting a typical training session
4. The team will consist of the author (Olivia Coope), Andrea Páez Calvente and Andrea Reales Salguero who will assist with data collection and physiotherapist Enrique Jordán who will monitor participants and their safety while performing the strength tests

5. Afterwards participants will be given a printout of the TQR questionnaire and the Hooper Index to complete for the next day. Participants will be given their course of supplementation, either placebo or KSM-66, and instructed to take one a day for 28 days
6. The following day, baseline measures for the muscle recovery will be collected remotely at midday via Whatsapp or Zoom, and at the same time participants will start supplementation of either KSM-66 or placebo
7. Fourteen days later, participants perform the muscle strength tests in the evening at the club gym. Similar to before, the team will consist of the author (Olivia Coope), Andrea Páez Calvente, who will assist with data collection and physiotherapist Enrique Jordán who will monitor participants' safety while performing the strength tests
8. Afterwards participants continue with the regular gym training session. Printouts of TQR and Hooper Index questionnaires are given to participants for the next day
9. The following day muscle recovery measures are collected remotely via Whatsapp or Zoom at midday
10. An additional fourteen days later, participants finish supplementation and perform the muscle strength tests in the gym with identical circumstances (staff, equipment, temperature) as the previous two gym sessions
11. The following day muscle recovery measures are collected remotely via Whatsapp or Zoom at midday. The trial finishes
12. The analysis of data begins
13. The process dissemination of results and findings begins

The study is designed with ease of participation in mind, but there will be inconveniences for participants who forget to take the intervention or fill out the food diary. To alleviate this, participants will be notified (with permission via the consent form) over Whatsapp with reminders during the 28 days supplementation. Additionally, there will be a check-in session a week after supplementation starts, to assess participant satisfaction and compliance.

Resources

Primary resources will consist of raw data through observation obtained from gym equipment and hand grip dynamometer and first-hand accounts from participants when answering

subjective questionnaires and food diaries. Secondary resources will consist of Excel and Google sheets to store the data, SPSS software to analyse the data, and Nutritics software to analyse the dietary intake.

The facility where the baseline and post-supplementation muscle strength tests are performed will take place at the club gym for CE Seagull (located at Travessera de Montigalà, 18, 08917 Badalona, Barcelona). Participants will be familiar with the facility and its equipment due to previous weekly gym training sessions prior to any measurements.

Applicability and limitations

Applicability

There is a high likelihood that the study may impact the target population of female footballers and to athletes as a wider population. The supplement of KSM-66 is easily accessible, cost-effective and has Informed Sport certification, making it safe to ingest without the risk of contaminated ingredients. The supplement is already highly used in the area of sports performance; importantly, this study aims to verify whether short-term supplementation has benefits and will inform the wider population on safe dosage guidelines. This benefits not only athletes, but practitioners on best procedure for their patients or clients. Weekly gym sessions are an integral part of footballer's training regime, and if benefits are found in muscle strength, RPE and wellness scores of sleep quality, muscle soreness, stress and fatigue, athletes may wish to explore supplementation with KSM-66 to improve their strength output, perception of difficulty, sleep quality and muscle soreness with a safe method. Additionally, if athletes or active individuals experience high levels of stress and it affects their performance and recovery as a result, the study is applicable as the intervention may potentially improve these areas.

Limitations

Regarding participants, accuracy of food reporting and following supplementation guidelines will be based on trust between the author and participant. The study has convenience sampling, which allows for ease of data collection but lacks generalisability in the sample. The focus of the study is on subjective results, without the validity of physiological tests that could measure metabolic markers, such as cortisol, from blood samples. There is also a lack of

previous research on ASH and exercise in females and athletes, which impacts result credibility.

Dissemination plan

Full transparency of the measurements will be published within each of the corresponding three papers. No measurements or scores will be omitted. The purpose of the outreach is to contribute to the scientific knowledge of ASH and its effects on muscle strength and recovery in female athletes. Additionally, this research will provide scientific knowledge to sports science and the industry of sports nutrition of the influence of short-term ASH, and whether it provides any benefits to female athletes. The methods of sharing the scientific knowledge will include presentations at conferences, exhibitions, social media and published articles in journals.

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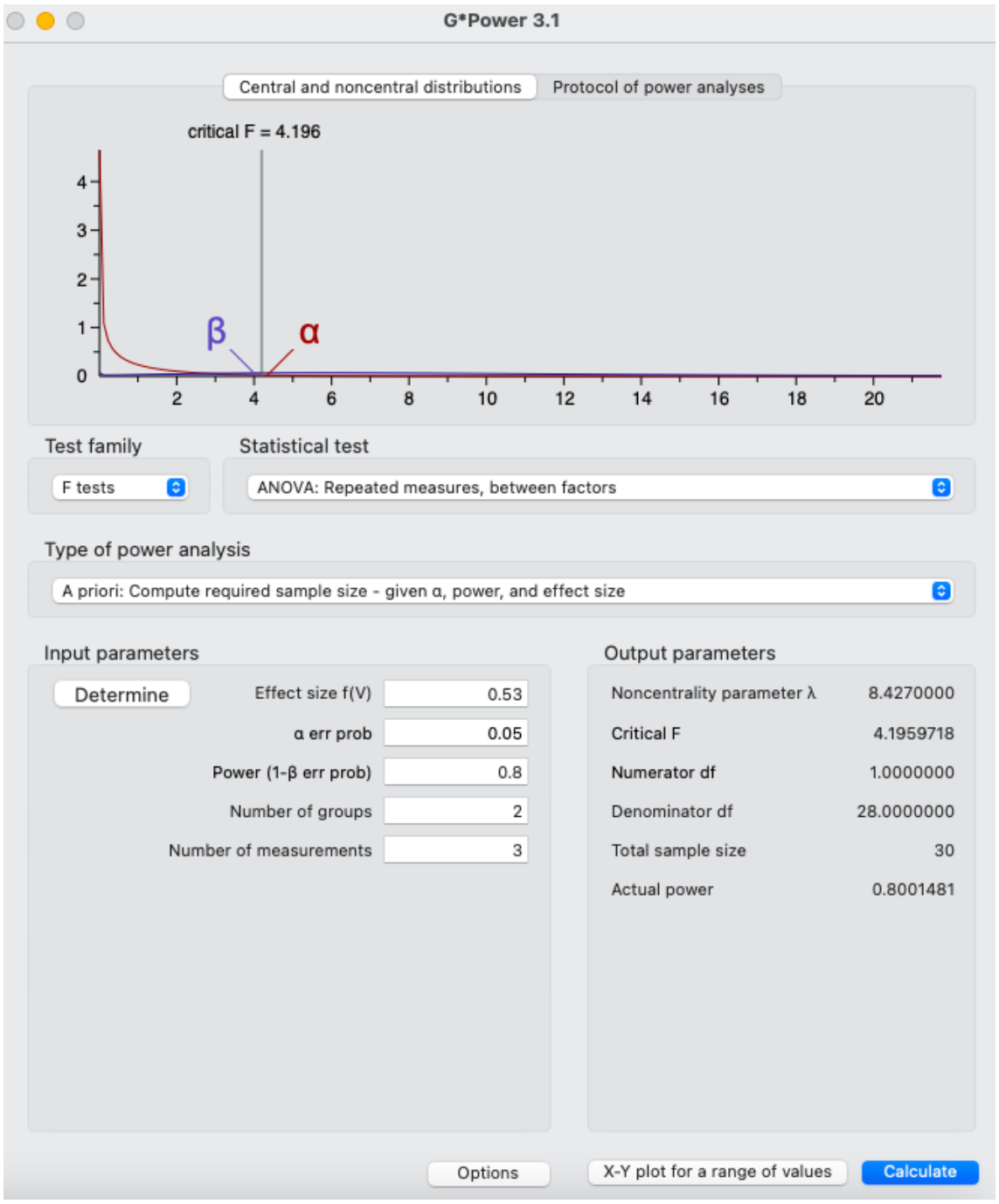
Appendix

Sample Size Calculation



Borg Scale

TABLA N°1: ESCALA DE BORG (CR-10) PARA LA PERCEPCIÓN DE ESFUERZO

Nivel indicador	Valor	Denominación	% contracción voluntaria máxima
	0	Nada en absoluto	0%
	0,5	Muy, muy débil (casi ausente)	
	1	Muy débil	10%
	2	Débil	20%
	3	Moderado	30%
	4	Moderado +	40%
	5	Fuerte	50%
	6	Fuerte +	60%
	7	Muy fuerte	70%
	8	Muy, muy fuerte	80%
	9	Extremadamente fuerte	90%
	10	Máximo	100%

(Vera & Rodríguez, 2022)

Total Quality Recovery questionnaire

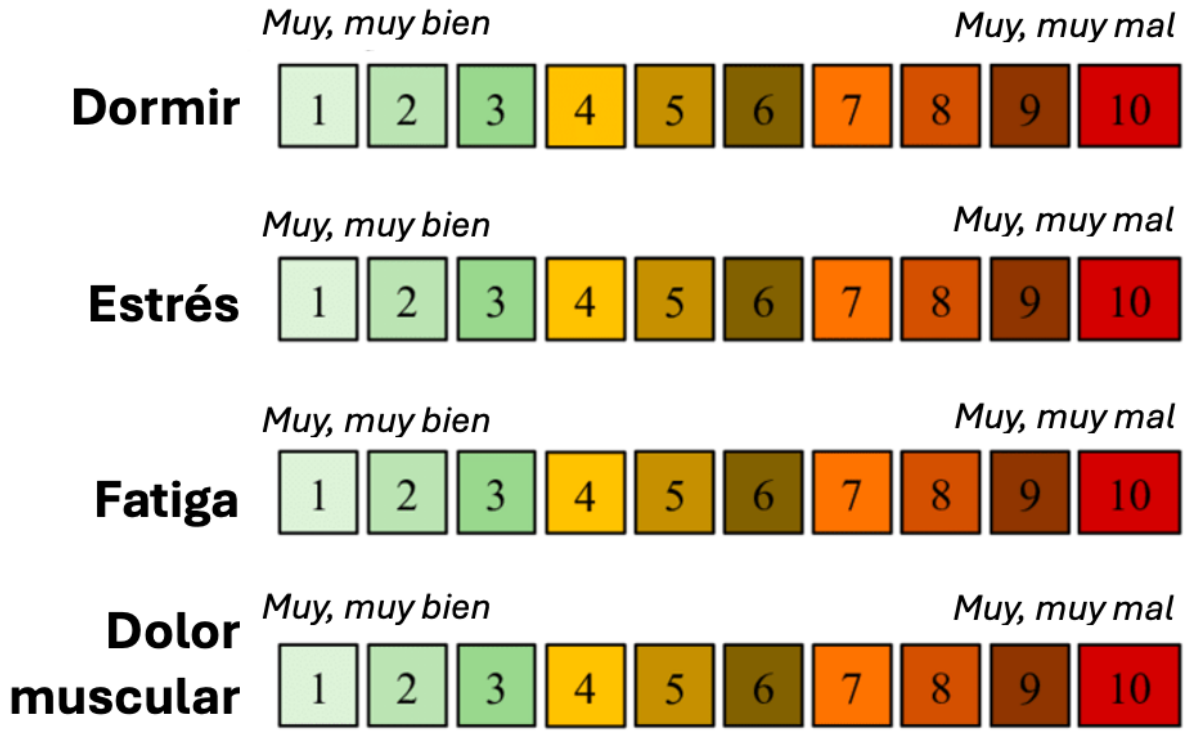
Puntuación de recuperación

Los atletas pueden completar esta guía en el transcurso de una semana para evaluar sus propias conductas de recuperación. Una vez que ha anotado un día completo, el total revela si están prestando una atención adecuada la recuperación física y mental necesaria. 17-20 puntos diarios es óptimo; 15-16 puntos son buenas pero muestra posibilidad de mejora; 14 o menos puntos significa que el atleta necesita una seria evaluación individual de conductas de recuperación.

	Puntos posibles	Dom.	Lun.	Mar.	Miér.	Jue.	Vier.	Sáb.	SCORING GUIDE ADJUSTMENTS
Nutrición	**8**								
Desayuno	1								Dar ½ punto para menos de un desayuno completo
Comida	2								Dar ½ punto para menos de una comida completa
Cena	2								Dar ½ punto para menos de una cena completa
Ingesta pre-entrenamiento	1								
Recarga de carbohidratos durante los siguientes 60 minutos al entrenamiento (recomendados: 1 a 1,5 g por kg de peso corporal).	2								Dar un punto por una recarga por debajo de la recomendada o por tardar más de 60 minutos.
Hidratación	**2**								
Orina antes del ejercicio: color claro o casi claro	1								
Orina después del ejercicio: color claro o casi claro	1								
Sueño y descanso	**4**								Dar dos puntos de 7 a <8 horas Dar un punto de 6-7 horas
8 horas de sueño reparador	3								
Siesta durante el día	1								
Relajación y estado emocional	**3**								
Después del entrenamiento 60 minutos completamente relajado o 30 minutos tumbado o en el sofá.	1								
No estrés psicológico-social	2								Dar un punto por un poco de estrés
Estiramientos/enfriamiento	**3**								
Adecuado enfriamiento post-entrenamiento	2								Dar un punto por enfriamiento parcial
Estiramientos al menos 10 minutos	1								
TOTALS	**20**								

(Martín, 2017; Francisco et al., 2020)

Hooper Index questionnaire

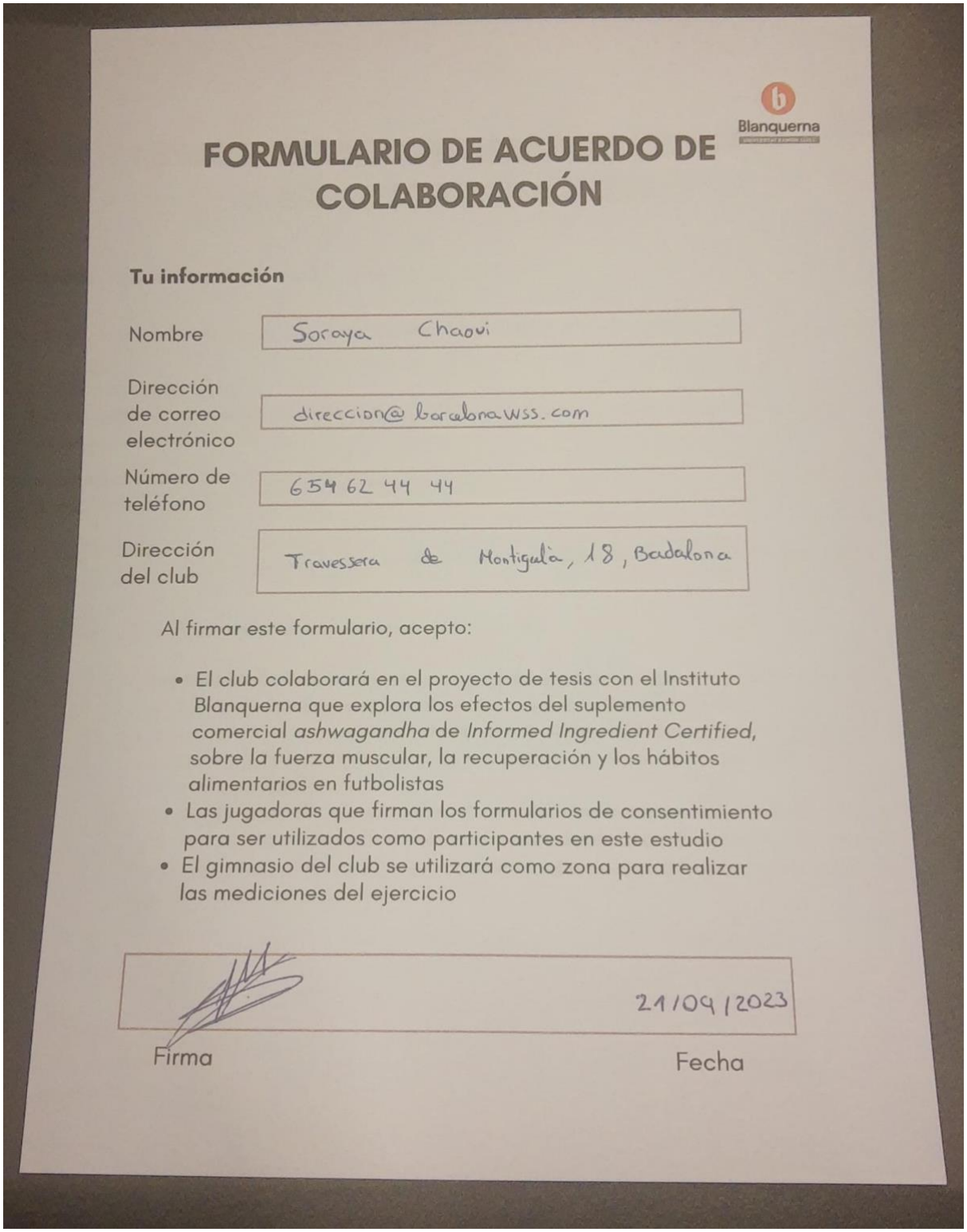


Supplement satisfaction questionnaire

Measure
Willingness to use ASH in future (1 = very unwilling; 10 = very willing)
Satisfaction - Pain Relief (1 = very satisfied; 5 = very dissatisfied)
Satisfaction - Overall (1 = very satisfied; 5 = very dissatisfied)

(Kuehl et al., 2010b)

Agreement document from club



Blanquerna

FORMULARIO DE ACUERDO DE COLABORACIÓN

Tu información

Nombre	Soraya Chaoui
Dirección de correo electrónico	direccion@barcelonawss.com
Número de teléfono	654 62 44 44
Dirección del club	Travessera de Montigalà, 18, Badalona

Al firmar este formulario, acepto:

- El club colaborará en el proyecto de tesis con el Instituto Blanquerna que explora los efectos del suplemento comercial *ashwagandha* de *Informed Ingredient Certified*, sobre la fuerza muscular, la recuperación y los hábitos alimentarios en futbolistas
- Las jugadoras que firman los formularios de consentimiento para ser utilizados como participantes en este estudio
- El gimnasio del club se utilizará como zona para realizar las mediciones del ejercicio

Firma

21/09/2023

Fecha

FORMULARIO DE ACUERDO DE COLABORACIÓN

Blanquerna

Tu información

Nombre: Soraya Chaoui

Dirección de correo electrónico: direccion@barcelonaWSS.com

Número de teléfono: 654 62 44 44

Dirección del club: Travessera de Montgalà, 18, Badalona

Al firmar este formulario, acepto:

- El club colaborará en el proyecto de tesis con el Instituto Blanquerna que explora los efectos del suplemento comercial *ashwagandha* de *Informed Ingredient Certified*, sobre la fuerza muscular, la recuperación y los hábitos alimentarios en futbolistas
- Las jugadoras que firman los formularios de consentimiento para ser utilizados como participantes en este estudio
- El gimnasio del club se utilizará como zona para realizar las mediciones del ejercicio

Firma

24/09/2025

Fecha