Study Protocol with SAP and ICF

NCT Number: not yet assigned
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Objetive:

The main objective of this research was to carry out an experimental study, triple blind, on the possible immunophysiological effects of a nutritional supplement (Synbiotic, Gasteel Plus®, Heel España S.A.U.), containing a mixture of probiotic strains, such as Bifidobacterium lactis CBP-001010, Lactobacillus rhamnosus CNCM I-4036, Bifidobacterium longum ES1, as well as prebiotic fructooligosaccharides, in both professional athletes and sedentary people.

Design:

This investigation was a triple blinded, randomized, placebo-controlled pilot study designed to identify the possible differing effects of the synbiotic Gasteel Plus® supplementation between sedentary individuals and soccer players. Subjects were asked to maintain their regular lifestyle and the participants were prohibited from consuming probiotics, prebiotics or fermented products (yogurt or other foods) to avoid unnecessary interference during the experimental periods. Presenting injury or illness would result in exclusion from the study. All participants were also asked to provide written informed consent before participating in the study, which had been previously approved by the ethics committee of the Catholic University of Murcia (Spain) following current legislation (CE031810).

On two separate days, the “baseline-tests” and “final-tests” were conducted. All participants performed a series of tests, before which they had to fast. The order and schedule (8 am) of the tests was the same for the "final-test" and the same materials and procedures were used. A period of 30 consecutive days elapsed between the base-line and final tests, during which the participants had to ingest their supplement (synbiotic or placebo). Accelerometers were distributed one week prior to the baseline test and the week after the final test. Blood and saliva sampling were taken early in the morning and questionnaires were filled out on the two testing days. The treatment was carried out during the last fortnight of May 2019 and the first fortnight of June of the same year, coinciding with situations of possible physical and mental stress in both participant groups, being at the end of an examination period, as well as the end of the soccer season.

Methods:

Objective determination of levels of physical activity, sedentary lifestyle and sleep quality: accelerometry

The accelerometer used was the Actigraph wGT3X-BT, which is a small and light triaxial accelerometer (4.6 x 3.3 x 1.5 cm, 19 g) with a response frequency of 30 to 100 hertz. This device was used to measure different objective parameters such as physical activity and its intensity, energy expenditure, metabolic equivalents rhythms (MET), weekly steps, sedentary bouts and sleep latency and efficiency. Participants wore the accelerometer held with an elastic band on the non-dominant wrist for 7 consecutive days and without interruption, except for those times of the day in which the correct operation of the device could be compromised (showers or any activity related to wa-ter). Subsequently, the files generated by the accelerometer were analyzed through a specific software called Actilife 6 (Actigraph, Pensacola, USA).

Determination of perceived levels of general health, stress, anxiety, fatigue, depression and sleep quality: questionnaires

Participants had to fill out a series of validated questionnaires to identify possible subjective health and mental states.

SF-36 Questionnaire. This is an instrument that provides results about the health status of a general population covering 8 scales: physical function, physical role, body pain, general health, vitality, social role, emotional role and mental health. The scales are ordered so that the higher the score, the better the health status (0 to 100).

Sleep Quality in Healthy Lifestyle and Personal Control Questionnaire (HLPCQ). The HLPCQ is a questionnaire whose main objective is to detect and quantify lifestyle patterns that reflect health empowerment, as evidenced by the levels of stress and of the internal locus of control. It also includes a section with various questions to measure sleep quality where higher scores indicate better sleep quality.

The State-Trait Anxiety Inventory (STAI). This questionnaire analyzes the degree of anxiety that each participant shows. It is divided into two parts: trait-anxiety (what they usually or generally felt) and state-anxiety (their expressed emotions at a specific moment), in which higher scores indicate a higher state of anxiety.

The Perceived Stress Scale (PSS). This questionnaire allowed the frequency at which individuals had experienced certain stressful feelings to be assessed, as well as their thoughts in the last month.

Brief Fatigue Inventory (BFI). Brief screening tool designed to assess the severity and impact of fatigue on daily functioning. The higher the score, the higher the degree of fatigue.

Beck´s Depression Inventory (BDI). This questionnaire was used to find out how the participants had felt during the last week, including the day of the test, with which it was possible to determine whether or not they presented signs of depression. Higher scores indicated higher signs of depression .

Blood and saliva sampling

Blood samples were collected from the subjects at 8 am and were deposited into collection tubes containing anticoagulant EDTA and coagulating agents to isolate plasma and serum, respectively. The plasma and serum were centrifuged, respectively, at 1.600 and 1800 x g for 10 minutes. Serum and plasma samples were coded and re-frigerated gradually at -20ºC as they were obtained. Finally, samples were stored at -80ºC until further analysis.

Saliva samples were obtained using a non-invasive method (Collection methods saliva Bio Oral swab, Salimetrics). Participants were asked not to ingest any type of food or drink with sugars, alcohol and/or caffeine, as well as tobacco, at least 12 hours prior to the tests. Volunteers were asked to open the packaging and remove the sterile swab for proper placement in the mouth under the tongue and were recommended to hold it for at least 2 minutes, to ensure against fluctuations in the volume of the sam-ple. Immediately after, samples were refrigerated at -20ºC and finally stored at -80ºC until further analysis.

Determination of metabolic profile

The determinations for obtaining the lipid and glycemic profile were carried out through standard techniques with the automatic analyzer of clinical chemistry BA 400 (BioSystems) in the SYNLAB laboratories (Diagnosticos Globales S.A.U., Badajoz, Spain).

Determination of inmuno-neuroendocrine parameters

For the determination of the pro-inflammatory and anti-inflammatory cytokines studied (IL-1β and IL-10), an instrument based on flow cytometry was used:the Lu-minexTM 200 System instrument (Luminex Corporation, Texas, USA) using the Pro-cartaPlex TM Multiplex Immunoassay. Catecholamines such as dopamine, epineph-rine and norepinephrine and also stress hormones like serotonin, cortisol, corticotro-pin-releasing hormone (CRH) and the adrenocorticotropic hormone (ACTH) were an-alyzed by competitive inhibition enzyme immunoassays (ELISA), using respectively: Dopamine Research Immunoassay, General Epinephrine (EPI) RD-EPI-Ge-96T and General Noradrenaline (NE) RD-NE-Ge-96T, General 5-Hydroxytryptamine (5-HT) RD-5-HT-Ge, The DetectX Cortisol Immunoassay Kit, Human Corticotropin Releasing Hormone (CRH) RD-CRH-Hu (Kelowna, BC, V1W 4V3, Canada) and Human ACTH (Adrenocorticotropic Hormone) ELISA Kit (Elabscience, USA). To determine immuno-globulin A in saliva, samples were analyzed by indirect enzyme immunoassay kit through the Salivary Secretory IgA Kit (Salimetrics LLC, USA). The procedures fol-lowed the instructions of the manufacturers, and the findings were measured using an ELISA auto analyzer to quantify color intensity (Sunrise, Tecan, Männendorf, Swit-zerland).

Statistical Analysis Plan (SAP):

Statistical analysis was performed with IBM statistics SPSS v20.0 software (SPSS Inc., Chicago IL, USA). To verify the normality of the data, the Shapiro-Wilk test was performed. The repeated two-way analysis of variance (ANOVA) was used, followed by Student's paired and unpaired t-tests to analyze the intervention effect. The values were expressed as mean ± standard deviation (SD) and the significance level was con-sidered when $p<0,05$.

Results:

Among the most important results we can observe:

the sedentary group administered with placebo obtained some significant differences with respect to baseline values ($p<0,05$). A decrease in calories, metabolic rate and intensity level of physical activity are reflected. The synbiotic seems to prevent this situation by avoiding the mentioned decreases and even increasing the consumption of Kilocalorie (Kcal) in sedentary individuals although without significant differences. Results from the athlete group show no significant changes ($p>0.05$) in those who consumed placebo, while those who followed the protocol with the intake of the synbiotic had significant improvements in sleep efficiency and latency, as well as increases in the consumption of Kcal and METS.

The baseline values are quite similar in the sedentary and athlete groups. Only in the athletes did the synbiotic intervention significantly improve ($p<0.05$) the perceived general health as determined by the SF-36 questionnaire, but no differences were found in perceived sleep quality, state anxiety, and fatigue.

No significant differences were found in the perceived stress, trait anxiety, and depression between sedentary people and athletes at basal status (before intervention). The results show a decrease in the levels of perceived stress ($p<0.01$) and anxiety ($p<0.05$) only in the athlete group administered with the synbiotic. Also shows a decrease ($p<0.05$) in perceived depression levels in both groups (sedentary and athlete) after the synbiotic treatment. Then, training only affected the behavior in response to the synbiotic intervention in stress and particularly in anxiety ($p<0.05$) also when evaluating by the two way ANOVA test). These effects were not due to a placebo effect.

The results corresponding to blood concentrations of glucose, cholesterol, and triglycerides as measurements of metabolic profile. Firstly, individuals in both the sedentary and athlete groups presented lipid and glycaemic levels compatible with normal and healthy ranges. Thus, as expected the consumption of the synbiotic did not provoke an appreciable or significant effect

It should be noted that both groups presented healthy baseline levels of the inflammatory and immune parameters analysed. No significant differences in the IL-1β concentrations were observed between the sedentary and athlete groups. However, a different behaviour ($p<0,05$) between the two groups was found in response to the synbiotic intervention: while the synbiotic increased ($p<0.05$) the systemic concentration of IL-1β in the sedentary group, it slightly decreased in the soccer player group.

No significant variations (except a potential placebo effect in the sedentary group) were found in the IL-10 concentration. There were also no significant differences in the levels of immunoglobulin A between groups, or as a consequence of the intervention.

A lower concentration without significant differences were found in the dopamine concentration of the soccer players group. However, training affected the response to the synbiotic intervention ($p<0.05$), since it induced a significant increase ($p<0.05$) in the dopamine concentration only in the athletes. This ef-fect cannot be attributable to a potential placebo effect of the intervention. However, the decrease ($p<0.05$) in epinephrine levels in the sedentary group administered with the synbiotic compared to their basal levels could potentially be attributed to a placebo effect of the intervention. In addition, there were also no significant changes in norepinephrine. Basal concentration of serotonin was, however, higher ($p<0.05$) in the athlete group than in the sedentary group, but statistical differences with the synbiotic intervention were not observed.

Finally, the results corresponding to CRH showed a lower systemic concentration of CRH than sedentary volunteers. In addition, the behaviour of CRH secretion in response to the synbiotic intervention was also different ($p<0.05$) between the athlete group and the sedentary group, decreasing significantly ($p<0.05$) in the sedentary group with respect to their baseline levels (as also found with the placebo) and increasing slightly in the athlete group.The latter cannot be attributable to a placebo effect of the intervention. There were no significant differences in levels of cortisol and ACTH between groups or as a consequence of the intervention.

Informed consent form:

I, ..., with ID: .....

DECLARE:

I have been informed of the study and research procedures of the Project entitled: Differential effects on health through the use of a synbiotic in professional athletes and sedentary people.

The researchers who will have access to my personal data and test results are: Carmen Daniela Quero Calero, Eduardo Ortega Rincón and Pedro Manonelles Marqueta. Likewise, I have been able to ask questions about the study, understanding that I voluntarily participate in it and that I can leave it at any time without any kind of prejudice.

I CONSENT:

1. To undergo the following exploratory tests (if applicable): blood plasma sampling, stool and urine sampling, intake of a food supplement (Gasteel Plus synbiotic) or placebo, anthropometry, accelerometry, heart rate variability measurements, validated questionnaires for the study of stress, anxiety and perception of quality of life.

2. The use of the data obtained as indicated in the following paragraph:

In compliance with Organic Law 15/1999, of December 13, on the Protection of Personal Data, we inform you that the information you have provided and that obtained as a result of the scans to which you will be submitted will become part of the automated file INVESALUD, whose owner is the FUNDACIÓN UNIVERSITARIA SAN ANTONIO, for the purpose of RESEARCH AND TEACHING IN THE AREAS OF KNOWLEDGE EXPERIMENTAL SCIENCES AND HEALTH SCIENCES. You have the right to access this information and cancel or rectify it, by contacting the address of the entity, in Avda. de los Jerónimos de Guadalupe 30107 (Murcia). This entity guarantees the adoption of appropriate measures to ensure the confidential treatment of such data.

In Guadalupe (Murcia) to ..... of ..... of 20

The participant	The investigator
Signed:……	Signed:……..