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#### Abstract

Introduction: Enviromental factors such as exercise participation and nutrition have often been linked to bone improvements. However, not all sports have the same effects, being non-osteogenic sports such as swimming defined as negative or neutral sports to practice regarding bone mass by some authors, similarly exercisediet interaction in especific groups is still not clear.

Objective: To present the methodology of the RENACIMENTO project that aims to evaluate body composition and more specifically bone mass by several techniques in adolescent swimmers and to observe the effects and perdurability of whole body vibration (WBV) and jumping intervention (JIN) on body composition and fitness on this population and explore posible diet interactions.

Design: Randomized controlled trial. Methods: $\mathbf{7 8}$ swimmers ( $\mathbf{1 2 - 1 7}$ y) and $\mathbf{2 6}$ sex- and agematched controls will participate in this study. Dual energy X-ray, peripheral Quantitative Computed Tomography, Quantitative Ultrasound, Bioelectrical Impedance Analysis, and anthropometry measurements will be performed in order to evaluate body composition. Physical activity, nutrition, pubertal development and socio-economical status may act as confounders of body composition and therefore will also be registered. Several fitness factors regarding strength, endurance, performance and others will also be registered to evaluate differences with controls and act as confounders. A 7month WBV therapy will be performed by 26 swimmers consisting of a training of 15 minutes 3 times per week. An 8 month JIM will also be performed by 26 swimmers 3 times per week. The remaining 26 swimmers will continue their normal swimming training. Four evaluations will be performed, the first one in order to describe differences between swimmers and controls. The second


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# REPERCUSIÓN DEL ENTRENAMIENTO Y LA PRÁCTICA DE LA NATACIÓN SOBRE EL DESARROLLO METABÓLICO Y ESTRUCTURAL DEL HUESO EN CRECIMIENTO; BENEFICIOS DE LA INCORPORACIÓN DE ENTRENAMIENTO PILOMÉTRICO O VIBRATORIO; EL ESTUDIO RENACIMIENTO 

## Resumen

Introducción: En la actualidad se ha demostrado que el ejercicio físico y la nutrición mejoran la masa ósea. Sin embargo, existen deportes de bajo impacto como la natación que no presentan efectos positivos en su desarrollo. Además, la interacción ejercicio-dieta y su efecto osteogénico sigue sin estar clara.

Objetivo: Presentar la metodología del proyecto RENACIMIENTO que tiene por objetivo evaluar la composición corporal del nadador adolescente y más concretamente la masa ósea a través de diversas técnicas. Además se pretenden determinar los efectos y la perdurabilidad que pueden tener el entrenamiento vibratorio (WBV) y una intervención con saltos (JIN) sobre la composición corporal y condición física de estos adolescentes, explorando la posible interacción con la dieta.
Diseño: Ensayo clínico aleatorizado.
Metodología: 78 nadadores (12-17 años) y 26 controles del mismo sexo y edad participarán en el estudio. Se utilizarán absorciometría dual de rayos-x, tomografía axial computerizada, ultrasonidos cuantitativo, bioimpedancia eléctrica, y medidas antropométricas para evaluar la composición corporal. La actividad física, nutrición, desarrollo puberal y status socio-económico podrían actuar como covariables de la composición corporal y por lo tanto también serán registradas. Se evaluarán diversos factores de la condición física relacionados con la fuerza, resistencia, rendimiento y otros para definir las diferencias con los controles y para que sirvan como covariables. 26 nadadores realizarán una intervención de WBV 7 meses 15 minutos 3 veces por semana. Además otros 26 nadadores realizarán una JIN 3 veces por semana durante 8 meses. Los 26 nadadores restantes continuarán con su entrenamiento habitual de natación. Se realizaran 4 evaluaciones, la primera de ellas para describir las diferencias existentes entre nadadores y controles, la segunda para describir los efectos de las intervenciones realizadas
one to describe the effects of the interventions and the third and fourth evaluations to describe the perdurability of the effects of the WBV and JIN.

Conclusion: The RENACIMIENTO project will allow to answer several questions regarding body composition, fitness, bone mass and interaction with diet of adolescent swimmers, describe swimming as a positive, negative or neutral sport to practice regarding these parameters and elucidate the effects and perdurability of WBV and JIM on body composition.
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Key words: Swimmers. Osteoporosis. Bone mass. pQCT. DXA.

## Abbreviations

BIA: Bioelectrical impedance analysis.
BMD: Bone mineral density.
BMC: Bone mineral content.
BUA: Broadband ultrasound attenuation.
CFQ: Calcium frequency questionnaire.
DXA: Dual energy X-ray.
JIN: Jumping intervention.
MICS: Maximum isometric cuadriceps squat.
pQCT: Peripheral quantitative computed tomography.

QUS: Quantitative ultrasound.
RCT: Randomized Controlled Trial.
SOS: Speed of sound.
SES: Socio-economical status.
WBV: Whole body vibration training.

## Introduction

It is well known that physical activity has a positive effect on bone mass, and that practiced during growth periods may improve bone acquisition. ${ }^{1}$ However, not all sports have the same effects on bone mass. Recent literature suggests that high impact sports such as volleyball or basketball submit bones to higher strains producing increments in bone mineral density (BMD) and bone mineral content (BMC) ${ }^{2}$ and may improve bone structure. ${ }^{3}$ Other sports like cycling ${ }^{4}$ or swimming that are known as non-impact sports have shown to be less beneficial for bone health than high impact sports being described by some authors as negative sports to practice regarding bone mass. ${ }^{5}$

Focusing on swimming, a recent systematic review ${ }^{6}$ with 52 studies included, concluded that swimmers presented similar BMD and BMC values than sedentary controls, but these results were not conclusive due to the heterogeneity of the included studies. Nevertheless, swimmers presented lower values when they were compared to high-impact sports in all of the studies included in the review. However, the few studies that
y la tercera y cuarta para evaluar la perdurabilidad de estas intervenciones.

Conclusión: El proyecto RENACIMIENTO permitirá contestar a diversas preguntas relacionadas con la composición corporal, condición física y masa ósea de los nadadores adolescentes y el posible efecto combinado dieta-ejercicio en esta población. Definirá la natación como un deporte positivo, neutral o negativo en relación con estos parámetros y esclarecerá los efectos y perdurabilidad de la WBV y JIN sobre la composición corporal.
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evaluated bone with peripheral quantitave computed tomography (pQCT) showed that swimmers presented a better structure than sedentary controls. Higher values of bone turnover were also found, suggesting therefore that although swimmers may present similar BMD and BMC values than controls both measured with Dual energy X-ray (DXA), swimmers might present a higher bone quality than sedentary controls.

Most of the studies included in the previous review had reduced sample sizes ( $\mathrm{n}=20$ in most of the studies) to compare with other sports, moreover the authors of the review state that several studies usign DXA did not adjust by any covariables and therefore results migth have been masking the real effects of swimming. Out of the 52 studies included in the review only 22 included adolescent swimmers and out of these, only 2 included more than 50 swimmers. Moreover, none of the studies including adolescents used pQCT to evaluate bone structure.

Therefore, we propose the following questions:

1. Is swimming negative for bone mass acquisiton during adolescence, or does it not have any effect at all in this population?
2. If it is negative, is there a threshold regarding hours of swimming when this activity becomes negative? And regarding years of swimming?
3. If bone structure is analyzed by two methods ( pQCT and ultrasound), will there be differences between methods and are swimmers bones stronger, similar or weaker than sedentary controls?
4. Is bone improved with 3 sessions of 15 minutes of whole body vibration training (WBV) or jumping intervention (JIN) complementary to swimming training?
5. If bone is improved with 45 minutes of WBV or JIN per week, what it the perdurability of these effects?
6. Is bone turnover affected by swimming?
7. How does diet interact with exercise and its combined effect on bone mass, estructure and metabolism?

These and other questions should be answered, specially in this age-population where bone is constantly developing. The idea that "senile osteoporosis is a paediatric disease" is increasingly accepted. ${ }^{3}$ In fact, the World Health Organization proposed prevention as the most powerful way to fight against the non-communicable diseases, i.e. osteoporosis. ${ }^{7}$ Adolescence is therefore a critical period for bone development and the effect of intense swimming training on bone should be carefully studied in order to evaluate and avoid bone diseases in this population later in life.

All of the previous questions and the need of describing the art-of-the-state in adolescent swimmers have been the beginning of the RENACIMIENTO project that aims to answer most of these interrogants and elucidate the real effect of swimming on bone mass measured by several different techniques.

With this report we aim to present the general methodology of this wide research project as a way to offer a commun comprehensive methodology in this research field.

## Material and methods

## Arguments for publishing a desing paper

The present paper is going to describe a randomized controlled trial (RCT) assessing a WBV and JIN in adolescent swimmers which will take place over a swimming season. Publishing the design and rationale of a RCT before the results are available has important benefits. The study can be critically evaluated for its methodological quality, irrespective of the results. Moreover, if a design paper is written and published, the results will most probably be published, even if they are negative. In addition, a design paper includes a more detailed description of the study techniques, the intervention and all outcome measures than what can be reported in the method section of a regular publication focusing only on part of the study results. This methological paper can help researchers from similar fields to compare methods and obtain a global view of the project.

## Ethical committee

The protocol study has been aproved by the Ethics Committee of Clinical Research from the Government of Aragón (C.I.PI11/0034; CEICA; SPAIN), and will follow the ethical guidelines of the Declaration of Helsinki 1961 (revision of Edinburgh 2000), demanding in all cases the signed informed consent by the adolescent participant and his parents or tutors.

## Study sample

The RENACIMIENTO study will evaluate 78 adolescent swimmers of both sexes aged from 12 to 17 years old
and 26 adolescent sedentary sex- and age-matched controls not involved in any specific sport participation. All the participants will be healthy adolescents that will not take any drugs affecting bone mass.

The sample size has been calculated in regard to the variable with most variability BMD. An independet ttest was performed in order to attain a power of $99 \%$ to detect differences in the contrast of the null hypothesis $H_{0}: 1=2$. Statistical level of significance was set at $1 \%$, and assuming that the mean from the reference group was 1.25 units, the mean of the experimental group 1.20 units and the standard deviation of both groups was 0.02 units it would be necessary to include 10 experimental units in the reference group and 10 in the experimental groups, making a total of 40 experimental units in the study. However, the number of participants to include in a study, also depends of the possible loss of participants: $n^{\prime}=n /(1-p)$, so if loss were of $30 \%$ (possible lost), the number of subjects to recruit would be $n^{\prime}=10 /(1-0.3)=12.5$ subjects $=13$ subjects in each group. As there are 4 groups (swimmers; swimmers+WBV, swimmers+JIN intervention, controls) and each group is duplicated by sex it makes a total of 104 adolescents (4 groups x 13 participants per group x 2 genders $=104$ participants) .

## Study design

The RENACIMIENTO project is a RCT with follow-up period (fig. 1) where swimmers and controls will be measured in 4 ocassions.

The first measurement was performed in SeptemberOctober 2012. The intervention has been performed, although no results from the first cohort nor the intervention have been published yet.
The second measurement took place in May-June (swimmers) 2013. The effect of WBV, JIN and swimming on bone and body composition during a whole season in adolescent swimmers will be described with this measurement.

The third measurement will take place in FebruaryMarch, 2014. This measurement will evaluate the perdurability of the effect of the WBV and JIN (if there were any) 9 months after the intervention.

The last measurement will take place in SeptemberOctober, 2014. With this last measurement we intend to elucidate the perdurability of the effects produced by WBV and JIN on body composition after 18 months.

## Equipment

All the used equipment is summarized in table I. Body composition is the main outcome that we intend to measure. However, other factors such as physical activity, nutrition, pubertal development or socioeconomical status may act as confounders and therefore must also be recorded.


Fig. 1.-Chronological design of the RENACIMIENTO project.

## Evaluation

## Body composition

One of the main aims of the RENACIMIENTO study is to evaluate bone mass which is the single most important determinant of future fracture. ${ }^{8}$ Several techniques have been designed for this purpose. Included bone mass measurements in this project have been DXA, pQCT and quantitative ultrasound.

## Dual energy X-Ray

DXA is the most common photon absorptiometry method used to evaluate bone mass, and has been defined by the WHO as the gold standard method for evaluating osteoporosis. It is a two dimensional measure highly influenced by body size. ${ }^{9}$ It therefore seems necessary to adjust by covariates to minimize the differences among participants when these are compared. The used equipment for the current project will be an Hologic QDR 4500 scanner (paediatric version of the software QDR-Explorer, Hologic corp., Software version 12.4, Bedford, MA, USA). This device uses two X-ray beams to distinguish between fat and lean tissues on the one hand and bone and soft tissues on the other, on the basis of the extent to which the pairs of tissues attenuate the two X-rays to different degrees. DXA equipment will be calibrated daily using a lumbar spine phantom as recommended by the manufacturer. All DXA scans will be completed with the same device and software and performed by the same technician who has been fully trained in the operation of the scanner, the positioning of subjects, and the analysis of results, according to the manufacturer's

| Table I |  |  |
| :--- | :---: | :---: |
| I |  |  |
| Meast intra-measures coefficient of variation |  |  |
|  | Zone <br> of evaluation | \% coeficient <br> of variation |
| Radius |  |  |
| Total area | 4.26 |  |
| Total density | $4 \%$ | 2.25 |
| Trabecular area | $4 \%$ | 4.27 |
| Trabecular density | $4 \%$ | 2.78 |
| Total area | $4 \%$ | 2.42 |
| Total density | $66 \%$ | 2.07 |
| Cortical area | $66 \%$ | 2.44 |
| Cortical density | $66 \%$ | 0.84 |
| Cortical thickness | $66 \%$ | 3.60 |
| Periosteal circumference | $66 \%$ | 1.21 |
| Endosteal circumference | $66 \%$ | 2.98 |
| Muscle area | $66 \%$ | 1.34 |
| Fat area | $66 \%$ | 7.81 |
| Tibia | $66 \%$ |  |
| Total area |  |  |
| Total density | $4 \%$ | 0.82 |
| Trabecular area | $4 \%$ | 0.67 |
| Trabecular density | $4 \%$ | 0.83 |
| Total area | $4 \%$ | 0.90 |
| Total density | $38 \%$ | 4.36 |
| Cortical area | $38 \%$ | 1.27 |
| Cortical density | $38 \%$ | 5.31 |
| Cortical thickness | $38 \%$ | 0.49 |
| Periosteal circumference | $38 \%$ | 4.61 |
| Endosteal circumference | $38 \%$ | 2.33 |
| Muscle area | $66 \%$ | 2.38 |
| Fat area | $66 \%$ | 1.69 |

guidelines. Fat mass, fat-free mass and bone mass are calculated using a computer algorithm provided by the manufacturer. A whole body scan will be performed
allowing a regional analysis (upper and lower extremities and pelvic region). The arm region from the regional analysis includes the hand, forearm and arm and is separated from the trunk by an inclined line crossing the scapulohumeral joint such that the humeral head is located in the arm region. The leg region includes the foot, lower leg and upper leg and is defined by an inclined line passing just below the pelvis crossing the neck of the femur. The head region comprises all skeletal parts of the skull and cervical vertebra above a horizontal line passing just below the jabone. In addition to this whole body scan a lumbar spine, hip and forearm scans will be performed. To save time and reduce x-ray exposure, only the nondominant hip and forearm will be scanned. Values for the femoral neck, Ward's triangle, greater trochanter, and intertrochanteric subregions are provided from the hip scan. The Ward's triangle is defined as the area (approximately $1.1 \mathrm{~cm}^{2}$ ) of the femoral neck with the lowest BMD. Values reported for the lumbar vertebrae L2-L4 are obtained from an anteroposterior lumbar scan and expressed as the mean BMD of the three vertebrae. Values reported for the forearm are ultradistal, mid and $1 / 3$ radius and ulna. The coefficients of variation of the DXA in our lab are published elsewhere. ${ }^{10}$

## Peripheral Quantitative Computed Tomography

In contrast to DXA, pQCT measures volumetric BMD and allows for separate assessment of trabecular and cortical bone of the appendicular skeleton, such as the radius and tibia. ${ }^{8}$ This device provides measures of cross-sectional areas related to bone size (area), mass (mineral content), apparent tissue density and geometry (spatial distribution of mass). Moreover, this device calculates strength indices, which combine bone cross-sectional geometry and tissue density measures. Our equipment is a XCT 2000 Peripheral QCT Scanner, Ortometrix, INC that allows measurement of the tibia and radius. For the present study we will use both non-dominant limbs. Coefficients of variation for pQCT in our laboratory have been calculated for several variables and are summarized in table 1 . For the calculation of the coefficient of variation the non dominant forearm and lower leg of 20 subjects (16-24 y) were scanned. Two consecutive scans were performed for each limb. All scans and image analysis were performed by the same technician.

## Quantitative Ultrasound (QUS)

QUS is a new technology for the assessment of bone strength that measures speed of sound (SOS) along the bone and is not affected by bone size, allowing for better comparisons between children of different sizes. ${ }^{11}$ QUS also provides broadband ultrasound
attenuation (BUA) which as SOS, is also related to bone density and structure and to the elastic modulus of bone, ${ }^{12}$ but not to cortical thickness. ${ }^{13}$ A Lunar Achilles Insight (Achilles Insight, GE, USA) device will be used to evaluate the calcaneus bone which is the most common measurement site due to its accessibility, suitable shape, and high trabecular content. ${ }^{14}$ QUS has been established as an alternative technique for the assessment of bone status, ${ }^{15}$ due to the low cost effectiveness and the absence of ionizing radiation. This measurement will be performed in the non-dominant calcaneus.

## Bioelectrical impedance analysis (BIA)

BIA is a popular and widely-used method for measuring body composition. ${ }^{16}$ This technique determines the electrical impedance of body tissues, which provides an estimate of total body water that is converted to an estimate of fat-free mass, with assumed constant values for the hydration of lean tissue. For our study, a TANITA BC-418 (Tanita, Tokyo, Japan) 8-contact electrode system will be used. Coefficients of variation for BIA in our laboratory have been calculated for weight ( $0.09 \%$ ) and body fat percentage ( $1.11 \%$ ). For the calculation of the coefficient of variation 15 subjects (16-30 y) were assessed two consecutive occasions.

## Anthropometry

Researchers were all level 1 or 2 anthropometrists ISAK before the study began. Skinfolds will be measured with a Holtain Harpender Skinfold Caliper (Holtain, Dyfed, UK) and circunferences with a Rosscraft Anthrotape (Rosscraft Innovations Inc, Vancouver, Canada) (table II). Two researchers will perform anthropometries. To avoid inter-observer error in the longitudinal study, a register will be performed indicating which researcher measured each participant so that the same researcher measures in the different cross-sectional moments of the study the same participant. The technical error of measurement inter and intra-observer will be between the limits recommended by ISAK ( $<5 \%$ for skinfolds and $<1 \%$ for the other measurements).

## Bone turnover

Osteoporosis is diagnosed based on an assessment of bone density. However, the results only provide a past history rather than an evaluation of how bone is currently developing. An examination of metabolic markers of bone metabolism can be used to provide an understanding of the dynamic course of bone remodelling. More specifically, serum markers of bone resorption and bone formation can be used to examine the

Table II
Methods included in the RENACIMIENTO project

| Outcome | Method | Measurement | Device |
| :---: | :---: | :---: | :---: |
| Body composition |  |  |  |
|  | Three-component model | Dual-energy X-ray | Hologic Corp., Software version 12.4, Bedford, MA, USA) |
|  | Two-component model | Bioimpedance | TANITA BC-418 MA |
|  | Skinfold thickness (7 sites) | Subcutaneaous fat at defined sites: Biceps, triceps, subscapular, supraspinale, abdominal, front thigh and medial calf | Holtain Harpender skinfold Caliper |
|  | Circumferences ( 6 sites) Quantitative Ultrasound | Arm relaxed, arm flexed and tensed, waist, hip, mid-thigh, calf | Rosscraft Anthrotape Lunar Achilles Insight |
|  |  | Calcaneus Speed Of Sound, Broadband Ultrasound Attenuation, and Stiffness Index. |  |
|  | Peripheral Quantitative | Bone structure and densities | Stratec XCT 2000 L |
|  | Computed Tomography |  |  |
|  | Bone turnover | Osteocalcin, dexopyrydinoline, N -Telopeptides (NTx) and C-telopetides (CTx) | ELISA <br> Inmunoassay |
| Physical activity |  |  |  |
| Fitness | Biaxial and triaxial accelerometers | Acelerometry | Actitrainer and Geneactive |
|  | Sedentarism questionnaires | Sedentary activities | HELENA screen time-based sedentary behaviour questionnaire ${ }^{20}$ |
|  | Physical activity | Practiced sports and hours of physical activity | Self-administered questionnaire |
|  | 20 m shuttle run test | Paliers reached in the 20 meter course-navette test | Course navette CD |
|  | Broad-jump | Horizontal distance reached jumping with both feet together | Measuring tape |
|  | 30 meter sprint | Time to perform a 30 meter distance | Timing gates (Byomedic fotoelectric cells, Barcelona) |
|  | Maximum isometric strength | Forearm strength | Takey TKK 5401, Tokyo, Japan |
|  |  | Shoulder strength | SignalframeUSB gauge |
|  |  | Cuadriceps strength (extensión) | SignalframeUSB gauge |
|  |  | Cuadriceps strength (squat) | Kistler force plate 9260AA |
|  | Jump evaluation | Squat Jump, Counter Movement Jump, Abalakov | Kistler force plate 9260AA |
|  | Maximum explosive strength | Squat at 20,30 and $40 \%$ of the maximum isometric cuadriceps strength | Kistler force plate 9260AA |
| Nutrition |  |  |  |
|  | Helena Dietary Assessment Tool | 3 day 24 hour recall | HELENA-DIAT tool |
|  | Calcium frequency questionnaire | Calcium intake | Calcium FFQ (Barr et al. ${ }^{26}$ |
|  | Knowledge questionnaire | General nutrition knowledge questionnaire | Self-reported HELENA questionnaire ${ }^{27}$ |
| Socio-economical status |  |  |  |
|  | Socio-economical questionnaire | General socio-economic status | Adapted SES questionnaire ${ }^{46}$ |

current and changing status of bone turnover ${ }^{(17)}$. For this purpose, Bone specific Alkaline Phosphatase, and Osteocalcin will be measured in order to evaluate bone formation. For bone breakdown, the measured biomarkers will be N -Telopeptide and C-telopeptide. In adittion deoxypiridinoline and N -Telopeptides (NTX) will be determined from inmunoassay and ELISA respectively, from a urine sample

## Physical activity

## AcCELEROMETRY

An Actitrainer accelerometer (ActiTrainer ${ }^{\mathrm{TM}}$, Florida, USA) is a small ( $8.6 \times 3.3 \times 1.5 \mathrm{~cm}$ ) and light device. It is
multi-functional composed of heart-rate monitor, solidstate accelerometer, electronic pedometer, inclinometer and an ambient light sensor. The validity and reliability of the Actitrainer-based step counting in non-laboratory conditions has been previously validated ${ }^{(18)}$. Accelerometers will be placed at children's waist at the right side of the body in an elastic belt with a selected epoch length of 15 seconds. Adolescents will be required to wear the accelerometer from the moment they wake up in the morning until bedtime in the evening during 4 consecutive days including a weekend day. However, this accelerometer is not waterproof and therefore will be removed by participants when they have to take a shower or perform aquatic activities.

In the third cross-sectional moment of the study each participant will wear 2 accelerometers. The previously
mentioned actitrainer accelerometer and the GENEA. The GENEA is a triaxial, $\pm 6 \mathrm{~g}$ seismic acceleration sensor (LIS3LV02DL; STMicroelectronics, Geneva, Switzerland). The small ( $36 \times 30 \times 12 \mathrm{~mm}$ ) and lightweight ( 16 g ) water-proof design of the GENEA allows it to be easily worn at multiple locations on the body (e.g., wrist, waist, ankle). The GENEA has 500 MB of memory to assist with the storage of the raw $80-\mathrm{Hz}$ sampling frequency and can store $\sim 8 \mathrm{~d}$ of data in raw mode with 12 -bit resolution. Users have the ability to select user-defined sample frequencies ranging from 10 to 80 Hz . Using the GENEA software (version 1.487 update 531), via USB-to-PC connection, 47 GENEA accelerometers were initialized to collect unfiltered, triaxial acceleration data at a sampling rate of $80 \mathrm{~Hz} .{ }^{19}$

## SEDENTARISM QUESTIONNAIRE

A sedendetarism questionnaire including hours of diary television, computer, videogames and several similar sedentary activities will be delivered for the adolescents to complete. This questionnaire has been described elsewhere. ${ }^{20}$

## Practiced Sports

We will provide a questionnaire asking the current practiced sports, hours per week of practice and years of participation. Past practiced sports that are no longer being practiced will also be questionned in order to have a complete sport history of the participants.

## Nutrition

Nutrition is key to bone mass ${ }^{21}$ and therefore several methods will be used in order to register participants nutritional intake.

## Helena Dietary Assessment Tool

The HELENA-DIAT ${ }^{22}$ is a computer program that allows participants to register a 24 -hour recall. The advantage of using an informatic program is that it allows to view pictures of the food in order to choose the appropiate portion size. A researcher will guide the participants through the program to remind them items that they normally forget such as bread accompanying meals or water. The programm, calculates the macroand micronutrients intake of the evaluated day. A total of three 24-hour recalls will be performed (1 of them of a weekend day). The 24 -hour recall method has been described as the best method to get population mean intakes and distributions for participants aged 10 and over in different European countries. ${ }^{23}$ Moreover, we
have previously used it in our laboratory ${ }^{24}$ and all researchers are familiar with this tool.

## Calcium Frequency Questionnaire (CFQ)

Several observational studies have suggested that increasing the calcium intake would promote a greater bone mass gain, and thereby a higher peak bone mass. ${ }^{25}$ This nutrient is an important factor to take into account when studying bone mass. Thus, a specific CFQ ellaborate by Barr et al. ${ }^{26}$ will be used.

## Nutrition knoweledge

Nutrition knoweledge will be assessed by the NKT questionnaire described elsewhere. ${ }^{27}$

## Socio-ECONOMICAL status (SES)

It has been shown that SES influences sport participation, nutrition and body composition. ${ }^{28}$ Therefore, SES is an important confounder that must be taken into account when evaluating these variables and comparing two groups. A questionnaire described elsewhere ${ }^{27}$ will be used for the evaluation of the SES.

## Fitness

Laboratory and field tests will be performed in order to measure physical fitness and observe the possible relations with body composition, nutrition and socioeconomical status.

## LABORATORY TESTS

The tests presented bellow are in the same order has we will perform them in our laboratory.

- Dynamic strength of the lower limbs. The generated forces will be measured with a KISTLER platform type 9260AA (Kistler instruments Ltd., Hampshire, UK) while the participants perform 3 different jumps: Squat jump, Countermovement jump and Abalakov jump. The inclusion of the 3 jumps has been decided in order to analize differences between jumps and evaluate maximal lower limb explosive strength. Participants will perform 3 attemps of each jump with at least one minute rest in between. The best performance will be selected for future statistical analyses.
- Maximum isometric cuadriceps extension strength. Subjects will be sitting on a table, with an anchorage placed on the distal third of the tibia. This anchorage will be connected to a strain gauge
(MuscleLab, Force Sensor, Norway) that will register the Newtons of isometric force generated during the 6 seconds that participants will have to perform the test. Two attempts will be allowed for each leg, with a minimum of 3 minutes between attemps with the same leg. The best performance will be selected for future statistical analyses.
- Maximum isometric shoulder flexion strength. Participants will be encouraged to perform the maximum isometric strength lying on a fitness bench with one arm extended in an overhead position simulating the downsweep phase of freestyle swimming. Participants will perform force against an anchorage connected to a MuscleLab gauge that will register the Newtons of isometric force generated. Two attemps will be performed with each upper limb. The best performance with each upper limb will be selected for future statistical analyses.
- Maximum isometric cuadriceps squat $90^{\circ}$ (MICS). Participants will be placed in a 90 degree squat position standing on the force platform. They will be encouraged to execute their maximum strength in order to stand up from the $90^{\circ}$ squat position, performing strength against a fixed bar that will unable the subject to move. All the performed strength will be registered by the strength platform and will later be analyzed. The best performance with each leg will be selected for future statistical analyses.
- Maximum isometric forearm strength. A digital handgrip dynamometer (Takei TKK 5401, Takei scientific instruments, Tokyo, Japan) will be used in order to evaluate strength of the forearm and hand muscles. Hand span to perform the test will be different for each participant according to their hand size. The dynamometer will be placed according to the optimal handgrip span suggested by Ruiz et al. ${ }^{29}$ Participants will perform two maximum strength trials with each hand. The best performance with each arm will be selected for future statistical analyses.
- Muscular power of the lower limbs at 20,30 and $40 \%$ of the MICS. For these tests, participants will start from a standing position. From there, participants will be encouraged to perform a half-squat loading a bar and weight plates added to the bar. With this test it is intended to measure the maximum power that a participant is able to perform during the concentric phase when performing the extension lifting 20, 30 and $40 \%$ of their MICS. This will be performed in a machinery in which the resistance bar will be attached at both ends with linear bearing on two vertical bars, thus allowing only vertical movements of the bar. A rotator encoder attached to the bar (Tforce dynamic meaurement system, model TF-100, Ergotech consulting S.L. Murcia, Spain) will be used to register the performed power.


## FIELD TESTS

- Standing Broad Jump. This jump will be performed in order to test explosive leg power. A two-feet take-off and landing will be demanded to the participants, allowing them to swing their arms and bend their knees to provide forward drive. Three attempts will be performed. The best performance will be selected for future statistical analyses.
- Thirty meters sprint. The purpose of this test is to determine maximum running speed. Timing gates (Byomedic fotoelectric cells, Barcelona) will be placed with 30 meters between them. Participants will start at one gate, and when the researcher gives the start indication, the participant will run has fast as possible to the other gate. This test has shown to have a high predictive value for bone mass and bone mass accumulation during growth. ${ }^{19}$
- Twenty meters shutle run fitness test. This test will be performed in order to evaluate $\mathrm{VO}_{2_{\text {max }}}$ of the participants, using the Leger equation. ${ }^{20}$ It involves continuous running between two lines 20 meters apart in time to recorded beeps. The speed starts at $8 \mathrm{~km} / \mathrm{h}$ and increases $0.5 \mathrm{~km} / \mathrm{h}$ per minute. The test will be stopped if the participant fails to reach the line for two consecutive beeps.


## Evaluation of pubertal development

Pubertal development will be evaluated by selfassessment following the Tanner stages, that has been described as a reliable method for this purpose. ${ }^{31}$

## Intervention

Swimmers will be randomly divided into 3 groups. One group will receive an intervention with WBV and another group and intervention based on pliometric training. The third group will consist of swimmers who will continue their habitual training routine and will act as swimming control group.

## Whole body vibration training

There has been a recent increased interest of WBV. ${ }^{32}$ This training methodology is considered benefitial for performance ${ }^{33}$ and rehabilitation. ${ }^{34}$ Previous studies suggest that mechanic vibrations applied directly on the muscle fiber, produce reflect muscle contraction due to the tonic vibration reflex. ${ }^{35}$ Although in nonsportive population it has generally been used in older populations, ${ }^{36}$ it has also prooved its effectiveness improving mobility, ${ }^{37}$ muscular function ${ }^{38}$ and bone mass ${ }^{39}$ in children and adolescents with different phatologies. ${ }^{40,41}$ Recent studies have demonstrated that

Table III
Whole body vibration protocol

| Month | Exercises | Total number of exercises | Frequency (Hz) | Amplitude (mm) | Duration <br> (s) | Rest <br> (s) | Total <br> (min) | G-Force |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| 0 (2 weeks) | 2 (A,B,C,D,E) | 10 | 30 | 2 | 45 | 45 | 14.30 | 2.6 |
| 1 (4 weeks) | $2(\mathrm{~A}, \mathrm{~B}, \mathrm{C}, \mathrm{D}, \mathrm{E})$ | 10 | 30 | 4 | 45 | 45 | 14.30 | 5.1 |
| 2 (4 weeks) | $2(\mathrm{~A}, \mathrm{~B}, \mathrm{C}, \mathrm{D}, \mathrm{E})$ | 10 | 32 | 4 | 45 | 45 | 14.30 | 5.8 |
| 3 (4 weeks) | $2(\mathrm{~A}, \mathrm{~B}, \mathrm{C}), 1(\mathrm{D}, \mathrm{E})$ | 8 | 34 | 4 | 60 | 60 | 15 | 6.6 |
| 4 (4 weeks) | $2(\mathrm{~A}, \mathrm{~B}, \mathrm{C}), 1(\mathrm{D}, \mathrm{E})$ | 8 | 36 | 4 | 60 | 60 | 15 | 7.4 |
| 5 (4 weeks) | 2 (A,B,C), 1(D,E) | 8 | 38 | 4 | 60 | 60 | 15 | 8.2 |
| 6 (4 weeks) | 2 (A,B,C), 1(D,E) | 8 | 40 | 4 | 60 | 60 | 15 | 9.1 |

$\mathrm{A}=$ Squat at $120^{\circ} ; \mathrm{B}=$ Squat at $90^{\circ} ; \mathrm{C}=$ Dynamic squat from 90 to $120 ; \mathrm{D}=$ Lunge right leg; $\mathrm{E}=$ Lunge left leg.

WBV performed at low frequencies and amplitudes is safe and effective on the musculo-skeletal system. ${ }^{42}$

One of the main advantages of WBV is that training sessions can be very short, being 10 minutes enough to produce osteogenic effects. In young women with low BMD, 12 months of WBV ( 10 minutes, $30 \mathrm{HZ}, 0.3 \mathrm{~g}$ ) produced an increase of trabecular bone in the lumbar spine and an increase of the cortical area of the femur bone. ${ }^{43}$

Adolescent swimmers included in our study are training an average on 10 hours per week, therefore we could not include a type of training that needed of another 2 or 3 extra-hours per week to improve bone mass. The short training times needed for WBV and the benefits on bone mass found in literature, made it our choice to try to improve swimmers bone mass.

## WBV DEVICE

The WBV platform used in the study is a Power Plate Pro 5,(PowerPlate, London, UK). The WBV market is extense, the election of this platform was based on the medical certificates supplied by this company and the previous experience of the research group with them.

## WBV PROTOCOL

The choosen protocol for the intervention has been designed by an expert of this field that has performed several studies using WBV. ${ }^{44,45}$ The protocol is summarized in table III. The first 2 weeks are an adaptation period, and the intense training begins in week 3 (month 1) and lasts for 6 months.

## High impact training

This programme will consist on a jumping intervention that will take place 3 times per week in 15 minute
sessions. For the design of this intervention easy exercises with accesible material have been choosed. The intervention program is summarized in table IV. A circuit of 4 stations that include high impact jumping (ground reaction forces higher than 3.5 body weight in the lower jumps and over 5 times body weight in the piometric jumps) will be prepared in each sesion, consisting of obstacle jumps with different positions and directions.

The intensity and number of jumps will progressively increase over 4 levels, having each level a duration of 8 weeks. Intensity will be modified increasing the hurdle height from 25 cm in level 1 to 35 in level 4 . The volume per sesion will also increase by increasing the jumps from 120 in level 1 to 160 in level 4 with a 1 minute rest between each activity station.

## High impact Protocol

Four main exercises will be performed in each sesion:

- Hurdle jumping: Hurdles will be separated by 6070 cm . Participants will jump 8 consecutive hurdles jumping and landing with both feet simultaniously. Jumps will be performed in a pliometric way, without allowing rest between hurdles. Once they have jumped the 8 hurdles, participants will walk back to the begining to repeat the exercise.
- One foot bench: Participants will jump from one side to another of a bench with one foot. Landing each time with a different foot and jumping with that same foot. Once they have ended the bench, participants will walk back to the begining to repeat the exercise.
- Hurdles back and forward: Participants will jump a hurdle back and forward jumping and landing with booth feet simultaniously ten times.
- Two feet bench: Participants will jump from one side to another of a bench with both feet together. When finished, they will walk back to the begining to repeat the exercise.



## Dissemination plan

Dissemination activities aim to promote international dissemination and exploitation of the RENACIMIENTO results. These results will be presented in national and international congresses and meetings focused on physical activity, swimming and overall sport sciences. Moreover, the likely impact of the results of this research will allow the publication of scientific papers in top journals of the sports science area. In addition to this scientific path, participants will also be informed of the results obtained in the current project. The Spanish Swimming Federation, swimming clubs and Ministry of Education, Culture and Sport will also be informed of the results found by the RENACIMIENTO study.

## Perspective

Therefore, the RENACIMIENTO project is aiming to answer as many questions of those proposed in the introduction as possible, in order to better understand the actual effects of intensive swimming training during adolescence on different variables of health, and the possible effects of a WBV and jumping interventions over a season.

These answers will be presented in the form of original research articles, leaded mainly by the three PhD students running the field and laboratory testing during the 3-year project. The different articles, and hence the 3 different PhD theses, will be focused in 3 main areas, previously explained, within the RENACIMIENTO project: 1) body composition, 2) performance and 3) nutrition.

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