1. *PROPOSAL DATA*

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Title of the project:

Profiling genome-wide DNA methylation changes in saliva after a two-month physical exercise intervention in young basketball players.

Agents involved:

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Basquet Girona, 17007 Girona, Spain.

Genome-wide DNA methylation analysis was conducted at the Genomics Unit of the Josep Carreras Leukemia Research Institute (Barcelona, Spain)

Raw data processing and downstream bioinformatic analyses were subsequently conducted by the Cancer Epigenetics Laboratory

2. *BACKGROUND, CURRENT STATUS AND JUSTIFICATION OF THE PROPOSAL*

BACKGROUND

Epigenetics and physical exercise (PE)

Epigenetics comprises the mechanisms that regulate gene expression without directly altering the DNA sequence. These mechanisms include DNA methylation of CpG islands, posttranslational modifications of histones and microRNAs[1,2].

Although patterns of DNA methylation are mainly established early in development and traditionally have been thought to be maintained throughout life, physical activity (PA) can induce changes in DNA methylation patterns. Those epigenetic modifications, have a potential impact on insulin sensitivity, glycolytic and oxidative metabolism, as well as muscle regeneration[3]. The impact of physical exercise (PE) on DNA methylation seems to depend on the intensity and duration of the exercise. However, a consistent finding across all interventional studies is that acute or chronic physical exercise impacts DNA methylation in a gene-specific manner. According to Soci et al. (2017)[4], it is important to investigate how epigenetic modifications can be influenced by specific training interventions to improve athletic performance.

Saliva: New and non-invasive bio-sample for DNA methylation analysis

The use of different non-invasive biospecimens in the field of epigenomics, specifically saliva, will provide new levels of insight in molecular mechanisms. Saliva, in comparison to blood and urine which are the most used diagnostic fluids in laboratory setting, stands as a lot more practical bio-sample to use. Saliva collection is easy and non-invasive, and it does not require specially trained personnel for sampling procedure.

A progress in salivary genomics has been favored by the availability of sufficient quantity of DNA in saliva, and reliable polymerase chain reaction (PCR) results. There is a number of independent studies which confirm that genome-wide DNA methylation profiles of saliva are more than 90% comparable to blood, both in adults, and younger populations[5].

Physical activity (PA) and Physical exercise (PE)

Physical activity (PA) is defined as any bodily movement produced by skeletal muscles that results in energy expenditure[6]. It is widely recognized as one of the most effective, affordable, and accessible strategies for maintaining overall health, enhancing well-being, and improving quality of life[7]. The benefits of PA extend across the lifespan, and regular engagement is recommended from early childhood through older adulthood to support physical, mental, and social health[7,8].

Despite its well-documented benefits, insufficient physical activity remains a major global public health concern. The economic burden associated with physical inactivity is substantial, with global healthcare costs estimated in the billions due to increased incidence of non-communicable diseases and related conditions[9]. These costs affect both public healthcare systems and private households, highlighting the urgent need for preventive strategies.

Physical exercise (PE) is a subcategory of physical activity that is planned, structured, repetitive, and aimed at improving or maintaining one or more components of physical fitness[6]. Promoting increased participation in PA and PE is therefore a critical public health priority. Tailored exercise programs have been shown to play a key role in the prevention and management of cardiovascular diseases, obesity, certain cancers, cognitive decline, and musculoskeletal disorders[10,11]. Consequently, encouraging active lifestyles through targeted interventions is essential for reducing disease burden and improving population health outcomes.

Basketball

Young people practice a wide range of different sports. Regarding the interest shown by young people, basketball can be considered one of the most popular. Basketball is an organized sport that involves high technical, tactical and physical demands performed in a very short period of time[12]. Playing basketball in a club increases the likelihood of achieving the PA recommendations in children and adolescents. A bulk of research agrees that basketball requires an important anaerobic component involving high intensity demands such us sprints, jumps or changes of direction alongside aerobic demands to be able to cover the game distances and to recover from the anaerobic intermittent efforts[13-15]. Therefore, basketball conditioning should prepare the athlete in order to run faster than the opponents, to develop enough strength and balance to cope with the contacts between players, and power to perform all the high intensity actions, to jump higher and faster and to be able to perform all the mentioned aspects better than the opponent avoiding fatigue accumulation and injury risk. For this reason, physical attributes and physiological performance will be essential determinants of success in youth basketball players. Interestingly, it has been of current research interest that most of the underpinning traits that contribute to elite status are partially heritable and different genetic variants has been studied in order to find their potential influencing sports performance[16].

Current status and justification of the proposal

Based on the authors' knowledge, epigenetic modifications that occur in young basketball players after a PE intervention have not yet been studied and could provide a new and innovative approach to youth's physical conditioning, health and development.

Despite the crucial role of PA in health the impact of physical training on DNA methylation in youth in basketball settings is largely unknown. We are certain that PE could change various physiological parameters, such as hormone production or blood flow, by altering the gene

expression. Therefore, identifying precisely how, when, and to what extent, different regimens of PE alter homeostasis is an important and necessary challenge to determine the optimal dose and specificity required to maximize health benefits and reduce physical inactivity.

Moreover, a better understanding of the epigenetic changes that influence basketball performance in young people could help coaches for a better understanding of the optimal training conditions for each player based on their epigenetics. The example of basketball could be useful to implement epigenetic research in other sports in the future.

3. OBJECTIVES, METHODOLOGY AND WORK PLAN

Hypothesis

This project is based on the following hypotheses:

- A weekly 30-minute intervention consisting of either high-intensity, power-focused strength training (SST) or sprint-based training (RST) over 8 weeks will induce significant changes in genome-wide DNA methylation in saliva cells of youth basketball players.
- These interventions will also lead to measurable improvements in physical performance, including:
    - Explosive strength
    - Aerobic fitness (VO_{2max})
    - Speed
    - Agility (T-test, V-cut test)
- The DNA methylation response will be intervention-specific, with distinct:
    - Differentially methylated positions (DMPs)
    - Enriched biological pathways depending on whether participants undergo SST or RST.

Main Objective

- To investigate genome-wide DNA methylation changes in response to 8 weeks of weekly 30-minute SST or RST sessions, performed alongside regular basketball training in youth athletes.

Specific Objectives

1. Methylation and Performance Changes

- To identify changes in DNA methylation (hypermethylation and hypomethylation) at DMPs following SST and RST interventions.
- To evaluate concurrent changes in:
  - Explosive strength
  - Aerobic fitness
  - Speed
  - Agility

2. Association Analysis
   - To examine the relationships between DNA methylation changes and performance adaptations.
3. Genomic Characterization
   - To characterize the genomic context of identified DMPs, including:
     - Proximity to transcription start sites (TSS)
     - Distribution across CpG features (e.g., islands, shores, shelves)
4. Pathway and Functional Analysis
   - To perform enrichment analyses to identify biological pathways associated with SST and RST interventions.
   - To compare pathway-level responses between training modalities.

4. METHODOLOGY

Study Design

This study is designed as a randomized controlled trial with two active intervention arms, comparing the effects of strength and power-focused training (SST) and sprint-based training (RST) on genome-wide DNA methylation and physical performance in youth basketball players. The inclusion of a non-intervention control group was deliberately avoided due to ethical considerations, as withholding a potentially beneficial training stimulus during the competitive season would not align with best practices in youth athlete development. Instead, both groups received structured training interventions in addition to their regular basketball practice. The study followed a parallel-group design with pre- and post-intervention assessments.

Sample Size Estimation

An a priori power analysis was conducted using G*Power (version 3.1), based on effect sizes reported in previous genome-wide DNA methylation studies in sport-related contexts[17]. Assuming

a significance level of 0.05, a statistical power of 0.80, and a two-tailed testing approach, the required sample size was estimated at 58 participants, with 29 participants per intervention group.

Participants

Participants were recruited from the youth academy of Bàsquet Girona, comprising competitive male basketball players aged between 13 and 18 years. All participants had a minimum of three years of structured training experience and were actively competing at the national level.

Following stratification by age category (U-13 to U-18), participants were randomly assigned to either the SST or RST group using a computer-generated allocation sequence. Randomization was performed by an independent researcher not involved in recruitment, and allocation was concealed until assignment.

Inclusion criteria required participants to be free from injury or illness at baseline and actively engaged in the club's training program. Exclusion criteria included the use of medication affecting study outcomes, injury during the intervention period, failure to complete at least 7 of the 8 intervention sessions, or attendance below 85% in training and competition activities.

A total of 66 participants were initially recruited. Following withdrawals due to injury or illness, the final sample consisted of 62 players (SST: n = 32; RST: n = 30).

Study Procedures

The study was conducted during the competitive season over an 8-week intervention period. Baseline assessments were performed prior to the intervention, and follow-up assessments were conducted after its completion.

All participants continued their regular training schedule, consisting of three weekly 90-minute team sessions and weekend matches. In addition, participants completed one 30-minute training session per week specific to their allocated intervention group.

To ensure consistency, all testing and training sessions were conducted at the same facility and at the same time of day (between 17:00 and 20:00). Participants were instructed to avoid strenuous physical activity in the 24 hours preceding testing and to maintain consistent dietary, hydration, and sleep habits throughout the study.

Biological Sample Collection and Processing (Primary Outcome)

The primary outcome of this study was genome-wide DNA methylation.

Saliva samples were collected using the Oragene Discover® system prior to physical testing. Participants provided approximately 1–2 mL of saliva under standardized conditions, including a

minimum fasting period of one hour to avoid contamination. Samples were stabilized immediately after collection according to manufacturer instructions to ensure DNA integrity during storage and transport.

Genomic DNA was extracted using standardized protocols, and DNA quantity and quality were assessed prior to downstream processing. Bisulfite conversion was performed to enable methylation analysis.

Genome-wide DNA methylation profiling was conducted using the Infinium HumanMethylationEPIC v2 BeadChip, which allows high-resolution quantification of methylation levels at over 900,000 CpG sites. Methylation levels were expressed as β-values, representing the proportion of methylation at each site.

Raw data were subjected to quality control, normalization, and probe filtering procedures. Differentially methylated positions (DMPs) were identified using linear regression models adjusted for age and batch effects. Statistical significance was defined using a false discovery rate (FDR) threshold of < 0.05, with additional consideration of biological relevance based on changes in methylation magnitude ($\Delta\beta > 0.10$).

Secondary Outcomes

Secondary outcomes included physical performance and anthropometric characteristics.

Anthropometric Data

Standing height, sitting height and body mass were measured pre- and post-intervention using a Portable Seca® Stadiometer 213 (Seca® 213, Germany) and a Tanita BC-545 scale (Tanita®, Japan). Sitting height was measured with participants seated on the ground against the stadiometer [18]. Maturity offset and age at PHV were calculated using the Moore Formula [19]. Following the approach from Malina et al. (2024), participants were classified as early, circa (average) or late matures based on their age at PHV. Early maturer were defined as those with age at PHV more than one SD below the sample mean, late maturer as more than 1SD above and circa maturer as within ± 1 SD. The cut-off values used for classification were < 13.35 years for early, 13.35–14.24 years for circa and > 14.24 years for late maturer, based on the sample mean age at PHV (13.80 ± 0.45 years).

Aerobic Fitness

The Yo-Yo intermittent recovery test assessed aerobic fitness following validated protocols [20]. U-13 and U-14 teams performed level 1, while U-15 to U-18 teams completed level 2. Maximal oxygen uptake (VO_{2max}) was estimated based on the distance covered during the test [20].

Explosive Strength

Lower body explosive strength was evaluated using the jump heigh on the CMJ test [21]. Measurements were taken using Chronojump® software (Version 1.6.1.0; Bosco Systems®, Barcelona, Spain). Participants completed two trials with a 2-min rest between jumps, and the best result was used for analysis.

Speed

Linear speed was assessed using the 20-m sprint test [22], with times recorded using two photocells (Witty gate, Microgate®, Bolzano, Italy). Each participant performed two trials with a 3-min rest in between, and the fastest time was used for analysis. Speed (m/s) was calculated by dividing the 20-m distance by the time taken to complete the sprint.

Agility

Agility was evaluated using two tests: The T-test [23] and the V-cut test [24]. The T-test, measured forward, lateral and backward movements with timing recorded by a single photocell (Witty gate, Microgate®, Bolzano, Italy). The V-cut test assessed change of direction ability with two photocells (Witty gate, Microgate®, Bolzano, Italy). Each participant performed two trials with a 3-min rest between them, and the fastest time was used for analysis.

Training Intervention

After the pre-test, participants were randomly assigned to the SS group (N = 32; age: 15.35 ± 1.67 years; maturity offset: 1.43 ± 1.78 years; body height: 180.41 ± 12.67 cm; body mass: 68.20 ± 13.67 kg) or the RST group (N = 30; age: 15.46 ± 1.72 years; maturity offset: 1.34 ± 1.53 years; body height: 179.59 ± 10.69 cm; body mass: 68.21 ± 14.74 kg). To ensure balanced distribution across developmental stages, randomization was stratified by age category, with participants within each age group (U-13 to U-18) randomly allocated to either intervention group. Each group then completed one 30-min session per week over an 8-week period. RST protocol consisted of 2 sets of 6 x 10 s all-out sprints with 20 s of active recovery between repetitions and a 6-min half-court shooting drill between sets [25-27]. Each sprint included four basketball-specific changes of direction to enhance neuromuscular and coordination demands (Thurlow et al., 2025). While court dimensions and work-to-rest ratios remained constant throughout the 8 weeks, change-of-direction patterns were progressively varied; one set was used in weeks 1–4, and a different set in weeks 5–8, introducing new angles and movement directions. This variation was introduced to increase movement variability and sustain neuromuscular load (Maggioni et al., 2019). The structure of the protocol remained consistent across age groups, but intensity was individually adjusted based on maximal effort to ensure that the load matched each participant's age-related capacity.

The SS protocol consisted of three sets of six basketball-specific multi-joint exercises (three paired supersets) at 30–50% 1RM, with the load adjusted to each participant's capacity through level of effort [28,29]. In every set, each player performed 5 to 6 repetitions with a weight they could lift for 14 repetitions, ensuring an individualized power-oriented stimulus [28]. The exercises were chosen to reflect basketball-specific movement patterns to enhance sport-specific strength [13]. The structure remained stable throughout the 8 weeks, targeting fight actions (push-pull), acceleration (frontal and lateral) and jumping (bilateral and unilateral). However, the specific exercises changed mid-intervention; one exercise program was used in weeks 1–4 and a different one in weeks 5–8, while maintaining the same movement goals (e.g. e.g., push to pull, front to side acceleration, unilateral to bilateral jumping). While the exercises remained consistent across all participants, the intensity was adjusted by modifying the weight according to the age-specific capacity of each group. Incomplete rest periods (60 s) were used to increase metabolic stress and overall training load [30].

Statistical Analysis

Changes in DNA methylation were calculated as the difference between post- and pre-intervention β-values. Performance changes were expressed as percentage differences between time points. Associations between methylation changes and performance adaptations were assessed using Spearman's rank correlation coefficients. Multiple testing was controlled using the Benjamini–Hochberg false discovery rate procedure, with statistical significance set at FDR-adjusted $p < 0.05$. Genomic annotation of DMPs was conducted to determine their location relative to transcription start sites and CpG regions. Functional interpretation was further explored באמצעות pathway enrichment analysis using Metascape, integrating multiple biological databases to identify significantly enriched pathways associated with each intervention.

COVID-19 measures:

The measures that will be applied are in accordance with current regulations that currently apply to Girona Basketball and will be adapted at all times to the changes that may occur depending on the evolution of the pandemic.

1. Study subjects will wear the mask at all times.

2. Study subjects will disinfect their hands when entering and leaving. Alcoholic disinfectant solutions are available at the entrance.

3. Each player will be given an appointment to collect the data. Punctuality will be requested to avoid the accumulation of players in the data collection area. In the event of crowds, participants will be asked to wait outside and will be advised when they can enter.

4. After each participant, the spaces and the material used will be cleaned and disinfected.

5. Regarding saliva samples, in no case will the researchers or any other study participant be in direct contact with the collected saliva. It will be the participant himself who will self-collect the sample and store it in sample storage boxes following the instructions provided by the researcher.

6. The samples will be processed in the laboratory following the appropriate biosafety standards for working with potentially infectious samples.

5. LIMITATIONS AND CONTINGENCY PLAN

There are other epigenetic marks that will not be studied in this project, such as histone acetylation and the expression profile of miRNAs (as they are beyond the scope of the present proposal). Using saliva, instead of skeletal muscle tissue is the second limitation, but performing skeletal muscle biopsies in youth will never be an option because of ethical reasons. However, the current proposal

goal is to identify epigenetic marks and core proteins from an easily available tissue to be widely used in sports club settings to monitor training adaptations.

The current proposal includes an interventional program which duration time is limited to two months. However, we are aware that chronic physical activity over a longer period of time, may have a different impact on DNA methylation. Thus, a good study design in future studies could be a long-term follow-up study on a cohort, whose changes in lifestyle related to physical activity are monitored, and whose DNA methylation levels, or protein concentrations are regularly assessed to ascertain whether DNA methylation changes are transient or stable over time in saliva.

Finally, all the youth male basketball players that take part in the study are from Basquet Girona basketball club and are healthy. However, the research team is well connected to other basketball clubs, which will ensure the possibility to include other basketball teams and female basketball players if necessary. Moreover, the sample size is small, but as it is a professional structure with a very controlled basketball sessions, this will guarantee the homogeneity of the results.

As it is well known, COVID-19 situation is also a limitation when collecting saliva samples in human population. However, young basketball players from this professional structure undertake the training sessions under a well supervised condition. If necessary, we can assess subgroups during the duration of the proposal to ensure data collection.

6. ETHICAL CONSIDERATIONS

The study protocols are approved by the Research Ethics Committee of the Dr. Josep Trueta hospital in Girona (basketball players: 2020.193). Before participating, all players, as well as their parents, teachers and coaches, will be carefully informed about the potential risks and benefits of the project, and will be asked to read and sign the approved informed consent document. The informed consent will be signed by all the study subjects and the families in case of underage players. The participation in this study is completely voluntary without any repercussions with any professional from Basketball Girona or University of Girona. The research will be conducted in accordance with the 1974 Helsinki Declaration for Human Research (last modified 2014, Hellman, Verdi, Schlemper, Caponi). All personal data and information collected or generated during the study will be protected in accordance with current legislation which includes Organic Law 3/2018, of December 5, on the Protection of Personal Data and guarantee of digital rights (LOPDGDD) and General Regulation (EU) 2016/679, of April 27, 2016, on data protection (RGPD).

The results of this study will be used for scientific purposes. To maintain confidentiality, the results and samples will be identified by a code, and only the principal investigator will be able to identify each sample or data. Therefore, the access to personal information will be restricted to the study principal investigator and the Clinical Research Ethics Committee, when required to verify the data and procedures of the study, but always maintaining confidentiality in accordance with current legislation. All data and samples will be kept until the study is considered completed, and will be eliminated according to Biomedical Research law 12/2007 of July 3.

Authorization is available by the researcher team of the Basquet Girona to use its facilities for monitoring and obtaining necessary data for the study.

7. CHRONOGRAM

TASKS	YEAR	MONTH											
		1	2	3	4	5	6	7	8	9	10	11	12
Sample Selection and inclusion.													
	2022												
	2023												
	2024												
	2025												
Measuring and Assessment procedures (pre-intervention).													
	2022												
	2023												
	2024												
	2025												
Collecting Biological Samples.													
	2022												
	2023												
	2024												
	2025												
Intervention.													
	2022												
	2023												
	2024												

	2025												
Measuring and Assessment procedures (post-intervention).													
	2022												
	2023												
	2024												
	2025												
DNA methylation array and selection of differentially methylated genes													
	2022												
	2023												
	2024												
	2025												
Validation of differentially methylated by pyrosequencing.													
	2022												
	2023												
	2024												
	2025												
Core proetin laboratory Analyses.													
	2022												
	2023												
	2024												
	2025												
Database maintenance, statistical analysis and dissemination of results.													
	2022												
	2023												
	2024												
	2025												

8. BUDGET:

GOODS AND SERVICES:

Pack of 50 GFX Saliva Collector	1.693,05 €	60 players will be assessed before and after the intervention, thus we will collect 120 saliva samples. Isohelix sell the collection kids (ref: GFX-02/50) in packs of 50. We will need 3

		packs at 564,35€ each pack. 564,35*3=1.693,05
DNA extraction kit from isohelix	1.085,37 €	We will need to extract DNA from 120 samples (pre and posttest). The extraction kit is for 50 samples, then we will need 3 kits at 361,79€ = 1.085,37€
EZ DNA Methylation-Gold Kit (Ref. D5006)	2.033,7 €	Extracted DNA must be bisfultitated in order to perform methylation analysis. We will have to bisulfite 500 samples (250 pre and 250 post; 190 from schooled children study and 60 from basketball study) and the kit is for 200 samples, then, we will need 3 kits at 677,9. In total 2.033,7€
Laboratory consumables	1.500 €	To extract DNA and perform PCR we will need tips, polypropylene tubes, etc.
ELISA quantification	24.000 €	Approximately 5 core proteins are expected to be identified. Thus, we will need to by 5 different ELISA kits to assess those 5 core proteins. All saliva samples (n=500) will be assessed, thus we will need 6 kits for each core protein. In total we will need a 30 ELISA kits. The approximate price for each kit is about 800€. 30 ELISA kits * 800€ = 24.000.
Pyrosequencing servicie	11.495 €	Pyrosequencing analysis will be outsourced (Servei Anàlisis d'ADN, Instituto de Biomedicina de Valencia-CSIC). The price for the methylation service costs 11.495€.
PCR Amplification analysis and electrophoresis gel	16.405 €	DNA will be processed at UdG laboratories before pyrosequencing. Thus, we will need to perform PCRs for a total of (15.345,092€): This price includes: 1) dNTP Set, PCR Grade, 4 x 100ul, 250 mM each_0,16ul per mostra Werfen Qiagen Ref. 509201913, 2) Hot Star Taq Polymerase 1000U Wefen Qiagen, Ref. 509203205; 3) MicroAmp Optical 96-well Reaction Plate Thermofisher Scientific Ref.

		N8010560; 4) MicroAmp™ Optical Adhesive Film Thermofisher Scientific ref. 4360954 and 5) primers from Condalab. All PCR products must be checked for integrity before pyrosequencing. This will cost 1.059,94, which includes: 1) UltraPure Agarose Thermofisher Scientific ref: 16500100; 2) TBE Buffer 10X Thermofisher Scientific 1 l; 3) Midori Green Advance 1ml Cultek ref. B4MG04 and 4) 100 bp DNA Ladder - 50 ug Werfen - New england biolabs ref: 174N3231S TOTAL 16.405
Array metilación WG-317-1002 Infinium MethylationEPIC BeadChip Kit (32 samples) and metylation service fees	32.588 €	We will perform the analysis of 850K in 32 samples, 8 from each group (See methods). The Infinium MethylationEPIC BeadChip Kit array for 32 samples (ref. WG-317-1002) costs 7.808€. The price for the methylation service at Epigenomic Unit, Instituto de Investigación Sanitaria la Fe, Valencia, Spain costs 24.780€.

TRAVEL EXPENSES:

AEI meetings	500€	In case we are receiving the requested findings it is expected to attend to some AEI meetings. Thus, travel expenses are estimated to be 500€.
National and international meetings	9.600€	Conferences expenses: 3 people at a national conference will cost 600€ per researcher: 3 researchers x 600€ x 2 years= 3.600€. And 3 people at a European conference (ECSS) will cost 1.000€ per researcher: 3 researchers X 1.000€ x 2 years= 6.000€): This will cover for travel, maintenance, registration and accommodation expenses for the principal investigator and at least one predoctoral student.

OTHER

Audit	1.200 €	

Translation and publication expenses	12.000 €	The publication of at least 6 papers in high impact journals is expected (open access): 6 publications x 2.000€=12.000€
Sending samples	1.000 €	Bisulfited DNA must be sent in optimal conditions (dry ice) to the external laboratory for methylation arrays and pyrosequencing.

* Outsourcing service fees are subject to annual reviews

It will be material used in the study for which no funding will not be requested:
Laboratory equipment: centrifuges, refrigerators, freezers, real-time PCR, spectrophotometer, etc.: will be provided by the research laboratory at the UdG for the development of the project. All necessary equipment to collect data from young basketball player will be provided by Basket Girona.

This project will be funded by Grant PID2021-124162OA-I00 funded by MCIN/AEI/ 10.13039/501100011033 and by "ERDF A way of making Europe", by the "European Union".

SUMMARY OF THE REQUESTED BUDGET

Concept		Cost €
Direct costs	Personnel	0€
	Travel expenses	10.100€
	Other expenses	11.200€
	Inventory acquisition	0€
	Inventory rental	0€
	Inventory maintenance	0€
	Goods and services	90.800,12
Total		112.100,12€

9. PREVIOUS RESULTS FROM OUR RESEARCH TEAM RELATED TO THE PROPOSAL

Dr. Anna Prats Puig have performed two postdoctoral stages to gain knowledge on epigenetics: one in the Montpelier Institute of Molecular Genetics (IGMM, CNRS) in France and at the University of Cambridge (United Kingdom). For this Cambridge stay, the Ministry of Education, Culture and Sports granted her with a José Castillejo grant (2014). The papers published from these stays and related to methylation assessment by pyrosequencing are: 1) Methylation of the

C19MC microRNA locus in the placenta: association with maternal and chilhood body size. **Prats-Puig A,** Xargay-Torrent S, Carreras-Badosa G, Mas-Parés B, Bassols J, Petry CJ, Girardot M, D E Zegher F, Ibáñez L, Dunger DB, Feil R, López-Bermejo A. Int J Obes (Lond). 2020 Jan;44(1):13-22. doi: 10.1038/s41366-019-0450-9. Epub 2019 Sep 25. PMID: 31554916 AND 2) The placental imprinted DLK1-DIO3 domain: a new link to prenatal and postnatal growth in humans. **Prats-Puig A**, Carreras-Badosa G, Bassols J, Cavelier P, Magret A, Sabench C, de Zegher F, Ibáñez L, Feil R, López-Bermejo A. Am J Obstet Gynecol. 2017 Sep;217(3):350.e1-350.e13. doi: 10.1016/j.ajog.2017.05.002. Epub 2017 May 11. PMID: 28502757

Related to the Infinium® MethylationEPIC BeadChips (Illumina), Dr Prats Puig, together with Dr. Raquel Font Lladó, Dr. Sergi García Retortillo and Fidanka Vasileva they work together in the analysis and interpretation of the array data to publish the paper entitled: DNA Methylation Reorganization of Skeletal Muscle-Specific Genes in Response to Gestational Obesity**. Prats-Puig A, García-Retortillo S,** Puig-Parnau M, **Vasileva F, Font-Lladó R**, Xargay-Torrent S, Carreras-Badosa G, Mas-Parés B, Bassols J, López-Bermejo A. Front Physiol. 2020 Jul 31;11:938. doi: 10.3389/fphys.2020.00938. eCollection 2020. PMID: 32848869

Dr. Prats-Puig, Dr. Font-Lladó and Dr. Barretina have previously work together on human cohort studies as can be seen on the following publication: The Aging Imageomics Study: rationale, design and baseline characteristics of the study population Josep Puig, Carles Biarnes, Salvador Pedraza, Joan C Vilanova, Reinald Pamplona, José Manuel Fernández-Real, Ramon Brugada, Rafel Ramos, Gabriel Coll-de-Tuero, Laia Calvo-Perxas, Joaquin Serena, Lluís Ramió-Torrentà, Jordi Gich, Lluis Gallart, Manel Portero-Otin, Angel Alberich-Bayarri, Ana Jimenez-Pastor, Eduardo Camacho-Ramos, Jordi Mayneris-Perxachs, Victor Pineda, **Raquel Font-Lladó**, **Anna Prats-Puig**, […] **Jordi Barretina**, Josep Garre-Olmo. Mech Ageing Dev . 2020 Jul;189:111257. doi: 10.1016/j.mad.2020.111257. Epub 2020 May 11.

Finally, we have all worked together with Jesús Escosa, Arnau Sacot, Ana Jódar and Dr. Julio Calleja González to write the young basketball players' proposal to get the approval from the Research Ethics Committee of the Dr. Josep Trueta hospital in Girona (which was approved in 2020; ref. 2020.193). What is ore, the following systematic revision is already under revision at The Journal of Sports Science: "Multidisciplinary neuromuscular and endurance interventions on youth basketball players: A systematic review with meta-analysis and meta-regression". **Arnau Sacot, Víctor López-Ros, Anna Prats-Puig, Jesús Escosa, Raquel Font-Lladó, Anna Jódar,**

Jordi Barretina, Fidanka Vasileva & Julio Calleja-González. Importantly, Dr. Prats Puig together with Dr. Víctor López Ros and Dr. Julio Calleja González are mentoring Arnau Sacot's doctoral thesis. And the IP of this grant is also the mentor of Fidanka Vasileva for her PhD. Thus, the present proposal will help them to improve the results of their doctoral thesis, otherwise they will perform gene directed analysis.

10. SCIENTIFIC-TECHNICAL IMPACT

Once protected intellectual property, the information obtained with the project will be disseminated. Dissemination plans include wide collection of activities, ensuring the visibility of the project and its impact on the enhancement of the state-of-the-art in the field: Participation in joint events organized on European levels, scientific journals, conference and workshop articles, publications of results in specialized technical press and production of publicity material. Given the importance of the proposed research, a high-level impact is expected for the results. Furthermore, the identification of new therapeutic targets and the development of patents and commercialization of new diagnostic tests can also be expected.

The members of the project are well located in the international scientific community in their disciplines. Active members are of different professional associations such as the European College of Sports Sciences (ECSS), ASEPREB and EPCA.

In addition, they are members of editorial boards or as associated publishers of different Top scientific journals in the field of nutrition and / or physiology such as Nutrients, Biology, Frontiers in Endocrinology and International Journal of Environmental Research and Public Health.

The results will also be disseminated with the scientific community with doctors / policy makers, genetic industry, coaches, sport scientist, among others, trough different channels such as through agreements with administration and professional associations. The messages and communication tools will be developed and identified carefully to effectively achieve those groups.

The technological benefits may involve: 1) To identify the conditional and physical performance of young basketball players and the changes associated with the intervention; 2) A possible identification of epigenetic modifications through specific training with different physical and conditional qualities; 3) To describe the degree of individual response to training in order to adapt the training conditions to each individual and to be able to offer the best options to develop certain abilities and physical qualities for a better overall development in youth; 4) To provide an innovative and practical point of view to coaches, sport scientists and strength and conditioning

coaches on the epigenetic characteristics of young basketball players; and 5) to identify core proteins in saliva that may be useful to assess physical performance in basketball players and to monitor the impact of PE intervention in each player.

11. SOCIAL AND ECONOMIC IMPACT

Based on the authors' knowledge, methylation changes that occur after a PE intervention in young basketball players have not yet been studied and could provide a new and innovative approach to youth conditioning, health, and development. With this proposal we could shed light into the individual response to a specific training program and detect how subjects respond to a training intervention. The final goal would be to reach coaches for them to adapt the PE interventions to make it optimal for players regardless of its socioeconomic background. To do this, a project web site with project documents, demonstrations, publications and all the non-confidential material produced in the project will be created. The results obtained will also be disseminated among the general society through the local media, and some of the webs on table 1.

Table 1. Potential webs to publish and disseminate our results

Webs	Dirección
ASEPREB	**https://www.asepreb.com**
SOLOBASKET	**https://www.solobasket.com/**
KIA EN ZONA	**https://kiaenzona.com**
ZONA DE BÀSQUET	**https://zonadebasquet.com**
REVISTA GIGANTES	**https://www.gigantes.com**
EN CANCHA	**www.encancha.com/**
SOBRENTRENAMIENTO	**https://g-se.com/**
FEDERACION ESPAÑOLA DE BALONCESTO	**http://www.feb.es/inicio.aspx**

12. TRAINING CAPACITY

Doctoral Theses mentored by Dr. Prats- Puig:

1) PhD Student: Helen Valenzuela Lea. Title: Influencia de la grasa epicárdica en la arritmia de fibrilación auricular, la cardioversión eléctrica, la recurrencia y el tratamiento. Year: 2019; Institution: UdG

2) PhD Student: Gemma Carreras Badosa; Title: Creixement recuperador postnatal secundari a restricció de pes prenatal en rates Wistar: canvis moleculars en l'expressió dels gens STK11, DLK1 i SIRT1 en els teixits adipós, hepàtic, muscular I placentari; Year: 2015; Institution: UdG

3) PhD Student: Pilar Soriano Rodriguez; Title: SFRP5, WNT5A y CRTC3: Nuevas adipoquinas relacionadas con la obesidad en la edad pediátrica; Year: 2015; Institution: UdG

13. REFERENCES

1. Jones PA. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nature reviews genetics*. 2012;13(7):484-492.

2. Zhang L, Lu Q, Chang C. Epigenetics in health and disease. *Epigenetics in allergy and autoimmunity*. 2020:3-55.

3. Zhao L-Y, Song J, Liu Y, Song C-X, Yi C. Mapping the epigenetic modifications of DNA and RNA. *Protein & cell*. 2020;11(11):792-808.

4. Soci UPR, Melo SFS, Gomes JLP, Silveira AC, Nóbrega C, de Oliveira EM. Exercise training and epigenetic regulation: multilevel modification and regulation of gene expression. *Exercise for Cardiovascular Disease Prevention and Treatment: From Molecular to Clinical, Part 2*. 2017:281-322.

5. Nishitani S, Parets SE, Haas BW, Smith AK. DNA methylation analysis from saliva samples for epidemiological studies. *Epigenetics*. 2018;13(4):352-362.

6. Caspersen CJ, Powell KE, Christenson GM. Physical activity, exercise, and physical fitness: definitions and distinctions for health-related research. *Public health reports*. 1985;100(2):126.

7. Organization WH. *WHO guidelines on physical activity and sedentary behaviour*. World Health Organization; 2020.

8. Janssen I, Leblanc A. Systematic Review of the Health Benefits of Physical Activity in School-Aged Children and Youth. *International Journal of Behavioural Nutrition and Physical Activity*. 2009;1:27-32.

9. Ding D, Lawson KD, Kolbe-Alexander TL, et al. The economic burden of physical inactivity: a global analysis of major non-communicable diseases. *The lancet*. 2016;388(10051):1311-1324.

10. Booth FW, Roberts CK, Laye MJ. Lack of exercise is a major cause of chronic diseases. *Comprehensive physiology*. 2011;2(2):1143-1211.

11. Warburton DE, Bredin SS. Health benefits of physical activity: a systematic review of current systematic reviews. *Current opinion in cardiology*. 2017;32(5):541-556.

12. Petway AJ, Freitas TT, Calleja-González J, Medina Leal D, Alcaraz PE. Training load and match-play demands in basketball based on competition level: A systematic review. *PLoS One*. 2020;15(3):e0229212.

13. Schelling X, Torres-Ronda L. An integrative approach to strength and neuromuscular power training for basketball. *Strength & Conditioning Journal*. 2016;38(3):72-80.

14. Stojanovic E, Stojiljkovic N, Scanlan AT, Dalbo VJ, Berkelmans DM, Milanovic Z. The Activity Demands and Physiological Responses Encountered During Basketball Match-Play: A Systematic Review. *Sports Medicine*. 2018;48(1):111-135.

15. Narazaki K, Berg K, Stergiou N, Chen B. Physiological demands of competitive basketball. *Scandinavian journal of medicine & science in sports*. 2009;19(3):425-432.

16. Pickering C, Kiely J, Grgic J, Lucia A, Del Coso J. Can Genetic Testing Identify Talent for Sport? *Genes*. 2019;10(12):972.

17. Pilotto A, Mazzolari R, Turner D, et al. Human skeletal muscle possesses an epigenetic memory of high intensity interval training affecting mitochondrial function. *Physiology*. 2023;38(S1):5734999.

18. Massard T, Fransen J, Duffield R, Wignell T, Lovell R. Comparison of sitting height protocols used for the prediction of somatic maturation. *SPSR*. 2019;66(1):1-4.

19. Moore SA, McKay HA, Macdonald H, et al. Enhancing a somatic maturity prediction model. *Medicine & Science in Sports & Exercise*. 2015;47(8):1755-64.

20. Krustrup P, Mohr M, Amstrup T, et al. The yo-yo intermittent recovery test: physiological response, reliability, and validity. *Medicine & Science in Sports & Exercise*. 2003;35(4):697-705.

21. Bosco C, Luhtanen P, Komi PV. A simple method for measurement of mechanical power in jumping. *European Journal of Applied Physiology and Occupational Physiology*. 1983;50(2):273-282.

22. Gottlieb R, Shalom A, Calleja-González J. Physiology of Basketball–Field Tests. Review Article. *Journal of Human Kinetics*. 2021;77(1):159-167.

23. Semenick D. Tests and measurements: The T-test. *Strength & Conditioning Journal*. 1990;12(1):36-37.

24. Gonzalo-Skok O, Tous-Fajardo J, Suarez-Arrones L, Arjol-Serrano J, Casajus J, Mendez-Villanueva A. Validity of the V-cut test for young basketball players. *International Journal of Sports Medicine*. 2015;94(11):893-899.

25. Buchheit M, Laursen PB. High-intensity interval training, solutions to the programming puzzle. *Sports Medicine*. 2013;43(5):313-338.

26. Maggioni MA, Bonato M, Stahn A, et al. Effects of ball drills and repeated-sprint-ability training in basketball players. *International Journal of Sports Physiology and Performance*. 2019;14(6):757-764.

27. Thurlow F, McLaren SJ, Townshend A, Weakley J. The Application of Repeated-Sprint Training. *Strength & Conditioning Journal*. 2025:10.1519.

28. Hernández-Belmonte A, Courel-Ibáñez J, Conesa-Ros E, Martínez-Cava A, García-Pallarés J. Level of Effort: A Reliable and Practical Alternative to the Velocity-Based Approach for Monitoring Resistance Training. *Journal of Strength and Conditioning Research*. 2022;(36 11):2992-2999.

29. Rodríguez-Rosell D, Yáñez-García JM, Torres-Torrelo J, Mora-Custodio R, Marques MC, González-Badillo JJ. Effort index as a novel variable for monitoring the level of effort during resistance exercises. *The Journal of Strength & Conditioning Research*. 2018;32(8):2139-2153.

30. Realzola RA, Mang ZA, Millender DJ, et al. Metabolic profile of reciprocal supersets in young, recreationally active women and men. *The Journal of Strength & Conditioning Research*. 2022;36(10):2709-2716.