Academic Clinical Trials Call. Proposal Application Form

Application Number: ICI21/00005

Project Title: Effects of half-dose spiomet treatment in girls with early puberty and accelerated bone maturation: a multi-centre, randomised, placebo-controlled study

Name of the Project Leader: Abel López Bermejo

Type of research project: ○ INDIVIDUAL ◉ MULTI-CENTRE

PROJECT ABSTRACT

Summary of the project's objectives. The potential impact, healthcare aspects and the scientific and social policies of the Strategic Action for Health as stated in this call. (Max. 1/2 page)

Background: a "mismatch" sequence of less prenatal weight gain and more postnatal weight gain may lead to ectopic lipid accumulation, trigger the development of early adrenarche/pubarche and the activation of the gonadotropic axis resulting in early puberty and ending up in full-blown adolescent polycystic ovary syndrome (PCOS). **Hypothesis:** the reduction of ectopic adiposity with Half-Dose (HD)-spiomet (spironolactone 25mg + pioglitazone 3.75mg + metformin 425mg) will slow down the process of accelerated maturation and pubertal *tempo*. **Objectives**: to ascertain whether a low-dose combination of generics that collectively reduce ectopic fat through different pathways (HD-spiomet), can slow down the accelerated maturation in "mismatch" girls with early puberty. **Study Design & Methods**: randomised, placebo-controlled, multi-centre, phase 2a, study (n=56 girls; power: 0.90; alpha: 0.05; **https://clincalc.com/stats/samplesize.aspx**) from two tertiary centres. Pharmacological intervention will be with HD-spiomet (a half-dose version of SPIOMET, a combination that reverts the PCOS phenotype in "mismatch" adolescents). HD-spiomet will contain spironolactone (25 mg/d, to raise brown adipose tissue activity), pioglitazone (3.75 mg/d, to raise adiponectin and insulin sensitivity), and metformin (425 mg/d, to raise AMPK activity and GDF15). *Inclusion criteria*: girls; age, 8.0-9.0 yr; birthweight (BW) for gestational age in lower tertile (Z-score <-0.44), and body mass index (BMI) in upper tertile (Z-score >0.44); early progressive puberty (Tanner B2 at 8.0-9.0 yr). *Exclusion criteria*: obesity (BMI Z-score >2.00 for age); evidence for a pathological cause of rapid maturation. Recruitment: 1 yr; double-blind treatment: 1 yr; open follow-up: 1 yr; analyses and reporting: 1 yr. Interventions: randomisation (1:1) for placebo *vs* HD-spiomet. *Primary outcome*: bone age advancement (0-1 yr) by BoneXpert; secondary outcomes: insulin, IGF-I, high-molecular-weight adiponectin (HMW-adip), SHBG, usCRP, androgens, LH, FSH, oestradiol, GDF15, CXCL14, safety parameters, quantification of hepato-visceral fat (MRI). *Analyses*: analysis of quantitative variables of independent groups, registered in the SPSS program (version 23) will be performed by ANOVA for repeated measurements in models adjusted for potential confounding variables. A statistical analysis will be done by intention to treat and another with the patients who complete the follow-up. P <0.05 will be considered statistically significant. **Implications**: pubertal modulation will delay age at menarche and epiphyseal closure, improve the metabolic profile and potentially reduce the comorbidities associated with early menarche, and thus, the derived economic burden. The proposed study falls into the **Strategic Action for Health policies 2021**, 1) thematic priorities: clinical and translational research based on evidence of scientific and technological knowledge; 2) priority lines of research: a) translational and clinical research on human health: sexual and reproductive health; b) research on medicines and health products: non-commercial clinical research: independent clinical trials in general and, in particular, in orphan drugs, and in the paediatric population.

KEYWORDS

Please indicate between seven and ten keywords that represent the scientific content of the study (medical domain, disease, etc), approaches (genetics, pathophysiology, diagnostics, etc.), tools (animal models, omics, etc.)

Prenatal weight gain, postnatal weight gain, early puberty, early menarche, PCOS, ectopic fat, bone maturation, spironolactone, pioglitazone, metformin

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INDEPENDENT CLINICAL TRIALS APPLICATION FORM
PROJECT ABSTRACT AND EXPECTED IMPACT

(PLEASE COMPLETE THIS ITEM IN SPANISH)

Please summarize the project and expected results in the population subject of the study, using a non-scientific language understandable to the general public. Describe:

- **General characteristics of the project, specially those related to relevance and vulnerability of the problem approached.**
- **Expected impact of the project's results, in terms of improve capacity in healthcare procedures towards an improvement of patients' health and life quality.**
- **Dissemination plan targeted to general population, planned actions aimed at segments of the population with special interest/ vulnerability within the area of the study.** (Max. 1 page)

Para mantener buena salud metabólica, debe existir un equilibrio entre ganancia de peso prenatal y ganancia de peso después del nacimiento. Las personas que nacen con poco peso para las semanas de embarazo tienen menos tejido adiposo subcutáneo, que es donde se pueden almacenar de forma saludable las grasas de la alimentación. Si este individuo después del nacimiento gana peso de forma moderada, mantendrá buena salud metabólica, porque podrá almacenar estas grasas. Sin embargo, si gana rápidamente peso, no tendrá suficiente capacidad de almacenamiento, y las grasas se depositarán en las vísceras, y sobre todo en el hígado, de forma "ectópica" (obesidad central). Esta secuencia favorece el desarrollo de resistencia a la insulina (más insulina en sangre), y determina que proteínas protectoras de la salud cardiovascular como la adiponectina, se secreten menos. La resistencia a la insulina y la obesidad central favorecen la maduración ósea, y aceleran la adrenarquia, que es el aumento de la síntesis de andrógenos en la glándula suprarrenal, sobre todo en niñas. La adrenarquia temprana puede manifestarse como pubarquia precoz (vello en el pubis y/o axilas) antes de los 8 años. Si la ganancia de peso persiste, puede aumentar la síntesis de las hormonas del ovario, y desarrollarse pubertad adelantada (desarrollo de mamas 8-9 años). En la adolescencia, estas pacientes tienen más riesgo de padecer síndrome del ovario poliquístico (SOPQ), con trastornos de las reglas, aumento del vello corporal y exceso de andrógenos en el ovario. Estudios previos muestran que el tratamiento con metformina (un fármaco que mejora la función de la insulina) en niñas con bajo peso al nacimiento que desarrollan pubarquia precoz y pubertad adelantada reduce la insulina en sangre y la grasa hepática y visceral, y desacelera la maduración ósea ralentizando la pubertad, retrasando la regla y aumentando la talla final. En adolescentes con SOPQ, la combinación a dosis bajas de espironolactona (un activador del tejido adiposo marrón que regula el metabolismo), pioglitazona (otro fármaco que mejora la función de la insulina, y aumenta la adiponectina), y metformina, regula la menstruación, normaliza los andrógenos y la insulina, y disminuye más la grasa hepática y visceral que la metformina sola, y en menos tiempo.

Objetivo: determinar si el tratamiento con espironolactona, pioglitazona y metformina en un comprimido único -a mitad de dosis de las utilizadas en adolescentes con SOPQ [Half-Dose (HD)-spiomet] reduce la grasa hepática y visceral, y normaliza los valores hormonales y la maduración acelerada en niñas con pubertad adelantada.

Metodología: Se incluirán 56 niñas entre 8-9 años con pubertad adelantada progresiva (desarrollo mamario bilateral), con peso al nacer para la edad gestacional en el tercil inferior (<-0,44 DE), e índice de masa corporal (IMC) actual en el tercil superior (>0,44 DE). Se excluirán pacientes con obesidad (IMC > 2 DE) o con patologías específicas. Las candidatas se distribuirán aleatoriamente en dos grupos. A la mitad se les administrará un comprimido de HD-spiomet (25 mg de espironolactona, 3,75 mg de pioglitazona, y 425 mg de metformina) diario durante 1 año. La otra mitad recibirá durante el mismo periodo, un comprimido idéntico (placebo) que sólo contendrá lactosa (azúcar). Los comprimidos serán codificados con números para que ni la paciente ni el médico que la trata sepan de qué producto se trata ("doble ciego"). A los 6 meses y al año, se comprobará si las pacientes que reciben HD-spiomet mejoran más que las que reciben placebo. Se continuará el control hasta 1 año después de finalizado el tratamiento, sabiendo cuál se ha administrado. La variable principal (que dirá si el tratamiento ha sido efectivo) será el avance de la maduración ósea (0-1 año) medida por un método automático (BoneXpert); las variables secundarias (complementarias), que se determinarán a los 0, 6 meses, 1 y 2 años serán: estadio puberal (Tanner); insulina, IGF-I, adiponectina de alto peso molecular (HMW-adip), andrógenos, PCRus, estrógenos, LH, FSH, GDF15, CXCL14, perfil lipídico, parámetros de seguridad [función hepática/renal, glóbulos rojos y blancos, vitamina B12, ácido fólico, ionograma, hormona estimulante de la tiroides (TSH)], medición de grasa hepática/visceral (resonancia magnética abdominal).

Beneficios esperados: el tratamiento con HD-spiomet retrasará la progresión de la pubertad y el cierre de los cartílagos de crecimiento, y se acompañará de mejor perfil metabólico. Potencialmente, reducirá las comorbilidades asociadas al desarrollo precoz (problemas conductuales y de ajuste psicosocial, mayor riesgo para desarrollar diabetes gestacional, diabetes tipo 2 y cáncer de mama y de endometrio). Dada la carga sociosanitaria que supone el tratamiento de estas comorbilidades por su cronicidad y coste económico, su prevención es fundamental.

Diseminación: se prevé la difusión de los resultados en cursos de actualización y guías clínicas dirigidas a pediatras de atención primaria y médicos de familia, así como a las asociaciones de alumnos y padres de primaria y secundaria de escuelas a nivel local y estatal.

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BACKGROUND

Previous results in clinical research in the project area

Max. 3 pages (15,700 characters)

Puberty underlines an important physical and psychosocial period of life where an individual develops secondary sexual characteristics and attains reproductive capacity. Puberty is signalled by the reactivation of the hypothalamic-pituitary-gonadal (HHG) axis, which triggers the release of kisspeptin and the onset of pulsatile hypothalamic gonadotrophin-releasing hormone (GnRH) secretion, which in turn drives pituitary gonadotrophin synthesis and downstream gonadal steroid secretion (1). The precise mechanism triggering the reactivation of the HHG axis is not fully known; however, GnRH neurosecretory activity and thus pubertal timing appear to be partly controlled by complex neuroendocrine pathways gathering genetic, nutritional, hormonal, metabolic and environmental signals.

Adiposity and pubertal timing

Over the past decades, there has been a worldwide trend towards younger ages of pubertal onset and menarche in girls (2,3). Nowadays, mean age at puberty start is set up below age 10yr, representing and advancement of almost three months per decade from 1977 to 2013 (2). In contemporary societies, the worldwide rise in childhood overweight/obesity appears to play a key role in the global decrease of the age at puberty.

The pivotal role of nutrition and adiposity on pubertal timing has been known since nearly five decades ago, where Frisch and Revelle framed the ``critical weight hypothesis'' as a key determinant of pubertal start in girls (4). Several cross-sectional and longitudinal studies have linked childhood adiposity with earlier pubertal onset, especially in girls (5,6). In some populations, the trend towards early puberty may be even more pronounced, in parallel with rapid gains of body weight (7), and has a sexual dimorphism, being more noticeable in girls (8). This important rise cannot be explained by other environmental factors such as exposures to endocrine disruptors (9). Also, a sudden weight gain over a short period of time associated to the lockdown for the coronavirus pandemic has been shown to associate to an increased incidence of precocious and accelerated puberty in Italian girls (10). The concept that fat mass in childhood is linked to pubertal timing has recently been endorsed by longitudinal data from >2000 English girls, showing that more fat mass in childhood is followed by an earlier pubertal growth spurt and earlier pubertal completion (11). Also, recent genome-wide association studies (GWAS) in humans have identified body mass index (BMI)-increasing alleles that associate with earlier age at menarche, pointing toward genetic co-regulation (12). In addition, Mendelian randomisation studies support a causal effect of increasing childhood BMI on the risk of early menarche (<12yrs) (13). Despite these strong epidemiologic and genetic links, the precise mechanism(s) underlying obesity-related early pubertal onset have remained elusive until the discovery of kisspeptin almost two decades ago, connecting the metabolic cues derived from adipose tissue and the regulation of GnRH secretion. It was shown that leptin, which has a permissive role in puberty onset, is able to up-regulate kisspeptin secretion in the hypothalamus, which in turn regulates the pulsatile secretion of GnRH (14). Recently, a central ceramide signalling pathway has been unveiled as a novel mediator of obesity-induced early puberty in female rats (15). Indeed, reduced signalling by ceramidase and also by AMP-activated protein kinase (AMPK) in the hypothalamus appears to link energy status and puberty-reproduction (15,16). Adiponectin -an adipokine with insulin sensitizing and cardiovascular protective properties- signals through its own transmembrane receptors to raise intracellular ceramidase activity, preventing the accumulation of unfavourable ceramide, for example, in the hypothalamus and in the liver (17).

Early puberty as an adaptive response to ectopic fat accumulation in "mismatch" girls

Earlier/faster maturation in girls has been hypothesised to be the clinical expression of an adaptive mechanism through which girls attempt to escape from ectopic lipid accumulation. This accumulation in turn, results from a mismatch between reduced prenatal weight gain (with reduced subcutaneous adipogenesis, and thus with a reduced capacity for safe lipid storage), and augmented postnatal weight gain (with augmented lipogenesis, and thus, an augmented need for lipid storage) (18). Such a mismatch may lead to ectopic lipid accumulation, particularly in the liver and viscera (central obesity), the degree of which may be also influenced by epi(genetic) factors (19). The endocrine expression of this mismatch tends to be the early development of insulin resistance, whereas its cardiovascular reflection is often a trend towards higher blood pressure starting in early childhood (18,20-25). There are close associations in childhood between the aforementioned mismatch and central fat and also between the mismatch and insulin resistance -as judged by homeostasis model assessment insulin resistance (HOMA-IR)- and between central fat and insulin resistance (24).

In prepubertal girls, the responses to central obesity include also a decrease in circulating sex hormone-binding globulin (SHBG) and adiponectin, which may be followed by an early and amplified adrenarche, with high levels of its

marker, dehydroepiandrosterone-sulphate (DHEAS), and by the appearance of pubic (pubarche) and/or axillary hair, acne and pubertal odour before age 8yr (26-28). These responses can be viewed as being adaptive since they result in accelerations of body growth and maturation that most likely represent a coordinated feedback mechanism to counteract ectopic adiposity (18). If the ectopic lipid accumulation continues, then girls may develop another acceleration of growth and maturation by activating their gonadotropic axis, conceivably again in a homeostatic attempt to escape from central adiposity. The reduced adiponectin concentrations may favour a reduced intracellular ceramidase activity and thus ceramide accumulation in the hypothalamus and liver triggering pubertal onset (15). These novel insights may largely explain the worldwide trends towards younger ages at puberty start and menarche in girls (2,3,5,11), which are known to associate to higher levels of delinquent and aggressive behaviour and to more susceptibility to negative peer influences (29), and also to future risk of gestational diabetes (30); type 2 diabetes (31), and breast and endometrial cancer (32).

In girls with early puberty, the presence of a mismatch can be easily estimated by calculating the upward change in Z-score (or centile) between birthweight-for-gestational-age and BMI at onset of puberty. This “mismatch” hypothesis has now been tested in a cohort of girls with isolated variants of central precocious puberty from a single centre in Paris (33), the majority of whom were found to have experienced an upward mismatch between prenatal and postnatal weight gain (34).

In the first years after menarche, when adult height is almost attained, the compensatory effect of body growth on central fat accumulation is lost. If the energy balance remains chronically positive, the underpinning drive of ectopic adiposity will also remain, and the endocrine-metabolic responses to this drive (insulin resistance, low adiponectin and SHBG) will persist, and potentially result in a full-blown phenotype of adolescent polycystic ovary syndrome (PCOS) including luteinizing hormone (LH) hypersecretion which in turn, can drive ovarian androgen excess and oligo-anovulation (16).

Reduction of ectopic fat in “mismatch” girls with accelerated maturation and in adolescents with PCOS: pilot studies

Previous pilot studies performed by our group in rapidly maturing mismatch girls with precocious pubarche and/or early puberty have disclosed that metformin in monotherapy over a period of 3-4yr (up to 850 mg/d), can reduce central adiposity in viscera and liver (35-37), slow down bone maturation (38), delay pubertal onset (39), and decelerate the progression of puberty to menarche (40,41) and to adolescent PCOS (42), while augmenting the gain of body height (40,41). In mismatch adolescents with PCOS, a low-dose combination of spironolactone (50mg), pioglitazone (7.5mg) and metformin (850mg) in three separate tablets (SPIOMET) was recently shown to be capable of reversing the entire PCOS phenotype after only one year of treatment, including menstrual irregularities, hyperandrogenaemia and insulin resistance, by decreasing hepato-visceral fat excess (43-46). Here, we propose to conduct a randomised, placebo-controlled, multi-centre study using only half of the SPIOMET tablet, i.e., with Half-Dose (HD)-spiomet (spironolactone 25mg; pioglitazone 3.75mg; metformin 425mg). Administering a triple combination instead of metformin in monotherapy will allow to decrease the dose of each component and to reduce the treatment period to 1 year. The rationale for using three different medications is that each targets a distinct mismatch-derived dysfunction.

Spironolactone is a steroidal aldosterone antagonist marketed as diuretic but serves as an anti-androgen at higher doses (up to 200 mg/d). Recently, it has been identified as a potent activator of brown adipose tissue (BAT), and thus as a potential driver of energy expenditure, and aims at fat repartitioning (47,48). Spironolactone was first approved in 1960, and it has been used for heart failure, and for other disorders (primary hyperaldosteronism, essential hypertension, oedematous conditions). It is licensed for oedema in the paediatric population in Europe at doses of approximately 3 mg/kg. No safety concerns related to the use of spironolactone have been raised since its approval (49). In Europe and in the USA, spironolactone has been the anti-androgen of choice in the treatment of hirsutism for decades, with an excellent safety profile (50). The only minor side effects reported at high dose (100 mg/d or more) are menstrual irregularities, and to a lesser extent, abdominal pain, polyuria and dryness of the mouth (50). There are essentially no safety concerns when dosed at only 25 mg/d (equal or less than 1 mg/Kg/d), as will be in this study. Similarly, epidemiologic data show no evidence of an increased risk of any cancer associated with spironolactone use (51).

Pioglitazone is a thiazolidinedione (TZD) acting as insulin sensitiser in adipose tissue, liver and muscle. It raises circulating adiponectin, a driver of intracellular ceramidase (15,52) and also insulin sensitivity via preferentially subcutaneous adipogenesis (53). Pioglitazone was first approved in 1999, and in 2006, a fixed-dose-combination (FDC) containing pioglitazone and metformin was registered in Europe (Competact®, Glubrava®). At low dose (7.5 mg/d), pioglitazone acts as an inhibitor of cyclin-dependent kinase 5 (CDK5)-mediated phosphorylation of peroxisome proliferator-activated receptor-rather than as a peroxisome proliferator-activated receptor-activator (54). The use of pioglitazone has been questioned due a purported higher risk for bladder cancer in older men with diabetes. A 10-year prospective study performed by the FDA to evaluate this connection concluded that it was non-existing (55); accordingly, this association is considered to have been a “red herring” (56). In adolescents with PCOS, low-dose pioglitazone (7.5mg) has an excellent safety profile (43,44); pioglitazone has been used in autistic children at a dose 10 times higher than the proposed here without significant side effects (57). Pioglitazone will be officially under investigation for a first paediatric indication within the SPIOMET context (see below). There are no clinically relevant drug-drug interactions between pioglitazone and spironolactone, when the latter is dosed at 50 mg/d (double than that in HD-spiomet) via inhibition of hepatic CYP2C8, which is the main isoenzyme involved in pioglitazone's metabolism (58). Pioglitazone and spironolactone induce the

expression of C-X-C motif chemokine ligand-14 (CXCL14) -a chemokine released by BAT that protects against insulin resistance- in human adipocytes (48). SPIOMET administration normalizes the low levels of CXCL14 in girls with PCOS, suggesting that CXCL14 may mediate the benefits of the combination.

Metformin is an insulin sensitizer that targets insulin resistance; in addition, it raises AMPK activity, and the circulating concentrations of Growth-and-Differentiation Factor 15 (GDF15), a peptide hormone that reduces hepatic steatosis and intestinal glucose utilisation promoting weight loss (59-61). Metformin was first approved in 1959, and since then several FDCs have been approved for the treatment of type 2 diabetes as first and/or second line therapies (62). Currently, metformin is the drug most widely used world-wide for the treatment of type 2 diabetes in adults and in children older than 10 years; its use has significantly increased in younger children and adolescents without diabetes, including on early maturation and PCOS (37-41,63). Extensive experience has been gathered over the last 60 years related to the clinical use and safety of metformin (64). In 2001, the European Medicines Agency (EMA) issued a favourable benefit/ risk ratio for metformin that outlines its safety in humans (62). The main side effects are gastrointestinal symptoms (~10%), that usually resolve after therapy start (64). Lactic acidosis has been only described in cases of renal, cardiac and hepatic failure, or after intentional overdose (65). A decrease of vitamin B12 serum levels may occur after long-term treatment but appears to be without clinical significance (<0.01%) (66). The combination of spironolactone and metformin is not associated with a higher incidence of adverse events compared to low-dose spironolactone or metformin in monotherapy (50). Based on the EMA Summary of Product Characteristics (https://www.medicines.org.uk/emc/product/594/smpc#POSOLOGY), the dose range studied for metformin in clinical trials is 200-850mg, with the maximum daily dose being 2000mg. Hence, the proposed dose of metformin (425 mg/d) will be in the lower recommended range, assuming that the expected weight for a girl aged 8-9yr will be at least 25Kg (67).

Regulatory context (on March 20, 2021)

SPIOMET is a potential first-in-class treatment for adolescent PCOS. In Q2 2021, SPIOMET treatment is expected to become patent-protected in Europe, and to enter into Phase 2b of clinical development with a multi-centre placebo-controlled study funded by the Horizon 2020 programme of the European Union (899671-SPIOMET4HEALTH; H2020-SCI-BHC 2018-2020) and endorsed by a Paediatric Investigation Plan (PIP) of the Paediatric Committee (PDCO) of the EMA. SPIOMET should thus be under paediatric investigation in adolescents in Q2 2022, when the present study in children would be initiated.

Summary

A mismatch sequence of reduced prenatal weight gain and augmented postnatal weight gain in girls may lead to ectopic lipid accumulation, particularly in liver and viscera. Accelerated body growth and maturation, clinically expressed as early puberty, represents a concerted feedback mechanism to reduce such ectopic adiposity. We hypothesise that a reduction of ectopic adiposity with HD-spiomet (spironolactone 25mg; pioglitazone 3.75mg; metformin 425mg) can slow down the process of accelerated maturation, and thus delay pubertal *tempo*. If our hypothesis is correct, we intend to set up a multinational study where the hypothesis can be tested in other ethnic groups and thus extend the applicability of the present results.

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REFERENCES

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1. Navarro VM. Nat Rev Endocrinol 2020; 16:407-20.
2. Eckert-Lind C, et al. JAMA Pediatr 2020;174:e195881.
3. Worthman CM & Trang K. Nature 2018; 554:451-7.
4. Frisch RE & Revelle R. Science 1970; 169:397-9.
5. Reinehr T & Roth CL. Lancet Child Adolesc Health 2019; 3:44-54.
6. Huang A, et al. Curr Opin Endocr Metab Res 2020; 14:160-8.
7. Kim YJ, et al. J Pediatr 2019; 208:221-8.
8. Liu Y, et al. Endocrine 2021; Feb 2. doi: 10.1007/s12020-021-02630-3. Epub ahead of print.
9. Jung MK, et al. BMC Pediatr 2019; 19:323.
10. Stagi S, et al. Ital J Pediatr 2020; 46:165.
11. O'Keeffe LM, et al. Hum Reprod 2020; 35:2784-92.
12. Perry JR, et al. Nature 2014; 514:92-7.
13. Mumby HS, et al. J Obes 2011; 2011:180729.
14. Avendaño MS, et al. Hum Reprod Update 2017; 23:737-763.
15. Heras V, et al. Cell Metab 2020; 32:951-66.e8.
16. Franssen D, et al. Metabolism 2020; 115:154460.
17. Holland WL, et al. Nat Med 2011; 17:55-63.
18. de Zegher F, et al. Trends Endocrinol Metab 2018; 29:815-8.
19. Umano GR, et al. Am J Gastroenterol 2018; 113:376-83.
20. Ibáñez L, et al. J Clin Endocrinol Metab 2006; 91:2153-8.
21. Ibáñez L, et al. J Clin Endocrinol Metab 2008; 93:925-8.
22. Sebastiani G, et al. Pediatr Obes 2016; 11:251-7.
23. Díaz M, et al. Diabetes 2017; 66:779-84.
24. de Zegher F, et al. Pediatr Obes 2019;14:e12533.
25. Ong YY, et al. Int J Epidemiol 2020;49:1591-603.
26. Ong KK, et al. J Clin Endocrinol Metab 2004; 89:2647-51.
27. Ibáñez L, et al. Pediatrics 2006; 117:117-21.
28. Ibáñez L, et al. J Clin Endocrinol Metab 2009; 94:3696-9.
29. Mrug S, et al. Pediatrics 2014; 133:7-14.
30. Petry CJ, et al. Acta Diabetol 2018; 55:1209-19.
31. Cheng TS, et al. PLoS Med 2020;17:e1003017.
32. Day FR, et al. Nat Genet 2017; 49:834-41.
33. Harbulot C, et al. Eur J Endocrinol 2021; 184:243-251.
34. Sloboda DM, et al. J Clin Endocrinol Metab 2007; 92:46-50.34.
35. Ibáñez L, et al. J Clin Endocrinol Metab 2008; 93:1841-5.
36. Ibáñez L, et al. J Pediatr 2010;156:98-102.e1.
37. Díaz M, et al. Pediatr Diabetes 2015;16:538-45.
38. de Zegher F, et al. Horm Res Paediatr 2018;89:136-40.
39. Ibáñez L, et al. J Clin Endocrinol Metab 2006;91:2888-91.
40. Ibáñez L, et al. J Clin Endocrinol Metab 2006; 91:2068-73.
41. Ibáñez L, et al. Fertil Steril 2011; 95:727-30.
42. Ibáñez L, et al. J Clin Endocrinol Metab 2011; 96:E1262-7.
43. Ibáñez L, et al. J Adolesc Health 2017; 61:446-53.
44. Ibáñez L, et al. J Endocr Soc 2020; 4:bvaa032.
45. de Zegher F & Ibáñez L. Hepatology 2020 Oct 12. doi: 10.1002/hep.31592. Epub ahead of print.
46. de Zegher F, et al. BMJ Open Gastroenterology 2021, in press.
47. Thuzar M, et al. Diabetes Obes Metab 2019; 21509-16.
48. García-Beltran C, et al. BMJ Open Diabetes Res Care 2020; 8:e001035.
49. Food and Drug Administration. Label information NDA nº 012151. Aldactone®.
50. Ganie MA, et al. J Clin Endocrinol Metab 2013; 98:3599-607.
51. Biggar RJ, et al. Cancer Epidemiol 2013; 37:870 -5.
52. Ibáñez L, et al. Clin Endocrinol 2008; 68:317-20.
53. White U, et al. Diabetologia 2021; 64:159-67.
54. Choi JH, et al. Nature 2010; 466:451 -6.
55. Lewis JD, et al. JAMA 2015; 314:265 -77.
56. Ryder REJ & DeFronzo RA. Diabet Med 2019; 36:1185 -86.
57. Capano L, et al. Mol Autism 2018; 9:59.
58. Walsky RL, et al. J Clin Pharmacol 2005; 45:68-78.
59. Coll AP, et al. Nature 2020; 578:444-8
60. Yang M, et al. Sci Rep 2021; 11:2529.
61. de Zegher F, et al. Sci Rep 2021, in press.
62. European Medicines Agency (EMA). CPMP/4082/00. Referral for Metformin. 2001.
63. Ibáñez L, et al. Nat Rev Endocrinol 2014; 100:499-508.
64. Flory J & Lipska K. JAMA 2019; 321:1926 -7.
65. Pasquel FJ, et al. Am J Med Sci 2015; 349:263 -7.
66. Liu Q, et al. PLoS One 2014; 9: e100379.
67. Sánchez-Infantes D, et al. Clin Pharmacokinet 2011; 50:735-8.

Application Number: ICI21/00005

Project Leader: Abel López Bermejo

HYPOTHESIS AND OBJECTIVES

Max. 1 page

Hypothesis

General

Accelerated maturation in girls with early puberty and with a "mismatch" sequence of less prenatal weight gain (resulting in a reduced subcutaneous adipogenesis and a reduced capacity for safe lipid storage), and more postnatal weight gain (resulting in more lipogenesis and more need for lipid storage), is an adaptive response to ectopic lipid accumulation in the liver and viscera (ectopic adiposity).

Specific

1) Pharmacological intervention with Half-Dose (HD)-spiomet, a low-dose combination of spironolactone (25 mg/d), pioglitazone (3.75 mg/d) and metformin (425 mg/d) within a single tablet and over 1 year, will significantly reduce hepatic and visceral fat compared to placebo, as assessed by abdominal magnetic resonance (MRI).

2) The reduction of hepatic and visceral fat in girls treated with HD-spiomet will significantly slow down their accelerated maturation *vs* those treated with placebo, as well as the pubertal *tempo*, and will normalise more circulating insulin, insulin-like growth factor-1 (IGF-I), inflammation markers, sex steroids, high-molecular weight adiponectin (HMW-adip), CXCL14, and GDF15 concentrations.

3) The benefits of HD-spiomet on ectopic adiposity and endocrine-metabolic parameters on-treatment will still be detectable one year after treatment discontinuation.

4) Treatment with HD-spiomet for 1 year will be well tolerated and well accepted by the patients and will have no detectable side effects on hepatic and liver function, or on the electrolyte panel and circulating concentrations of vitamin B12 and folic acid.

Objectives

General

To ascertain whether a low-dose combination of generics within a single tablet known to collectively reduce ectopic adiposity through different pathways, can slow down the accelerated maturation in girls with early puberty and a "mismatch" sequence of less prenatal weight gain (resulting in a reduced subcutaneous adipogenesis and a reduced capacity for safe lipid storage), and more postnatal weight gain (resulting in more lipogenesis and more need for lipid storage).

Specific

1) To determine whether pharmacological intervention with a low-dose combination of spironolactone (25 mg/d), pioglitazone (3.75 mg/d) and metformin (425 mg/d) within a single tablet (HD-spiomet) over 1 year in "mismatch" girls with early puberty, reduces hepatic and visceral fat significantly more than placebo, as assessed by MRI.

2) To ascertain whether in girls treated with HD-spiomet, the reduction of hepatic and visceral fat associates to 1) a greater deceleration of bone maturation [as evaluated by Δ bone age (BA)/ Δ chronological age (CA) ratio over 1 year]; 2) a slower pubertal *tempo* (as judged by the progression of Tanner stage); 3) more normal levels of insulin, IGF-I, inflammation markers, sex steroids, HMW-adip, CXCL14 and GDF15; as compared to girls who received placebo.

3) To assess whether the benefits of HD-spiomet on hepato-visceral fat and endocrine-metabolic markers on-treatment are still detectable one year after treatment discontinuation.

4) To evaluate the tolerance and safety of HD-spiomet over 1 year (by assessing safety parameters), as well as the acceptance of the tablet (through a specific visual questionnaire).

Application Number: ICI21/00005

Project Leader: Abel López Bermejo

SCIENTIFIC AND/OR TECHNICAL METHODOLOGY

Work plan: overall strategy, highlighting the originality and innovation of the proposal. Please include a proposal for Data Management Plan (DMP).

Max. 3 pages (15,700 characters)

Study design

Randomised, placebo-controlled, multi-centre, phase 2a, study in "mismatch" girls from two tertiary centres in Spain: Dr. Josep Trueta Hospital (HJT, Girona) and Sant Joan de Déu Hospital (HSJD, Esplugues, Barcelona). Girls will be randomly assigned to receive a low-dose combination of three generics within a single tablet, namely, HD-spiomet (spironolactone 25 mg + pioglitazone 3.75 mg + metformin 425 mg; n=28) or placebo (n=28) for 1 year. All girls will be followed after finalisation of treatment the year thereafter. Six months after completion of the clinical visit in year 2, the girls will receive a phone call to ascertain whether they have reached menarche or not yet.

Patients will be randomised (placebo/ HD-spiomet, 1:1) with minimisation by centre, CA, birthweight (BW) and BMI. Randomisation will be performed by an independent investigator, with no contact with the patient and blind to the treatment allocation (http://www.sealedenvelope.com). The study will test the efficacy of HD-spiomet *vs* placebo on BA advancement, endocrine-metabolic and inflammation variables and abdominal fat distribution, both on treatment and post- treatment (see primary and secondary endpoints and see **ANNEX 2** for more details).

Subjects

Girls (n=56), age 8.0-9.0 years, consecutively seen at the Paediatric Endocrinology Department from HJT (n=28) and HSJD (n=28) with a "mismatched" sequence of reduced prenatal weight gain and augmented postnatal weight gain followed by early, progressive puberty.

Sample size calculation: In a previous study in girls with a history of low-BW and rapid postnatal catch-up in weight, metformin treatment for 4 years (425 mg for 2 years, then 850 mg for 2 years) slowed down the accelerated bone maturation (1). The progression of bone maturation, as assessed by the mean ratio of Δ BA over Δ CA, was faster in untreated girls [≈1.20 ± 0.2 (SD)] as compared to metformin-treated girls [≈1.00 ± 0.2 (SD)] (P≤0.05). To obtain a significance level of 0.05% and power of 90%, a total of 42 girls (21 in each study arm) must be included to detect the minimal relevant difference in bone age progression (*https://clincalc.com/stats/samplesize.aspx*). The number of patients has been further increased (n=28 in each study arm) to guarantee the necessary sample size in case of dropouts.

Inclusion criteria

1) age at study start 8.0-9.0 years; 2) BW for gestational age in lower tertile (Z-score below -0.44), and BMI for CA in upper tertile (Z-score above 0.44) (2); 3) early progressive puberty [bilateral breast development (Tanner stage 2)] starting between 8.0-9.0 years, with a minimum of 4 months of progression) (3,4).

Exclusion criteria

1) obesity (BMI Z-score above 2.00 for CA) (2); 2) evidence for a pathological cause of rapid maturation (i.e., congenital adrenal hyperplasia due to 21-hydroxylase deficiency); 3) thyroid, kidney or liver dysfunction; 4) treatment with glucocorticoids, sexual steroids, or other medications that can impair glucose tolerance or insulin resistance; 5) acute infections or intake of antibiotics or anti-inflammatory medications in the last 15 days.

Endpoints

Primary endpoint: BA advancement 0-1 yr (hand and wrist X-ray of the left hand) by an automated method, BoneXpert (Visiana, Denmark) (1).

Secondary endpoints

- *Clinical variables:* weight, height, BMI, waist and hip circumference and their ratio (WHR), systolic blood pressure (SBP), diastolic blood pressure (DBP), and Tanner stage (4).
- *Endocrine-metabolic variables:* 1) insulinaemia [fasting glucose, insulin, HOMA-IR (5)]; 2) IGF-I; 3) gonadotrophins (LH, FSH); 4) sex steroids [circulating androgens (total testosterone, androstenedione, SHBG, FAI) and oestradiol]; 5) lipids [total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides]; 6) markers of inflammation, insulin sensitivity and brown adipose tissue activity [ultra-sensitive C-reactive protein (usCRP), GDF-15, HMW-adip, CXCL14].
- *Safety markers:* blood count, circulating levels of alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), thyroid-stimulating hormone (TSH), urea, creatinine, electrolyte panel, vitamin B12, folic acid.
- *Abdominal fat partitioning (subcutaneous and visceral area) and hepatic fat:* abdominal fat distribution and intra-hepatic fat will be analysed by MRI.
- *Additional secondary outcomes:* 1) Dietary habits; 2) Acceptability of the tablet; 3) Study adherence; 4) Report of adverse events (see the section **DESCRIPTION OF ACTIVITIES** for extended details).

Medication

- HD-spiomet is a half-dose of the SPIOMET tablet, which is a fixed-dose combination within a single tablet containing the active pharmaceutical ingredients spironolactone (50 mg), pioglitazone (7.5 mg) and metformin (850 mg), developed according to the formula that has been tested in a previous phase 1 clinical trial (6) and will be used in a phase 2 clinical trial within a Horizon 2020

multi-centre, double-blind, placebo-controlled project in adolescents with PCOS (899671-SPIOMET4HEALTH). The proposed excipients are Povidone (polyvinylpirrolidone) k-30, Microcrystalline cellulose, Croscarmellose sodium, Polyglykol 4000 PS, Magnesium stearate and purified water.

- Placebo is a single tablet containing Povidone K-30, Microcrystalline cellulose, Croscarmellose sodium, Polyglicol 4000, Magnesium stearate.
- The tablets containing placebo and those containing the active ingredients (SPIOMET) will be scored to facilitate the split into two halves immediately before intake, will have the same size, sharp and colour, and thus will be indistinguishable from one another, to ensure the double-blind nature of the study.

Treatment security and withdrawal of medication

The aspects related to the treatment security are widely described in the section ***Reduction of ectopic fat in mismatch girls with accelerated maturation and in adolescents with PCOS: pilot studies*** of the **BACKGROUND.** For each patient, safety markers will be assessed at treatment start (month 0 of the randomised treatment), after 6 months and after 1 year on treatment and 1 year off-treatment, as well as in the event of reporting possible adverse events. Glucose blood levels <65 mg/dL and the progressive increase of creatinine, urea, transaminase and potassium levels, will be a reason for discontinuing the medication. In addition, the existence of signs and/or symptoms of hypoglycaemia, the appearance of a persistent skin rash, abdominal pain, persistent nausea and/or vomiting, headaches, sinusitis and severe bacterial infections, will be also a reason for discontinuation. In those cases, hepatic and renal function will be periodically monitored until the disappearance of the symptoms or the normalization of the altered parameters.

Allowed and incompatible concomitant treatments

Allowed: acetaminophen. Incompatible: anticoagulants, anti-inflammatories, oral hypoglycaemic drugs, anti-androgens, oestrogens, progestogens, digoxin.

Strengths and limitations

The main strengths are the double-blinded, placebo-controlled design of the study, the assessment of bone maturation by and automatic method, and the longitudinal assessment of abdominal fat partitioning and of novel markers of brown adipose tissue and insulin sensitisation. The main limitation is the lack of ethnic variability in the target study population.

Database and analyses

The study variables will be recorded in a REDCap (Research Electronic Data Capture) database. The statistical analyses will be performed with the SPSS program, version 23.0 (SPSS, Chicago, Illinois, USA). The analysis of quantitative variables of independent groups will be performed using ANOVA for repeated measurements in models adjusted for potential confounding variables. The association between BA, clinical variables, endocrine-metabolic and imaging variables will be sought by correlation and multiple regression analysis. A statistical analysis will be done by intention to treat and another with the patients who have completed follow-up. P <0.05 will be considered statistically significant.

Data management plan

The purpose of the data collection/generation is to assess the clinical health of the subjects under treatment (HD-spiomet or placebo) in order to evaluate the objectives of the project. The main objective (primary endpoint) is to test whether HD-spiomet treatment can slow down the accelerated maturation (by assessing bone age) in girls with early puberty and a “mismatch” sequence of less prenatal weight gain and more postnatal weight gain. The project will also ascertain the benefits of HD-spiomet on hepato-visceral fat (by MRI) and endocrine-metabolic markers (by blood sampling) and evaluate the tolerance and safety of HD-spiomet over 1 year (by assessing safety parameters in blood samples), as well as the acceptance of the tablet (through a specific visual questionnaire).

1. General description of the data

Three types of data will be collected:

a) clinical data: will consist of numerical data that will be obtained by the staff during the clinical visit or after blood analysis (that will be downloaded from the clinical history as a .pdf file)

b) imaging data: will consist of images (.tiff or .psd files) that will be obtained by MRI and X-ray and that will be analysed with the corresponding program softwares.

c) information from patient questionnaires (Kidmed nutrition questionnaire)

eCRFs (electronic case report forms) will be developed using REDCap for data capture directly at the trial sites. Patient data will be recorded in pseudonymised form (i.e., without reference to the patient's name) using exclusively the patient's identification code. In order to facilitate the documentation as per protocol in case of malfunction of the electronic system or any of its components, a paper version of the CRF will also be provided. The data transfer of the data entered into this paper version to the eCRF will be done as soon as the electronic system is available again. The information from the imaging data and from the questionnaires will be transferred to the eCRF by one member of the study.

The consistency and quality of the data will be controlled to ensure that no data are accidentally changed and the accuracy of data is maintained over its entire life cycle. This process will include repeated samples or measurements, standardised data capture, data entry validation and peer review of data.

2. Ethical and legal compliance

The study will be registered as a clinical trial (with an EudraCT number) and will be approved by the Institutional Review Boards of HJT and HSJD, and the Spanish Agency of Medicines and Health Products -AEMPS (Ministry of Health). Informed written consent will be obtained

from the parents. Informed consent statements will not include language that would prohibit the data from being shared with the research community.
Investigators in the recruiting trial centres will initially collect all data. Together with information on the trial, eligible patients will be informed about data capture, transmission and analysis processes. Once a patient is eligible, and the parents/legal tutors have given informed consent to trial participation and data collection, the investigator will assign the patient a unique patient identification code. Patient identification code lists will remain at the recruiting sites and be stored separately from the trial data. These data will be only used for the re-identification of the patients, if needed.
The research groups and their institutions will be the owners of the data and hold the copyright for the research data they generate. The PI will conclude contracts of ownership and rights of use of data within the research team and with the collaborators, with the support of the IDIBGI legal advisors. The publication policies will be thoroughly discussed with the team members and collaboration partners with signed agreements. The principal investigator will have overall responsibility for data management over the course of the research project and will monitor compliance with the plan.

3. Data Storage
The research data of this project will be stored in a REDCap database. The data will be collected and entered in the eCRF database by the members of the research group (using a username and password). There is no risk for pseudonymisation failure as far as the eCRFs are concerned, because no identifying data will be entered in the eCRF and no imaging data will be uploaded (only the numerical data obtained after analyzing the images)
At the end of the project, data will be recovered from REDCap and an SPSS file (.sav) will be created to perform the statistical analysis of the results. No sensitive data will be present in this file.

4. Data sharing and long-term preservation
All relevant data concerning the manuscripts will be associated with the manuscript document and made openly available upon publication, using established field-specific public repositories, according to the journal recommendations. A standard citation with Digital Object Identifier (DOI) will be provided to facilitate attribution. The DOI provides permanent identification for the data and ensures that they will always be found at the URL specified. Researchers will be able to contact the PI for access to other data (not directly related to the manuscript) they would need.
All relevant trial documentation will be stored for 30 years by the investigating sites after the trial's completion. With this time span all local rules and legal requirements regarding archiving at all sites will be addressed. Research has shown that multiple locally and geographically distributed copies of digital files are required to keep information safe. Accordingly, each institution will store a master copy of each digital file (i.e., research data files, documentation, and other related files) in the Hospital Archival Storage.

Innovation and originality
Early puberty is a prevalent entity and is the second reason for consultation in paediatric endocrinology clinics. The trend has significantly increased in the last decade, concomitantly with the increase in overweight/obesity in children. Early puberty may result in psychosocial maladjustment and risky behaviours in adolescence and may associate with several comorbidities in adulthood. The proposed novel treatment with a low-dose combination of generics (HD-spiomet) is the first one targeting all pathophysiological events of the entity, and as a consequence, holds the potential of reducing the short- and long-term associated comorbidities and thus may have a beneficial impact on the economic burden of health care systems.

References
1- de Zegher F, et al. Horm Res Paediatr 2018; 89:136-40
2- Ferrández-Longás A, et al. In: ERGON, ed. Patrones de crecimiento y desarrollo en España. Madrid: Atlas de gráficas y tablas 2004; 61 -115
3- Lindhart Johansen M, et al. J Clin Endocrinol Metab 2017; 102:1001-8
4- Marshall WA, et al. Arch Dis Child 1969; 44:291-303
5- Levy JC, et al. Diabetes Care 1998: 21:2191 -2
6- Ballester et al. 2nd International Conference on Medicinal Chemistry & Drug Design and 2nd International Conference on Bio Equivalence & Bio Availability; Barcelona 2019 (pp53 Abstract book); https://scientificfederation.com.icbeba-2019

Application Number:
ICI21/00005

Project Leader: Abel López Bermejo

SCHEDULE/TIMELINE

(List and timing of the different activities)

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Recruitment and inclusion.	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year												
	3rd Year												
	4th Year												

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Clinical follow-up.	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3rd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	4th Year	✓	✓	✓	✓	✓	✓						

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Bone age (Hand and wrist X-ray and bone age assessment).	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3rd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	4th Year												

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Blood sampling. Processing and storage of biological samples.	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3rd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	4th Year	✓	✓	✓	✓	✓	✓						

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Assessment of endocrine-metabolic and safety markers.	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3rd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	4th Year	✓	✓	✓	✓	✓	✓						

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Abdominal fat distribution (subcutaneous and visceral) and hepatic fat assessment (by MRI).	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3rd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	4th Year												

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Assessment of dietary habits. Assessment of safety parameters. Assessment of treatment compliance.	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3rd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	4th Year	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

ACTIVITY		MONTH J	F	M	A	M	J	J	A	S	O	N	D
Database maintenance. Statistical analyses. Elaboration of manuscripts and communication of final results	1st Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	2nd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	3rd Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
	4th Year	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

Application Number:
ICI21/00005

Project Leader: **Abel López Bermejo**

DESCRIPTION OF ACTIVITIES

Clearly define the responsabilities and workloads of each participating research group, including the time management plan.

Max. 5 pages (26,100 characters)

Centres

1) Josep Trueta Hospital (HJT) and Institut d'Investigació Biomèdica de Girona Dr. Josep Trueta (IDIBGI) (Girona); 2) Sant Joan de Déu Hospital (HSJD) and Institut de Recerca Sant Joan de Déu (IRSJD) (Esplugues, Barcelona).

Time management plan

- Recruitment: 1 year
- Active treatment (double-blind): 1 year
- Post-treatment follow-up (open): 1 year
- Measurements, analyses and reporting: 1 year

Study time points

Enrolment (time -1-0 year): candidate girls will be recruited at the Outpatient Clinic of HJT and HSJD among those referred by paediatricians of primary care for early onset of puberty. Confirmation of eligibility will require a clinical visit where inclusion and exclusion criteria will be carefully screened. A baseline blood test will be performed to assess circulating concentrations of 17-hydroxyprogesterone (17-OHP) in order to rule out congenital adrenal hyperplasia due to 21-hydroxylase deficiency (which is an exclusion criterium), and abnormal thyroid, kidney or liver function (which are exclusion criteria). If the patient fulfils all the inclusion criteria and none of the exclusion criteria, she will be considered as eligible. Then, the objective of the study will be explained to the patients and their parents/ legal guardians and written informed consent will be obtained (see **ANNEX 1** for more details). The written informed consent will include the full name of the patient and the full name and signature of the patient's parent/ guardian, together with the date and the signature of the local investigator, and a contact phone number that will be available throughout the study duration.

Baseline evaluation (time 0): will include the clinical visit [to collect clinical variables: height, weight, BMI, Tanner stage, waist and hip circumference and their ratio (WHR), SBP, DBP, a hand and wrist X-ray (to assess bone age), MRI (to assess abdominal fat partitioning and hepatic fat) and blood sampling in the fasting state (to assess endocrine-metabolic and inflammation variables and safety markers). Dietary habits will be also recorded through a standard questionnaire (Kidmed).

Randomisation: the patients will be randomised to receive placebo or HD-spiomet 1:1 (with minimisation by centre, for CA, BW and BMI) for 1 year.

Evaluation on treatment (time 6 months and 1 year): a clinical visit with the same parameters collected at baseline, a MRI and a blood sample extraction (to assess endocrine-metabolic and inflammatory variables and safety markers) will be performed after 6 months and after 1 year on treatment. Dietary habits, study adherence, acceptability of the tablet and adverse events will be also recorded. After 1 year on treatment, a hand and wrist X-ray will be performed for bone age assessment.

Evaluation post-treatment (time 2 years): will include a clinical visit 1 year after treatment discontinuation, collecting the same parameters as at time 0, 6 months and 1 year; in addition, a MRI scan and a blood sampling (to assess endocrine-metabolic and inflammation variables and safety markers) will be performed. Dietary habits, study adherence, acceptability of the tablet and adverse events will be also recorded. Six months later a phone call will be made to ascertain the presence or absence of menarche.

Bone age

A hand and wrist X-ray of the left hand will be taken at the Radiology Department either at HJT or HSJD, at treatment start and after 1 year on treatment. The same investigator at each centre will assess bone age by using the BoneXpert programme [version 3; February 2021 (Visiana, Denmark)] (1). This method will yield bone ages by both Greulich & Pyle (GP) and Tanner-Whitehouse 2 (TW2). Bone age maturation will be shown as the ratio between the increase of BA over the increase in CA (ΔBA/ ΔCA) (2). The expected ΔBA/ ΔCA ratio will be 1.20 ± 0.20 in the placebo group and 1.00 ± 0.20 in the HD-spiomet

subgroup, respectively.

Clinical characterisation
Clinical variables will be obtained during the clinical visits with the endocrinologist at HJT and HSJD. The same investigator at each centre will assess the clinical variables at times 0, 6 months, 1 year and 2 years. Thereafter, girls will be contacted 6 months later by phone to ascertain the presence or absence of menarche.

Blood sampling
10 mL of peripheral blood in the fasting state will be drawn in the early morning at each clinical centre, at baseline (time 0), after 6 months on treatment, after 1 year on treatment, and 1 year off treatment. Blood samples will be stored (at -80ºC) at the Paediatric Endocrinology lab (IDIBGI) and at the Metabolic Endocrinology lab (IRSJD) until analyses. Safety parameters (blood count, electrolyte panel, thyroid, liver and renal function, vitamin B12, folic acid) as well as the lipid profile and fasting glucose and insulin will be assessed at each study visit; the remaining parameters will be assessed at the end of the intervention period and at the end of the study.

Endocrine-metabolic variables analysis
- The real-time biochemical lab at both HJT and HSJD will be responsible for analysing: 1) glucose (by the glucose oxidase method); 2) fasting insulin, IGF-I, SHBG and usCRP (by immuno-chemiluminescence); 3) LH, FSH and oestradiol (by immuno-chemiluminescence); 4) total cholesterol, LDL-cholesterol, HDL-cholesterol, and triglycerides (by molecular absorption spectrometry).
- Circulating testosterone and androstenedione concentrations will be centrally assessed at the Bioanalysis Research lab [Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Hospital del Mar], by liquid chromatography-tandem mass spectrometry. The free androgen index (FAI) will be calculated with the following formula: testosterone (nmol/L) x 100/ SHBG (nmol/L)
- The analysis of circulating HMW-adip, GDF-15 and CXCL14 concentrations will be centralised at the Metabolic Endocrinology lab (IRSJD). Circulating levels of HMW-adip will be analysed by ELISA (Millipore, St Louis, MO), with intra- and inter-assay coefficients of variation (CVs) <9%. Serum GDF15 will be assessed using a specific human ELISA kit (R&D Systems, Minneapolis) with intra- and inter-assay CVs <6%. Serum CXCL14 will be assessed using a specific ELISA kit (Ray Biotech, Norcross, GA, USA), with a sensitivity of 0.7 ng/ ml, and inter- and intra- assay CV less than 12%.

Safety markers analysis
The real-time biochemical lab at HJT and HSJD will analyse: 1) blood count (by flow cytometry and colorimetric assay; 2) circulating levels of ALT, AST, GGT, TSH, urea, electrolyte panel and creatinine (by molecular absorption spectrometry); 3) vitamin B12 and folic acid (by immuno-chemiluminescence).

Abdominal fat distribution (subcutaneous and visceral) and hepatic fat assessment
Abdominal fat distribution and intrahepatic fat will be analysed by MRI at both clinical sites with the same scan [multiple-slice 1.5 Tesla scan (Signa LX Echo Speed Plus Excite, General Electric Healthcare, Milwaukee, WI)]. The assessments will be performed at Clínica Girona (Girona) and CETIR Medical Centre (Barcelona). Scans will be performed by the same operator at each centre, blinded to the treatment allocation, at times 0, 6 months, 1 year and 2 years. A central reader based at HSJD and blinded to treatment allocation will be appointed for handling and reading the imaging studies from both participating centres (QUIBIM, Valencia, Spain).

Dietary habits
The quality of the dietary habits will be assessed by a modified version of the Kidmed questionnaire (http://www.sspa.juntadeandalucia.es/servicioandaluzdesalud/distritomalaga/docs/cuidados/anexos/Anexo%205.%20Cuestionario%20Kidmed%20modificado.pdf). Girls will complete the test during the clinical visits at the corresponding centre (at times 0, 6 months, 1 year and 2 years).

Acceptability of the tablet
The patient's opinion regarding the palatability and swallowability of the tablet will be recorded by using a simple questionnaire with numerical rating scales and the integrated 100 mm visual analogue scale (VAS)/ facial hedonic scale (3,4). Patients will answer the questionnaire during the clinical visits at each centre at time 6 months and 1 year.

Study adherence
Study adherence will be calculated as the ratio between the number of tablets prescribed and dispensed for the period between two hospital appointments and the number of half tablets returned by the patient at the following appointment (the patients will split every evening one tablet into two halves with a cutter, swallow one half and store the other half in a

box kept for this purpose). The Pharmacy Department at HJT and HJD will dispense the medication and receive the tablets returned by the patients.

Report of adverse effects

The nature, severity, treatment and outcome of any potential adverse event will be recorded by the clinical investigator seeing the patient at each centre during the clinical visits (at 6 months, 1 year and 2 years).

Medication

Reig-Jofré (RJF) is the pharmaceutical company that will lead the product development and manufacturing of the study medication. RJF will prepare and distribute the kits of the study to the participating clinical centres recruiting patients. The study medication will be packed in primary and secondary packaging to allow preparing sufficient medication kits for all study subjects. Each kit will be labelled in the primary and the secondary packaging and codified according to randomisation codes provided by the Sponsor. Re-labelling of the packed study medication or second batch manufacturing will be considered in case of need depending on stabilities of the drug product and placebo. RJF will be responsible for the distribution of the medication to the pharmacies of the clinical trial sites, together with certificates of analyses, with tracking of shipments. Each study centre will have a back-up stock of study medication in case of emergency. The shipments will be performed under temperature control. RJF will perform the shipments to clinical sites having into account the stabilities of the drug product and placebo.

The Pharmacy Department at HJT and HSJD will dispense the medication (SPIOMET or placebo) to the patients at each hospital appointment. The number of tablets dispensed will cover the daily dose needed until the next scheduled hospital visit plus one week. Parents will split the scored tablet of SPIOMET or placebo into two halves with a cutter that will be also provided by the respective hospitals. Patients will swallow one half of the tablet (containing 25 mg of spironolactone, 3.7 mg of pioglitazone and 425 mg of metformin) immediately and will keep the other half in a box also provided by the Pharmacy Department. This box will be delivered to the Pharmacy Department during the next hospital appointment as an account for adherence to treatment. To ensure the uniformity of the two halves, dose uniformity studies have been performed (general monograph Ph.Eur. 2.9.40) by Reig Jofre as part of drug development and this parameter will be added as part of release specifications. The medication will be taken on a daily basis, orally and at dinner time, during the meal. Adherence to treatment will be screened by history at each clinical visit and by tablet counts in the pharmacy dispensing the study medications.

Associated risks

The current project includes the assessment of different variables with potential pathophysiological weight, through well-established techniques and methodologies. The execution of the study implies the extraction of blood samples from subjects and the use of imaging techniques.

- Blood sampling: peripheral blood extraction is a common clinical practice that will be executed only by trained personnel. Extractions will be performed in a quiet environment, after applying an anaesthetic cream on the skin (EMLA cream, commonly used in paediatrics). The extraction of 10 mL of blood at age 8-9 years implies no significant health risks.
- X-ray: the assessment of bone age by hand and wrist X-ray is widely used in paediatric endocrinology. The amount of radiation used in a bone age study is small and is not considered dangerous.
- MRI: will be used to assess abdominal fat partitioning and hepatic fat. The MRI scanner is widely used in clinical practice and no side effects from the magnetic fields and radio-waves have been reported. The test takes between 30-60 min and a trained professional will continuously monitor the subject and device.

Clinical Research Organization (CRO)

ADKNOMA will provide support during the trial development process as a clinical regulatory specialist and will be responsible of the regulatory documents, trial monitoring and data audit, and of the coordination of security activities.

Task distribution

HJT and IDIBGI:

- Abel López-Bermejo (MD, PhD): is the IP of the project and will coordinate and supervise all the tasks. He will be responsible for the recruitment, inclusion and clinical follow-up of patients, bone age assessment, collection of clinical data and record any adverse effects at HJT. He will be responsible of the analysis of the data and will write future manuscripts.
- Elsa Puerto (MD): recruitment, inclusion and clinical follow-up of patients, bone age assessment and analysis of clinical

data.

- Judit Bassols (PhD): database maintenance, statistical analyses and preparation of manuscripts.
- Gemma Carreras Badosa (PhD): processing and storage of biological samples. Determination of markers of inflammation and insulin sensitivity.

HSJD and IRSJD:

- Lourdes Ibáñez (MD, PhD): will lead the study at HSJD. She will be responsible for the recruitment, inclusion and clinical follow-up of patients, bone age assessment and collection and analysis of clinical data. She will record any adverse effects of the treatment, write future manuscripts, and coordinate the project with Dr. López-Bermejo.
- Paula Casano (MD, PhD): recruitment, inclusion and clinical follow-up of patients, bone age assessment and analysis of clinical data.
- Marta Díaz (PhD): processing and storage of biological samples. Determination of markers of inflammation and insulin sensitivity.
- Cristina Garcia (MSc): processing and storage of biological samples. Determination of markers of inflammation and insulin sensitivity.
- Cristina Plou (research nurse): will organize and schedule the clinical visits, perform the blood sampling at each study visit, and collect and analyse the dietary questionnaires and the questionnaires regarding the acceptability of the tablet. She will also schedule the periodical MRI's at CETIR in Barcelona.

References

1- Thodberg HH. J Clin Endocrinol Metab 2009; 94: 2239 -44
2- de Zegher F, et al. Horm Res Paediatr 2018; 89:136-40
3- Bastiaans DET, et al. Br J Clin Pharmacol 2017; 83:2789 -97
4- Kozarewicz P. Int J Pharm 2014; 469:245-8

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Project Leader: **Abel López Bermejo**

MANAGEMENT/COORDINATION OF WORK PACKAGES

Please provide a written description or a diagram. Max. 1 page

Diagram showing the different work packages to complete throughout study duration:

	Recruitment†	On Treatment			Post treatment		
Study months	**-12 - 0**	**0**	**6**	**12**	**24**	**30**	**36**
Inclusion and exclusion criteria	√						
Informed consent	√						
Randomisation	√						
0 mo visit – start randomised treatment		√					
6 mo visit – on randomised treatment			√				
12 mo visit – stop randomised treatment				√			
24 mo visit – follow up off treatment					√		
Phone interview ^						√	
Bone age assessment		√		√			
Clinical variables *		√	√	√	√		
Endocrine – metabolic- inflamation variables &		√	√	√	√		
Safety markers ¶		√	√	√	√		
Abdominal fat partitioning and hepatic fat (MRI) ¤		√	√	√	√		
Dietary habits		√	√	√	√		
Acceptability of the tablet			√	√	√		
Study adherence			√	√	√		
Report of adverse events			√	√	√		
Measurements, analyses and reporting							√

† Recruitment will be performed over a year (months -12 to 0).
^ Patients will receive a phone call to ascertain the presence or absence of menarche.
* Clinical variables: weight, height, body mass index (BMI), waist and hip circumference and their ratio (WHR), systolic (SBP) and diastolic (DBP), Tanner stage.
& Glucose, insulin, homeostasis model assessment insulin resistance (HOMA-IR), Insulin-like growth factor 1 (IGF-I), luteinizing hormone (LH), follicle stimulating hormone (FSH), testosterone, androstenedione, sex hormone-binding globulin (SHBG), free androgen index (FAI), oestradiol, total cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), triglycerides, ultra-sensitive C-reactive protein (usCRP), growth differentiation factor-15 (GDF-15), high molecular weight adiponectin (HMW-adip), C-X-C motif chemokine ligand 14 (CXCL14).
¶ Safety markers: blood count, alanine transaminase (ALT), aspartate transaminase (AST), gamma-glutamyltransferase (GGT), thyroid-stimulating hormone (TSH), urea, electrolyte panel, creatinine, vitamin B12 and folic acid.
¤ MRI, magnetic resonance imaging.

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ADDED VALUE OF SCIENTIFIC COLLABORATION AMONG THE DIFFERENT RESEARCH GROUPS

Please describe the synergies and advantages of the collaboration. Max. 1 page

The research group on **Paediatric Endocrinology of the IDIBGI**, led by Dr. López-Bermejo (http://www.idibgi.org/es/grups/pediatric-endocrinology-es) has extensive experience in the study of cardiometabolic risk markers in children. During the last years, the group has characterized several cardiovascular risk markers in a longitudinal population-based cohort of children with and without obesity and developed several projects for the early detection of metabolic abnormalities as a result of prenatal programming in two longitudinal population-based prenatal cohorts of pregnant mother-father-infant trios with postnatal follow-up in the newborns.

The research group on **Metabolic Endocrinology of the IRSJD,** led by Dra. Ibáñez (https://www.irsjd.org/en/research/10/metabolic-endocrinology) is integrated in the Centre for Biomedical Research Network in Diabetes and Associated Metabolic Disorders (CIBERDEM).The group has extensive experience in the study of the biological, biochemical and genetic mechanisms underlying endocrine-metabolic disturbances in small-for-gestational-age children (SGA). The prospective follow-up of longitudinal cohorts since 2006 has contributed to a significant advance in clinical and molecular knowledge in this field. He has also developed therapeutic strategies with combinations of insulin sensitisers and antiandrogens in adolescents with polycystic ovary syndrome (PCOS).

During the last years, both groups have maintained a close relationship and collaborated in several projects to investigate the epigenetic markers and mechanisms that explain the metabolic risk associated with intrauterine growth retardation, obesity, or metabolic abnormalities during pregnancy. The strategic research lines shared by both groups are the following:

1) **Pathophysiological bases of SGA and PCOS**: Study of the pathophysiological bases related to the outcome of SGA children and of adolescents with PCOS (Polycystic Ovary Syndrome). We have identified novel markers associated with the comorbidities of SGA and PCOS disorders, including: circulating GLP-1 (IJO 2015), liver volume (IJO 2018), hepatic adiposity, cIMT (Ped Obes 2016) and PUFAs (Horm Res Paed 2018) in SGA and AGA children, and the miRNA profile in PCOS vs control girls (JCEM 2020).
2) **Pharmacological reversibility of metabolic programming:** Development of therapeutic strategies aimed at preventing, reducing or avoiding the comorbidities associated with several metabolic diseases including PCOS, SGA and childhood obesity. We have developed two clinical trials (EC08/00056; EC10/00252) with metformin in SGA children with catch-up growth and children with obesity to study their benefits in reducing the endocrine-metabolic effects associated with these diseases (PlosOne 2019; Pediatr Diab 2015).

Both research groups are multidisciplinary (paediatricians, endocrinologists, biologists...) and have extensive experience in the generation of longitudinal cohorts and in conducting clinical trials in the area of metabolic endocrinology, pre- and postnatal growth, and the consequences of low birth weight on adrenal and ovarian function. The groups' researchers are complementary in terms of areas of connection and experience, which allows for an optimal interdisciplinary approach. In this sense, Dra. Ibañez is leading the European clinical trial 899671-SPIOMET4HEALTH in PCOS girls (H2020 project) and will lead a new proposal to test HD-spiomet in girls with early puberty in four European countries (that will be submitted to the European Society for Paediatric Endocrinology Research Unit in the following months), while Dr. Abel López-Bermejo will lead the current proposal to test HD-spiomet in Spanish girls with early puberty.

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AVAILABLE RESOURCES

Indicate the available resources to carry out the research study. Max. 2 pages (10,000 characters)

Dr. Josep Trueta Hospital of Girona: The outpatient paediatric rooms (8 offices) provide an appropriate physical space for conducting the clinical visits of the children included in the study. On the 4th floor of the "government area building", the Paediatric Endocrinology group has an office equipped with a computer, printer and telephone, from where the research nurse will contact the patients and schedule complementary examinations. The Clinical Laboratory of the hospital will be the physical framework for the assessment of the metabolic parameters and the Radiology Department will be responsible for the assessment of the child's hand and wrist X-ray.

Institute of Biomedical Research of Girona (IDIBGI): The IDIBGI is located in the Parc Hospitalari Martí i Julià de Salt (Girona). The building holds an area of 11,000 m^2 consisting of two floors, separated into different types of modules: offices, laboratory, as well as core services and meeting rooms. The laboratory is located on the ground floor of this building. It holds more than 500 m^2 of space that includes a common work area, a cell culture room (with 3 laminar flow hoods and 6 CO2 incubators), a PCR room, an immunohistochemistry room, a microscopy room, several equipment rooms and a cold room. The laboratory has the necessary equipment to carry out the studies: Genetics: Real-time PCR (ABI Prism 7000 and 7700), thermocyclers, fume hoods, standard DNA electrophoresis devices, apparatuses for high resolution electrophoresis, spectrophotometer, centrifuges and freezers. The samples obtained are stored in the IDIBGI laboratory in a freezer at -80°C (Nirco). The freezer is equipped with a temperature probe with local and remote alarm functions and data processing software to view graphs and register all events. The processing and storage of the biological samples is run by the IDIBGI Biobank, through the Noraybio software that allows for the registration of all the information of biological samples and processes, ensuring traceability at all times. Each laboratory worker has his/her own workspace and has a Lenovo laptop. These computers are connected to Internet, the IDIBGI intranet and the Hospital de Girona Dr. Josep Trueta. Researchers have access to online resources, including PubMed, Scopus and ScienceDirect. The study variables will be recorded in a REDCap (Research Electronic Data Capture) database. The statistical analyses will be performed with SPSS program, version 23.0 (SPSS, Chicago, Illinois, USA).

Clinica Girona: The magnetic resonance unit has the necessary equipment (magnetic resonance imaging devices coupled with specific software) for studies of abdominal fat distribution and hepatic fat.

Sant Joan de Déu Hospital (HSJD): The outpatient endocrinology rooms (6 offices) provide an appropriate physical setting for receiving patients, and for conducting clinical visits. The Clinical Laboratory of the hospital will be the physical framework for the assessment of the metabolic parameters and the Radiology Department will be responsible for the assessment of the child's hand and wrist X-ray.

Institut de recerca Sant Joan de Déu (IRSJD): The group has its own laboratory "Metabolic Endocrinology Laboratory" located on the 4th floor of the "teaching building", with an area of 60 m^2. The laboratory is equipped with the necessary technological resources to carry out biochemical and genetic determinations (nanodrop, spectrophotometer for reading plates, fluorimeter, electrophoresis cells, power supplies, thermomixer, stirrers, -20ºC and -80ºC freezer equipped with probes of temperature recording and monitoring and connected to an alarm system and real-time PCR required for genotyping and gene expression studies. Likewise, it has instruments in common use among the different research groups located on the 3rd and 4th floors of the teaching building: laminar flow cabinet, centrifuges, ultracentrifuge, transilluminator, Kodak system for image capture and analysis, hybridization oven, PCR machines, cold room, dark room, freeze dryer, cell culture rooms, autoclave, milli-Q water, baths, ice machine, liquid nitrogen, vortex and refrigerators). In addition, on the 4th floor of the "teaching Building", the group has an office equipped with two computers, printer and telephone, from where the research nurse will summon the patients and schedule complementary examinations.

CETIR Medical Centre: They have the necessary equipment (magnetic resonance imaging devices coupled with specific software) for studies of abdominal fat distribution and liver fat.

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IMPACT

Describe the potential impact of the study and the expected scientific and technological contributions; detail the adequacy of the dissemination plan and the strategy for the technology transfer. This section should include: Potential impact on health population and National Health System; patents and other industrial transfer products. (Please write in Spanish).

Max. 3 pages (15,700 characters)

La pubertad adelantada es una entidad prevalente y es el segundo motivo de consulta en las visitas de endocrinología pediátrica. Su prevalencia ha aumentado significativamente en la última década, de forma concomitante con el aumento del sobrepeso/obesidad en la población pediátrica. La pubertad adelantada puede resultar en desajuste psicosocial y conductas de riesgo en la adolescencia y puede asociarse con varias comorbilidades en la edad adulta. El nuevo tratamiento propuesto con una combinación de dosis bajas de genéricos (HD-spiomet) es el primero que se dirige a todos los eventos fisiopatológicos de la entidad y, como consecuencia, tiene el potencial de reducir las comorbilidades asociadas a corto y largo plazo y, por lo tanto, puede tener un impacto beneficioso sobre la carga económica de los sistemas de salud.

<u>El impacto potencial del estudio incluye:</u>

ALTA PREVALENCIA -- ALTO IMPACTO
En todo el mundo, la pubertad adelantada es una entidad común en las niñas, con una incidencia anual media de aproximadamente 2,6 a 9,2 por 1000 niñas (1). Se prevé que esas cifras aumentarán aún más, ya que los datos europeos sugieren una tendencia secular hacia un inicio más temprano de la pubertad en la población general.

ALTAS COMORBILIDADES ASOCIADAS -- ALTO IMPACTO
Una edad más temprana en la pubertad puede estar asociada con dificultades psicosociales, es un factor de riesgo para el adelanto de la primera relación sexual y tiene implicaciones negativas para la salud a largo plazo, incluido un mayor riesgo de diabetes gestacional, diabetes tipo 2, mayor adiposidad, aumento de peso y obesidad, síndrome del ovario poliquístico (SOPQ), enfermedad cardiovascular, depresión y muerte prematura. Además, los datos genéticos a gran escala sugieren que la pubertad adelantada también se asocia con un mayor riesgo de padecer varios tipos de cáncer (endometrio y mama).

ELEVADA CARGA ECONÓMICA -- ALTO IMPACTO
No existen datos precisos sobre la carga económica de la pubertad adelantada en Europa; sin embargo, como hemos mostrado anteriormente, esta entidad se asocia con múltiples comorbilidades y tiene un impacto económico negativo significativo.

ELEVADA CARGA PSICO-SOCIAL -- ALTO IMPACTO
El inicio de la pubertad en etapas tempranas se ha relacionado con los problemas de salud mental de los adolescentes, los cuales incluyen depresión, ansiedad, trastornos alimentarios, delincuencia, consumo de sustancias, y fracaso escolar o abandono escolar. En un estudio de 7802 mujeres, la menarquia más temprana se asoció con tasas más altas de síntomas depresivos y comportamientos antisociales que persistieron hasta la edad adulta (2).

INTERVENCIÓN TEMPRANA -- ALTO IMPACTO
Este proyecto se dirige a niñas de 8-9 años, en parte porque una intervención temprana (aún dentro de una "ventana crítica") tiene el potencial de tener un impacto beneficioso a largo plazo, no solo en la paciente con pubertad avanzada, sino también en su futura descendencia (perfil de alto riesgo de padecer SOPQ, diabetes gestacional, obesidad…). La intervención temprana no solo es vital para evitar la sub-fertilidad o el riesgo de cáncer de endometrio pre-menopáusico y/o hiperplasia endometrial atípica entre otras comorbilidades asociadas al fenotipo del SOPQ, sino también para minimizar la enorme carga psicosocial.

COMPRIMIDO ÚNICO -- ALTO IMPACTO
Este proyecto va a facilitar el tratamiento de la pubertad adelantada, al utilizar un solo comprimido que contiene 3 medicamentos que, en muchos países, pueden no estar disponibles por separado en las dosis adecuadas. Además, el uso de un solo comprimido facilitará la adherencia al tratamiento. Teniendo en cuenta que la mala adherencia a la medicación es un desafío generalizado y no resuelto entre los pacientes, el uso de un solo comprimido en lugar de tres diferentes, se

espera que aumente el cumplimiento dentro de este grupo de edad.

HACIA UN PRIMER TRATAMIENTO APROBADO PARA pubertad adelantada -- ALTO IMPACTO
En la actualidad, no existe un tratamiento aprobado para tratar la pubertad adelantada. Este proyecto obtendrá datos preliminares y proporcionará la base para una mayor investigación y desarrollo a nivel europeo (ensayos clínicos de fase 3) y, en última instancia, para la comercialización y explotación del fármaco.

La diseminación y transferencia tecnológica esperada del estudio incluyen:

DIFUSIÓN CIENTÍFICA: El conocimiento generado será publicado en revistas indexadas en el Journal Citation Reports (JCR) y reportado en congresos nacionales e internacionales en el campo de la endocrinología pediátrica [Sociedad Española de Endocrinología Pediátrica (SEEP), European Society for Paediatric Endocrinology (ESPE)]. Los resultados y datos del proyecto serán de libre acceso (los manuscritos y los datos asociados se almacenarán en repositorios gratuitos).

DIFUSIÓN MÉDICA: Los conocimientos generados se incluirán en cursos de actualización y guías clínicas con el fin de dotar a la comunidad médica, concretamente pediatras de atención primaria y médicos de familia, con guías de práctica para el diagnóstico y tratamiento de esta entidad.

DIFUSIÓN DE LA SOCIEDAD: El conocimiento generado también se difundirá entre la sociedad general a través de notas de prensa, charlas informativas, *newsletters*, webs, radio y televisión. Se hará especial énfasis en la difusión de los resultados en las escuelas, incluyendo las asociaciones de alumnos y padres de primaria y secundaria de escuelas a nivel local y estatal.

EXPLOTACIÓN COMERCIAL: El proyecto generará los datos necesarios para lanzar un ensayo clínico internacional multi-céntrico de fase 3. El principal resultado comercial explotable del proyecto será el desarrollo del comprimido de HD-spiomet, específicamente con la dosificación empleada en este estudio, en el que se utilizará la mitad del comprimido SPIOMET, ya desarrollada. El Laboratorio Reig-Jofré tiene un gran interés en la futura comercialización de HD-spiomet mediante una explotación directa o mediante la comercialización de la nueva terapia a través de otros acuerdos comerciales con otras grandes compañías farmacéuticas. Se realizará la patente del producto y demás productos de transferencia industrial necesarios.

Los planes de difusión y explotación del proyecto van a permitir sensibilizar a la población; extender el impacto del producto; involucrar a las partes interesadas y los grupos destinatarios; compartir soluciones y conocimientos técnicos e influir en las políticas y la práctica hospitalaria.

Referencias
1- Bräuner EV, et al. JAMA Netw Open 2020; 3:e2015665.
2- Mendle J, et al. Pediatrics 2018; 141:e20171703.

Application Number: ICI21/00005

Project Leader: Abel López Bermejo

REQUESTED BUDGET

1. Personnel costs	Euros
	0
Subtotal personnel costs :	**0**

2. Execution costs

A) Goods and services (Equipment, consumables and other expenses).

Clinical trial: Project management (regulatory issues, monitorisation, insurance)	41.705
Clinical trial: Medication	27.545
Biochemical and hormonal parameters	18.780,99
Sevices: Blood sampling	448
Services: LC-MS/MS (testosterone and androstenedione)	6.720
ELISA assays: HMW-adiponectin ELISA kit, GDF15 ELISA kit; CXCL14 ELISA kit	13.699,62
Imaging: MRI Scan (Abdominal fat distribution and liver fat assessment)	28.000
Imaging: MRI Scan (Central reading of the images [QUIBIM])	8.769
Imaging: X-ray (Hand and wrist X-ray)	6.720
Laboratory consumables	5.000
Other Costs: Statistical Studies	3.000
Other Costs: Publication of Manuscripts Open Access	9.000
Other Costs: Transport of Samples	2.800
Subtotal goods and services costs :	**172,187.61**

B) Travel and allowance

Attendance at a national congress (X 2)	1.300
Attendance at international congress (X2)	2.200
Subtotal travel and allowance costs :	**3,500**
Subtotal execution costs :	**175,687.61**
Total Budget Requested :	**175,687.61**
Total Budget Requested + 10% Overhead :	**193,256.371**

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Project Leader: Abel López Bermejo

BUDGET JUSTIFICATION

Scientific justification of requested budget.

Max. 3 pages (15,700 characters)

1. PERSONAL COSTS

No funding for personal is requested because the current structural organization of the two centres allows to organize the blood sampling, the clinic visits, the imaging assessment and the completion of questionnaires with the personnel already ascribed to the project.

2. EXECUTION COSTS

A) Goods and services

The sample size will consist of 56 "mismatch" girls with early puberty who will be included in a randomised, double-blind, placebo-control study to compare the effects of HD-spiomet (spironolactone 25 mg/d + pioglitazone 3.75 mg/d + metformin 425 mg/d; n=28) *vs* placebo (n=28) for 1 year on bone maturation, endocrine-metabolic and inflammation parameters and abdominal and hepatic fat. All girls will be followed for 2 years (1 year of treatment followed by 1 year off treatment).

Clinical trial:

- ADKNOMA Health Research SL is a Clinical Research Operator (CRO) that will perform the necessary tasks related to periodical monitorisation, regulatory issues and pharmacovigilance of the study. The tasks will also include the submission of the protocol to the Agencia Española del Medicamento y Productos Sanitarios (AEMPs) and to the local Ethical Committees for Research with Medicinal products (CEIm) for approval, as well as the registration of the study in ClinicalTrials.gov (https://clinicaltrials.gov). ADKNOMA is a company with more than 15 years of experience in clinical trials including those with paediatric populations.
- The insurance fee (Confide) will cover the potential side effects associated to the medication during the active treatment phase and during the year of follow-up thereafter.
- The costs associated to taxes include AEMPS (Agencia Española de Medicamentos y Productos Sanitarios) clinical trial evaluation fees, CEIm's evaluation costs, document printing and courier costs.
- The budget allocated to the medication has been estimated taking into account a total amount of 41 tablets per patient per month (31 days + 10 spare tablets), accounting for 365 + 120 tablets per patient per year, and a total of 27.160 tablets of HD-spiomet or placebo for the whole duration of the intervention. The tablets will be dispensed in bottles containing the monthly medication (41 tablets each) x 6 months, which is the time allocated between clinical visits. The estimated costs include: a) the product development and manufacturing by Reig-Jofré (RJF); b) the distribution of the study kits to the participating clinical centres recruiting patients, together with certificates of analyses, with tracking of shipments by RJF; c) the primary and secondary packaging of the study medication and the labelling and codification which RJF will perform according to randomisation codes provided by the sponsor; d) the dispensation of the medication to the patients by the pharmacies at both HJT and HSJD at time 0 and at time 6 months, coincident with the hospital appointments while on treatment.

Biochemical and hormonal parameters:

- Blood tests will be performed in all girls at 0, 6, 12 & 24 months (56 subjects x 4 times; n=224 samples) for assessment of endocrine-metabolic and inflammation parameters. Circulating free thyroxine (T4L) and 17-OH-progesterone will only be measured once at baseline (n=56 samples) as part of the assessments needed to rule out exclusion criteria.

Services:

- Circulating total testosterone and androstenedione concentrations will be assessed by liquid chromatography - mass spectrometry (LC-MS/MS) at the Institut Hospital del Mar d'investigacions Mèdiques (IMIM), because HJT and HSJD do not have the necessary equipment. LC-MS/MS is now a well-accepted, simple, sensitive, specific and robust method for simultaneous quantification of steroids, with a precision far superior than the traditional immunoassays. Nowadays, the LC-MS/MS technology is considered to be the gold standard for steroid measurement mainly in children, as it allows to detect very low concentrations of steroids (1); consequently, its use is highly recommended by endocrine societies. The IMM has previously collaborated with HJT and HSJD in the assessment of circulating steroids by the LC-MS/MS method in adolescents with polycystic

ovary syndrome (J Adolesc Health 2017; Ibáñez et al.; J Endocr Soc 2020). The LC-MS/MS assessments will be performed in two times: at the end of the active treatment phase, where all samples (n=56 x 3; n=168) will be measured in the same batch, and at the end of the follow-up phase, where all samples (n=56) will also be measured in the same batch.

ELISA assays:

- The budget includes the assessment of n=224 samples in duplicate for three markers: HMW-adiponectin, GDF15 and CXCL14. The proposed ELISA assays have been used by HJT and HSJD in previous studies, and thus, both research groups have experience in these assessments. The measurements will be performed at HSJD in two times: at the end of the active treatment phase, where all samples (n=56 x 3; n=168) will be measured in the same batch, and at the end of the follow-up phase, where all samples (n=56) will also be measured in the same batch.

Imaging:

- Abdominal fat distribution and intrahepatic fat content by MRI will be analysed both at Clínica Girona (Girona) and at CETIR Medical Centre (Barcelona) for the following reasons: 1) the two centres will use the same multiple-slice 1.5 Tesla scan (Signa LX Echo Speed Plus Excite, General Electric Healthcare, Milwaukee, WI); 2) the two centres have previous experience in performing those measurements in paediatric populations, and specifically, they have collaborated previously with both HJT and HSJD in other clinical trials (Díaz et al; Pediatric Diabetes 2015; Ibáñez et al; J Adolesc Health 2017; Ibáñez et al.; J Endocr Soc 2020).
  The budget allocated to the central reading of the images will guarantee the objectivity of the results and will include the following services: a) precise definition of the protocol to perform image acquisition; b) follow-up of the outcome of the extracted images; c) central reading of the images by the same individual, blinded to treatment allocation; 4) activation of the security protocol in the case of detection of incidental findings. The central reader will also keep a central repository (QUIBIM.com; Valencia, Spain), where the images will be stored for analysis. This central reader will be based at HSJD.
  Bone age will be assessed with a software for automated measurement using the BonExpert method (https://bonexpert.com). The software will be provided for free for this study by Visiana ApS (Hørsholm, Denmark). The company started the development of BonExpert in 2004, in collaboration with researchers at University Children's Hospital in Tübingen, Rigshospitalet in Copenhagen, Academic Medical Centre in Amsterdam, University Children's Hospital in Zurich and the Foundation for Growth Science in Tokyo. Since then, about 150 hospitals have incorporated the method to their clinical practice.

Laboratory consumables:

- The budget includes consumable needles, syringes, tubes, pipettes, and other material needed for blood sampling and processing of samples until assessment, including storage.

Other costs:

- Statistical studies will be requested for the final analysis of the data which will remain blinded for the entire duration of the active treatment phase (0-12 months) of the study.
- Publication of manuscripts includes the payment of processing fees for open access availability and other publication expenses.
- Transportation of samples will include shipments from HJT to HSJD for HMW-adiponectin, GDF15, and CXCL14 measurements. In addition, the shipments from HJT and HSJD to IMIM for testosterone and androstenedione assessments by LC-MS/MS.

B) Travel allowance

Travel expenses have been estimated to attend national and international conferences by members of the team: 2 subjects attending a national conference and 2 subjects attending a European conference. These expenses account for travel, subsistence allowance, registration and accommodation.

Application Number: ICI21/00005

Project Leader: Abel López Bermejo

ANNEXES (Text)

Max. 2 pages (10,000 characters)

ANEXO 1: INFORMACIÓN PARA LA FAMILIA

Tratamiento con pioglitazona + espironolactona + metformina a dosis bajas (HD-spiomet) para reducir la grasa hepática y visceral y la maduración acelerada en niñas con pubertad avanzada

En los primeros años de vida, es importante que exista un equilibrio entre la ganancia de peso prenatal y el aumento de peso postnatal, para prevenir la aparición de problemas endocrino-metabólicos. Cuando se produce un desajuste en este equilibrio (aumento importante de peso antes de la pubertad comparado con el peso al nacimiento), pueden aparecer alteraciones metabólicas que se manifiestan en la infancia como sobrepeso, aumento de la grasa visceral y hepática (obesidad central), resistencia a la insulina, pubarquia precoz (aparición de vello púbico antes de los 8 años) y pubertad adelantada (inicio del desarrollo mamario entre los 8-9 años) de evolución rápida. El exceso de grasa hepática y visceral y la resistencia a la insulina parecen ser los factores clave que determinan la aceleración del crecimiento y de la maduración ósea y el inicio más temprano de la pubertad con el consiguiente adelanto de la menarquia (primera regla). Estos factores también incrementan el riesgo de desarrollar síndrome del ovario poliquístico (SOPQ) en la adolescencia, caracterizado por trastornos de las reglas, aumento del vello corporal y producción excesiva de andrógenos en el ovario, y de padecer síndrome metabólico en la edad adulta, que comúnmente se manifiesta en forma de diabetes tipo 2 y enfermedades cardiovasculares.

Estudios realizados en el Hospital Sant Joan de Déu en colaboración con el Hospital de Girona Dr. Josep Trueta han demostrado que el tratamiento con metformina (un medicamento que mejora la función de la insulina) en niñas que tuvieron bajo peso al nacimiento que desarrollan pubarquia precoz y pubertad adelantada, reduce las cifras de insulina en sangre y la grasa hepática y visceral, y desacelera la maduración ósea ralentizando la progresión de la pubertad, retrasando la regla y aumentando la talla final. Otros estudios realizados en adolescentes con SOPQ han mostrado que la combinación a dosis bajas de metformina (850 mg/día), pioglitazona (otro medicamento que mejora la función de la insulina, a dosis de 7,5 mg/día), y espironolactona (un medicamento que activa el tejido adiposo marrón, que regula el metabolismo, a dosis de 50 mg/día) regula los ciclos menstruales, normaliza los niveles de andrógenos y de insulina, y disminuye la grasa hepática y visceral mucho más que la metformina en monoterapia, y en un período más corto.

La **metformina** es un medicamento de los denominados sensibilizadores a la acción de la insulina. Este producto se utiliza desde hace muchos años para el tratamiento de la diabetes tipo 2, y su efecto principal es reducir la producción de glucosa por parte del hígado y hacer que la insulina sea más efectiva (trabaje mejor). La metformina, a dosis bajas, no tiene efectos secundarios conocidos; a dosis elevadas (unas 20 veces superiores a las que se van a utilizar en este estudio), y en adultos con diabetes mal controlada y con insuficiencia renal, puede dar lugar a un trastorno conocido como acidosis láctica, cuyas consecuencias pueden ser potencialmente graves para la salud. Esta situación no es previsible en este estudio por las dosis bajas de metformina que se utilizarán.

La **espironolactona** es un medicamento del grupo de los denominados anti-andrógenos, que impide que los andrógenos fabricados en exceso puedan favorecer el crecimiento excesivo del vello y del acné. Es un medicamento utilizado desde hace décadas en mujeres con hirsutismo, a dosis más elevadas que las que se van a utilizar en este estudio, asociada en ocasiones a anticonceptivos orales. También activa el tejido adiposo marrón, lo que regula de forma beneficiosa el metabolismo, porque permite mayor gasto energético. A dosis al menos quince veces superiores a las previstas para este estudio, este medicamento puede producir aumento de la diuresis, o aumento de potasio en la sangre.

La **pioglitazona** es otro medicamento sensibilizante de la acción de la insulina, y también se utiliza en el tratamiento de la diabetes tipo 2. Aumenta la eficacia de la insulina, y transforma la grasa visceral en grasa subcutánea (que no es nociva); aumenta el "colesterol bueno", reduce las cifras de triglicéridos, y aumenta las concentraciones de una proteína producida por el tejido adiposo (adiponectina) que tiene propiedades protectoras del aparato cardiovascular. La pioglitazona (asociada o no a metformina) a dosis hasta 14 veces superiores a las que se pretende utilizar en este estudio no tiene efectos nocivos sobre la función hepática. En el 5% de pacientes tratados con dosis al menos ocho veces superiores a las de este estudio, pueden aparecer dolores musculares y ligera hinchazón de las piernas que suelen desaparecer en unos días.

En este estudio pretendemos determinar si el tratamiento con metformina, pioglitazona y espironolactona -a mitad de dosis de las utilizadas en adolescentes con SOPQ- reduce la grasa hepática y visceral y las concentraciones de insulina y de esta manera enlentece la maduración acelerada en niñas con pubertad avanzada.

Las pacientes que se consideren candidatas para el ensayo serán distribuidas aleatoriamente en dos grupos. A la mitad de ellas se les administrará un comprimido único (HD-spiomet) que conteniene 25 mg de espironolactona, 3.7 mg de pioglitazona, y 425 mg de metformina, que debe tomarse diariamente durante 12 meses. La otra mitad recibirá durante el

mismo periodo, una cápsula similar pero que contendrá sólo el excipiente (celulosa); a este comprimido se le denomina placebo. Transcurridos 12 meses, se comprobará si las pacientes que reciben HD-spiomet mejoran más que las que reciben placebo. El control de la paciente se continuará hasta 12 meses después de finalizado el tratamiento. Los comprimidos que contienen una u otra medicación serán codificados con números para que ni la paciente ni el médico que la trata sepan de qué producto se trata. Este tipo de procedimiento se denomina "doble ciego" y es la manera más eficaz de juzgar la eficacia de los medicamentos. Existe la posibilidad de que los datos obtenidos en este estudio, de forma totalmente anónima, se incorporen a los obtenidos en un proyecto similar que se realizará en varios países europeos. La finalidad es obtener información aún más extensa sobre los beneficios del tratamiento con HD-spiomet.

ANEXO 2: EXPLORACIONES Y ANÁLISIS

El protocolo incluirá la recogida de información clínica previa (en consulta con el endocrinólogo pediátrico) y unas pruebas -detalladas a continuación- que se realizarán antes de iniciar el tratamiento, a los 6 y 12 meses de tratamiento y de nuevo, 12 meses después de finalizar el tratamiento.

1) Edad, peso y altura, estadio puberal.
2) Analítica: recuento de glóbulos rojos y blancos, colesterol, glucosa, insulina, cifras de electrolitos, función renal y hepática, hormonas masculinas y femeninas, y niveles de vitamina B12 y ácido fólico. En el caso de que se detecte una alteración mínima de la función hepática, renal, glucosa, cifras de electrolitos o de glóbulos rojos y blancos se suspenderá inmediatamente la medicación, y se tomarán las medidas necesarias. Parte del suero se guardará a -80ºC para realizar la determinación de otros parámetros relacionados con la eficacia del tratamiento al final del estudio. La extracción se realizará por la mañana tras un ayuno de 8 horas.
3) Determinación de la edad ósea: Esta prueba sirve para determinar la madurez del esqueleto ya que no siempre se corresponde con la edad cronológica. Consiste en una radiografía de la muñeca y mano izquierda que se realizará en el servicio de Radiología del hospital. Se trata de un procedimiento seguro e indoloro que dura 5 minutos y no necesita preparación previa. La radiación que se imparte es 10 veces inferior a la recibida en una radiografía de tórax convencional, entre 30-40 veces inferior a la recibida en un vuelo transatlántico, y muy próxima a la dosis de radiación ambiental, por lo que se considera que no conlleva riesgos significativos.
4) Análisis de la grasa abdominal y hepática: se realizará en la Clínica Girona o en el CETIR por un profesional entrenado mediante resonancia magnética, una técnica de imagen que permite estimar la cantidad de grasa abdominal diferenciando la grasa subcutánea de la visceral (ésta última responsable de los problemas metabólicos) y determinar la cantidad de grasa en hígado. Esta prueba dura 30-40 minutos, no requiere preparación previa y no supone ningún riesgo para la salud. La resonancia magnética es una técnica ampliamente utilizada en la práctica clínica, no irradia, y no tiene contraindicaciones.

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ANNEXES (Images)

Max. 1 figure

Diagram of the study design: