Universitat de Girona
Facultat de Medicina

Final Degree Project

SPIRONOLACTONE, PIOGLITAZONE AND METFORMIN COMBINATION THERAPY TO SLOW BONE MATURATION IN MISMATCH GIRLS WITH EARLY PUBERTY

A randomised placebo-controlled clinical trial

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ABSTRACT

BACKGROUND	Currently, puberty is occurring earlier, particularly in girls who have experienced a "mismatch" sequence of less prenatal growth and more postnatal weight gain. This sequence may lead to ectopic lipid accumulation, which may trigger the activation of the gonadotrophic axis, resulting in advanced puberty. An earlier and faster tempo of puberty can lead to full-blown adolescent polycystic ovary syndrome, decreased adult height and numerous psychological consequences.
JUSTIFICATION	At present, there is no valid treatment available for girls experiencing advanced puberty (8-9 years). Metformin has demonstrated little efficacy in delaying puberty and slowing bone maturation in low-birth-weight (LBW) girls with precocious pubarche (PP). This trial is designed to evaluate a low dose combination of three generic drugs in girls without LBW or PP but with a "mismatch" sequence.
OBJECTIVES	The study aims to assess whether a low-dose combination of spironolactone-pioglitazone-metformin therapy (*mini-spiomet*) can slow down the accelerated bone maturation in "mismatch" girls with early puberty. Secondary objectives include evaluating the treatment's efficacy in slowing down pubertal tempo, improving metabolic-endocrine markers, and reducing hepato-visceral fat.
DESIGN	This study is a multicentre, randomized, double blind, placebo controlled, 2-year duration clinical trial.
PARTICIPANTS	Subjects will be Caucasian girls between the ages of 8-9 years old in the beginning of puberty (Tanner B2) with history of slightly decreased birth weight (BW) (between the 3rd and 33rd percentile) and a current body mass index (BMI) slightly above the mean (between the 66th and 97th percentile). A total of 64 girls will be selected (32 taking *mini-spiomet* and 32 taking placebo) according to inclusion and exclusion criteria.
MAIN OUTCOME MEASURES	Primary outcome: bone age. Secondary outcomes: Tanner breast stage; fasting glucose, fasting insulin, HOMA-IR, IGF-I, high-molecular-weight adiponectin (HMW-adip), sex hormone binding globulin (SHBG), ultra-sensitive C-reactive protein (usCRP), LDL-cholesterol, HDL-cholesterol, total cholesterol, triglycerides, testosterone, androstenedione, luteinizing hormone (LH), follicle-stimulating hormone (FSH) and estradiol; quantification of hepato-visceral fat, uterine and ovarian volumes.
INTERVENTION AND METHODS	Participants will be randomly assigned to receive either *mini-spiomet* or placebo in a 1:1 ratio. They will take one tablet a day for a period of two years. Follow-up assessments will be conducted every six months during regular visits.
SETTING	Multicentre. The study will be performed in two centres: Hospital Dr. Josep Trueta (Girona) and Hospital Sant de Déu (Barcelona).
KEY WORDS	Prenatal weight gain, postnatal weight gain, early puberty, advanced puberty, PCOS, ectopic fat, bone maturation, spironolactone, pioglitazone, metformin.

ABREVIATIONS

AEMPS	Agencia Española de Medicamentos y Productos Sanitarios
AMPK	AMP-activated protein kinase
BA	Bone age
BAT	Brown adipose tissue
BMI	Body Mass Index
BW	Birth Weight
B(nº)	Breast number (Breast stage of Tanner)
CA	Chronological age
CEIC	Comitè d'Ètica d'Investigació Clínica
CRO	Contract Research Organization
CXCL14	C-X-C motif chemokine ligand-14
DHEAS	Dehydroepiandrosterone-sulphate
EMA	European Medicines Agency
FAI	Free androgen index
FSH	Follicle stimulating hormone
GH	Growth hormone
GnRH	Gonadotropin-releasing hormone
HDL-cholesterol	High-density lipoprotein
HJT	Hospital Dr. Josep Trueta
HMW-adip	High-molecular-weight adiponectin
HOMA-IR	Homeostatis Model Assessment of insuline resistance
HPG axis	Hypothalamic-pituitary-gonadal axis
HSJD	Hospital Sant Joan de Déu
IGF-I	Insulin-like growth factor I

LBW	Low birth weight
LDL-cholesterol	Low-density lipoprotein
LH	Luteinizing hormone
MRI	Magnetic resonance imaging
NPY	Neuropeptide Y
PCOS	Polycystic ovary syndrome
PP	Precocious pubarche
SD	Standard deviation
SGA	Small for gestational age
SHBG	Sex hormone binding globulin
TSC	Trial Steering Committee
usCRP	Ultra-sensitive C reactive protein

INTRODUCTION

"Early" or "advanced" puberty is a normal variant of puberty that occurs when pubertal development begins between the ages of 8 and 9 years in girls. This condition is not strictly pathological but can result in negative consequences for final height or social considerations (1). Over the past decades, there has been a worldwide trend towards younger ages of pubertal onset and menarche in girls (2,3). At the same time, there has been a rise in childhood overweight and obesity in contemporary societies, which has appeared to play a key role in the global decrease of age at puberty, especially in girls (4–6). It is known that a certain amount of body fat is necessary for puberty to begin (7,8), and there is strong evidence suggesting that body fat and the initiation of the hormonal events of puberty are in some way related (4,5,9–11).

MODULATION OF GnRH NEURONS

The onset of puberty is controlled by the hypothalamic-pituitary-gonadal (HPG) axis (7,10). The HPG axis is firstly activated during neonatal development and early infancy. Then, it experiments a quiescent period during childhood when it remains inactive, until it is restarted at the beginning of second decade of life (10). What occurs when the HPG axis is reactivated is that gonadotropin-releasing neurons (GnRH) located in the hypothalamus initiate the pulsatile release of GnRH. This GnRH pulses control gonadotrophins production and release (LH and FSH) by the pituitary gland, which finally regulates the gonadal secretion of sexual steroids (estrogens and androgens) and gametogenesis (7,10,12). In girls, the most reliable sign of estrogenic activity, and therefore, the most direct sign of ovarian activation, is the appearance of glandular breast tissue (10). This clinical feature is known as thelarche and its development is assessed using the clinical breast Tanner scale (see **annex 1**).

An essential requirement to initiate reproductive function is the modulation of the pulsatile secretion of GnRH. The precise mechanism triggering the HPG axis reactivation, and the underlying connection between adipose tissue and regulation of puberty onset have remained unknown until the discovery of kisspeptin neurons (7,10). These neurons

are located in the hypothalamus and serve as the main conveyor of metabolic cues derived from adipose tissue to control the reproductive axis. Kisspeptin neurons are subjected to a plethora regulatory factors and depend on a large number of peripheral signs, among which leptin, ghrelin and insulin, have been recognised to be the main peripheral modulators (7).

Leptin's role in the pubertal onset

Leptin is a peptide hormone synthesised in adipocytes and secreted at levels proportional to existing adipose tissue deposits (12). It acts as a mediator between energy storage and the HPG axis during puberty onset, informing to the brain that body fat reserves are adequate to cover the energy required for reproduction (10,13). The leptin surge promotes and up-regulates kisspeptin secretion, triggering GnRH pulsatile secretion (10,14,15). Higher concentrations of leptin in the serum have been observed in overweight and obese individuals when compared to those with a normal weight. These significantly elevated leptin levels are strongly correlated with the individual's BMI and percentage of body fat mass, with the latter being the main reason for early pubertal development (10).

Grhelin's role in the pubertal onset

Ghrelin is a gastric peptide hormone that increases appetite. Besides acting as an additional central hunger signal (7), it is also known to act by upregulating ceramide synthesis (16). Ceramides are a large family of lipid signalling molecules involved in a wide range of biological processes, and recent studies have demonstrated a relevant role for ceramides as transmitters for the central actions of leptin and ghrelin. High-fat diet-induced obesity, favoured by the action of ghrelin, may contribute to the early onset of puberty by increasing hypothalamic ceramide content (16).

Insulin's role in the pubertal onset

Insulin is a peptide hormone synthesised in the endocrine pancreatic cells which contributes to the control of glucose homeostasis. It promotes fat storage, and like

leptin, it circulates in the periphery at levels proportionate to fat body content (8). Insulin resistance is often associated with hypogonadotropic hypogonadism. Although the exact mechanism underlying this association is unknown, insulin has been shown to be essential for gonadotropin release in the brain. Kisspeptin neurons express insulin receptors, suggesting a likely direct effect of insulin on these neurons to transmit postprandial glucose information to the HPG axis (7). In addition, insulin has been shown to activate GnRH neurons indirectly (8). There are two different targets by which insulin indirectly activates GnRH secretion. The first one is the inhibition of hypothalamic neuropeptide Y (NPY) expression. Since NPY itself inhibits GnRH secretion, when insulin inhibits NPY expression, GnRH secretion is induced. The other target to the action of insulin are GABAergic neurons, which have an inhibitory effect on GnRH expression and secretion. Thus, insulin has the potential to remove this inhibition and induce GnRH production (8).

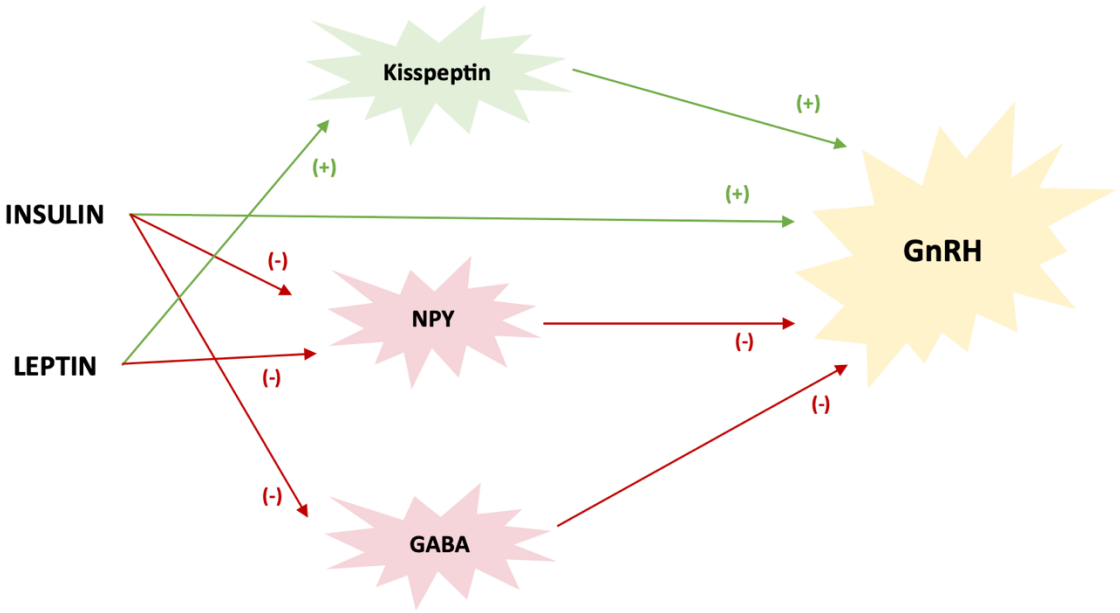


***Figure 1**. Adapted schematic representation of possible hypothalamic pathways involved in insulin and leptin signaling for neuroendocrine reproductive modulations, from "Insulin and NPY pathways on the control of GnRH function and puberty onset"* (8)

Importantly, the evidence for an indirect mode of action of such peripheral main factors on kisspeptin neurons illustrates the complexity of the central circuits linking metabolism and reproduction. Therefore, multiple levels of regulation and overlapping pathways are likely to be involved.

Recently, new central signaling pathways have been identified as novel mediators of adiposity-induced early puberty. One of these involves AMP-activated protein kinase (AMPK), the fundamental cellular sensor that is activated under conditions of energy deficit. AMPK has been shown to be involved in the control of kisspeptin neurons by driving an inhibitory valence in situations of energy deprivation during puberty (17).

CENTRAL FAT AS A RESULT FROM OPPOSSED PRENATAL AND POSTNATAL GROWTH

All of these previously described findings, support the idea that body adiposity, potentially influenced by the hyperleptinemia and insulin resistance, is a key contributor to an earlier variation in pubertal timing (18,19). Excess fat storage in prepubertal girls occurs primarily in the liver and the viscera, and it is the result of two major pathways (20):

- One begins with normal prenatal growth (and normal subcutaneous fat storage capacity), followed postnatally by a chronic positive energy balance with supranormal weight gain.
- The other major pathway starts with reduced prenatal growth (and consequently reduced subcutaneous fat storage capacity), followed by normal weight gain.

In summary, the combination of these two pathways often results in a mismatch between prenatal and postnatal weight gain, which can be estimated by calculating the Z-score change from birth weight (BW) to childhood body mass index (BMI), ultimately contributing to this excess of hepato-visceral fat and earlier pubertal timing (18,19).

AN ADAPTATIVE RESPONSE TO ECTOPIC FAT ACUMULATION IN MISMATCH GIRLS

It has been hypothesised that the clinical expression of early and rapid pubertal maturation in girls is an adaptive mechanism to escape ectopic fat accumulation (21).

The homeostatic responses to counteract and reduce the central adiposity have been shown to accelerate body growth and maturation (21). These responses include a decrease in circulating sex hormone-binding globulin (SHBG), followed by an early and

amplified adrenarche with higher levels of its marker, dehydroepiandrosterone-sulphate (DHEAS), and by the appearance of pubic and axillary hair, acne and pubertal odour (22,23). The onset of pubic hair is primarily due to the onset of adrenal gland androgen production rather than being directly related to the ovarian activation (10), and its development is assessed using the pubic hair Tanner stages (see **annex 1**). Another adaptive response is a decrease in circulating adiponectin levels. Adiponectin is an adipokine with insulin-sensitising and cardiovascular protective properties. A reduced serum adiponectin concentration may not only be associated with an increased risk of cardiovascular disease and insulin resistance, but it may also lead to a reduction in intracellular ceramidase activity (24). This will contribute to ceramide accumulation in the hypothalamus and liver, triggering the onset of puberty (16).

Therefore, it has been shown that these opposing influences of pre- and postnatal weight gain not only reflect forces that physiologically point towards body adiposity, but are also associated with relative insulin resistance in early childhood, which may be reflected in higher circulating IGF-I levels (18), as well as in the homeostasis model assessment of insulin resistance HOMA-IR (19). Insulin resistance has traditionally been defined as a reduced ability of insulin to mediate metabolic effects on glucose uptake and glucose production, resulting in an increased requirement for insulin to achieve the given metabolic effect. Under normal conditions, the effect of insulin on theca cell androgen production is only apparent at supraphysiological concentrations of insulin. For these reason, theca cells (found in the ovarian follicles) of girls who are insulin resistant may be more responsive to the androgen-stimulating effects of insulin, so that they have elevated serum circulating androgen levels (testosterone, androstenedione) (25).

UTERINE AND OVARIAN CHANGES DURING PUBERTAL DEVELOPMENT

The female reproductive system comprises the uterus and ovaries. Although evidence has demonstrated growth of these organs before the appearance of breast development (thelarche) and pubic hair (pubarche) (26), they undergo significant changes during pubertal development, particularly in terms of shape and volume. It is important to note

that this growth is objective, assessed by pelvic ultrasound, and not influenced by subjective evaluations.

Uterus

The shape of the uterus varies depending on the age. During neonatal period and infancy, it is drop-shaped and a small decrease in size can be seen. Then, in later childhood low increase in length and volume is found, and normally by the age of 8 years, it acquires a tubular form. During puberty, the typical pear shape is reached, and both length and volume start to increase significantly until they reach adult dimensions at about the age of 16 years. Usually, uterine length and volume are related to age or to Tanner breast classification. Another noticeable change is the relation of cervix to corpus. It is 2:1 in the prepubertal stage and 1:2 postpubertally, and the typical angle between the uterine corpus and the cervix can be seen only after puberty. Uterus contour in prepubertal girls is smooth and its structure is fine, with low echodensity seen in the ultrasound examination. Then, after puberty, a zone of higher echodensity in the centre that represents the mucosa is apparent and is clearly seen, especially premenstrually (27).

Ovaries

The shape of the ovary is oval. It has a smooth contour and a homogenous structure with low echodensity. Frequently, small cysts can be seen with the ultrasound pelvic examination, which correspond to the ovarian follicles. These can be detected from the age of early infancy onward, and they increase progressively in size and number after the age of 8.5 years, considering the pubertal onset at the age of 10. Three to four small cysts can be considered normal at any age (27).

Ovarian volume is relatively stable in early childhood and shows only little increase before puberty. Because of this relative stability, it is correct to define all ovaries <1ml in volume as prepubertal. During puberty, a steep increase in volume occurs and ovarian growth continues even after menarche. The average volume of the ovaries in a young adult is approximately 6.5 ml (27).

Ovarian maturation is influenced by age, although it exists is a positive correlation between the Tanner breast score and ovarian volume.

Together with uterine size, ovarian volume is a reliable indicator of the degree of development in girls (27).

Tanner score	Uterine volume, ml	Ovarian volume, ml
B1	1.0 (0.3)	0.7 (0.4)
B2	2.3 (0.6)	1.6 (0.9)
B3	10.3 (6.1)	3.5 (1.8)
B4-5	24.6 (14.4)	7.4 (4.8)

***Table 1**. Uterine and ovarian volumes (mean (SD)) in relation to Tanner score (breast stage)*

ADVANCED PUBERTY AND BONE MATURATION

Growth in height is driven by bone maturation through two processes: enchondroplasia and chondral osteogenesis at the epiphyseal plate, also known as the growth plate. Puberty can be defined as the transitional period from childhood through the development of secondary sexual characteristics to the achievement of final height in adulthood (28). During childhood, there are differences in the maturation status of the bones with enchondroplasia, such as the radius and ulna (long bones) and metacarpals and phalanges (short bones), and bones with chondral osteogenesis, such as the carpal bones. The maturation status of the carpal bones is an important variable in the status of other cuboid bones (mainly the vertebrae), which make an important contribution (about 40%) to adult height (29).

Physiologically, bone maturation is organised by a complex network of nutritional, cellular, paracrine, and endocrine factors. The most important of these are the close interactions between the estrogens and the growth hormone (GH). Both contribute to the regulation of growth and puberty through their roles at the growth plate. GH stimulates the synthesis and secretion of IGF-I in the liver and epiphyseal plate. IGF-I in turn, promotes chondrocyte proliferation, differentiation and hypertrophy, and ultimately contributes to the chondral osteogenesis in the growth plate (28). The effect

of estrogens on longitudinal bone growth is biphasic: at low levels, they stimulate growth (involving prepubertal and early pubertal stages), whereas higher levels of estrogens have strong inhibitory effects on longitudinal growth by accelerating epiphyseal closure (30,31).

Thus, as in advanced puberty, there is a premature exposure to high levels of estrogens, it contributes to accelerate the skeletal maturation, ultimately resulting in a short growing period and a decreased final height (6).

IMPACT OF EARLY PUBERTY IN HEALTH

In the first years after menarche, when adult height is almost attained, the compensatory effect of pubertal maturation and body growth on central fat accumulation is lost. If the energy balance remains chronically positive, the underlying drive for ectopic adiposity will persist, and the endocrine-metabolic responses to this drive such as insulin resistance, low adiponectin and SHBG, will also persist. This could potentially lead to a complete phenotype of adolescent polycystic ovarian syndrome (PCOS) characterised by hypersecretion of luteinizing hormone (LH), resulting in excess ovarian androgen and oligoanovulation (17). The cardiovascular reflection of this ectopic fat accumulation often follows a trend towards higher blood pressure starting in early childhood (19,21,32), and earlier pubertal development is known to be associated with higher future risk of gestational diabetes (33), type 2 diabetes (34), and breast and endometrial cancer (35).

In addition, puberty advancement can also lead to psychosocial discomfortable consequences such as embarrassment, higher levels of delinquent and aggressive behaviour and more susceptibility to negative peer influences (36).

PREVIOUS STUDIES

Attempts to treat advanced puberty with GnRH analogues

Depot forms of GnRH agonists are currently the standard treatment for progressive central precocious puberty. The mechanism of action of these treatments is to suppress the gonadotropic axis, with the aim of alleviating the clinical symptoms of precocious pubertal development, as well as its psychological and growth consequences. Precocious puberty is a heterogeneous condition and strict criteria should be used to define it, both in terms of age and in terms of potential for progression. Although the age limits have been debated, most physicians consider the onset of pubertal development in girls before the age of 8 years to be "precocious puberty". It is important to emphasise this because, unfortunately, in girls with onset of puberty in the lower half of the normal age distribution (8-10 years) - including advanced puberty in this lower half of normality - GnRH agonists have shown negative results in terms of treatment benefit (37,38).

Metformin therapy in rapidly maturing girls with precocious pubarche

Some years later, several studies have been performed in rapidly maturing mismatch girls with precocious pubarche which have shown favourable results in reducing central adiposity with metformin monotherapy (39–41). Additionally, they have demonstrated the efficacy of metformin in slowing down bone maturation (6), delaying pubertal onset (42), decelerating the progression of puberty to menarche (43) and to adolescent PCOS (44), while increasing height gain (43). The efficacy of metformin monotherapy for all these outcomes has been demonstrated over a period of 4-years of treatment period at doses up to 850 mg per day.

"SPIOMET" therapy in mismatch adolescents with PCOS

Other studies have been carried out in adolescent girls with PCOS. PCOS is a condition characterised by androgen excess and oligoamenorrhea, often caused by ectopic fat accumulation (45). These trials have shown efficacy of spironolactone-pioglitazone-metformin (SPIOMET) combination therapy in reversing the entire PCOS phenotype after only 1 year of treatment. The mechanism by which this triple pharmacological

combination reverses menstrual irregularities, hyperandrogenaemia, and insulin resistance is by reducing the hepato-visceral fat excess (45–48), which, as previously mentioned, is thought to be the main driver of such early maturation ending up in full-blown PCOS.

COMPONENTS OF TRIPLE DRUG COMBINATION *SPIOMET*

Spironolactone

Mineralocorticoids, such as aldosterone, regulate salt and water balance by activating mineralocorticoid receptors, which are widely expressed in many tissues. The role of mineralocorticoid system in adipose tissue is not fully understood. What is known is that the metabolic syndrome is commonly found in patients with primary hyperaldosteronism (49). In addition, aldosterone concentration is associated with obesity and metabolic risk in the general population (50,51). These observations suggest that the mineralocorticoid system may play a role in energy and substrate homeostasis.

Brown adipose tissue (BAT) is a thermogenic energy dissipating organ that protects against hypothermia and obesity, and plays an important role in energy balance in humans (52). Cold temperature is a potent stimulus of BAT function, with prolonged exposure improving insulin sensitivity and reducing fat mass. As cold exposure was unlikely to be widely accepted, there was considerable interest in developing pharmacological therapies to stimulate BAT function for the treatment of obesity. This, favoured the development of several studies to demonstrate efficacy of mineralocorticoid antagonists in improving BAT function, which eventually end up being demonstrated (53). An example of a mineralocorticoid antagonist is spironolactone.

Spironolactone is an aldosterone antagonist commonly used as a diuretic to serve as an antihypertensive agent. In addition, as noted above, it has recently been identified as a potent activator of brown adipose tissue, and thus as a potential driver of energy expenditure (54). Spironolactone has also been used as the antiandrogen of choice in the treatment of hyperandrogenism (primarily hirsutism), one of the main clinical features in girls with PCOS. The antiandrogenic effect of spironolactone is due to its

affinity for androgen receptors, and it has been employed for decades with an excellent safety profile. The only minor adverse effects reported at high doses (100 mg/day or more) are menstrual irregularities, abdominal pain, polyuria and dry mouth (55).

Pioglitazone

Pioglitazone is a thiazolidinedione that enhances insulin sensitivity and is currently used as a therapeutic agent for type 2 diabetes (56–58). One of the mechanisms by which pioglitazone acts as an insulin sensitizer is through the redistribution of adipose tissue, resulting in a reduction in visceral adipose tissue and an increase in lower-body fat, thereby promoting adipose tissue expansion (adipogenesis) in subcutaneous depots. Lower-body fat depots are associated with lower health risks and are metabolically protective (59–61). Conversely, preferential accumulation of ectopic fat, mainly visceral and abdominal adipose depots, is associated with obesity-related diseases (62,63). This is supported by data from various clinical and epidemiological studies (64,65), which suggest that there are inherent differences in the physiology and characteristics of fat depots (66). Thus, pioglitazone treatment may have a greater effect on the adipogenic rate in the metabolically protective subcutaneous depot, while inducing a significant decrease in visceral fat and improving insulin sensitivity (67–69).

Moreover, low-dose pioglitazone therapy (7.5 mg/day) has been shown to increase serum high molecular weight adiponectin (HMW-adip) levels in young women with hyperinsulinemic androgen excess. Adiponectin has anti-therogenic, anti-inflammatory and insulin-mimetic properties, and its circulating levels are generally associated with improved insulin sensitivity and metabolic health (70). The use of low-dose pioglitazone has demonstrated an excellent safety profile in adolescent girls with PCOS (45), and no adverse effects have been reported in children (71).

In addition, pioglitazone has demonstrated to induce the expression of CXCL14, a chemokine released by BAT that protects against insulin resistance. This metabolic effect occurring in adipocytes, has also been shown to occur with low-dose spironolactone therapy (50 mg/day) (54).

Metformin

Hyperinsulinemic insulin resistance, along with total, visceral, and hepatic adiposity is believed to be a significant contributor to early and rapidly progressive pubertal maturation (43). Therefore, metformin, the main insulin sensitizer and the biguanide most widely approved for the treatment of type 2 diabetes, has significantly increased in use among younger girls for the treatment of early maturation and PCOS. Metformin increases insulin sensitivity by reducing IGF-I circulating levels. It also increases serum levels of SHBG. This, may lead to a lower fraction of circulating free estrogens, which in turn, may be followed by a later closure of the epiphyses. Additionally, it reduces visceral and hepatic fat, leptin, and fasting insulin levels, while increasing HMW adiponectin, resulting in a more favourable lipid profile (40,43). Metformin therapy is also involved in rising AMPK activity, which, as mentioned above, is considered to be one of the most important conservative regulators of the cellular response to low energy (72). When activated, AMPK switches cells from an anabolic to a catabolic state, shutting down the ATP-consuming synthetic pathways and restoring energy balance (73). Thus, AMPK acts indeed as a signal for energy deprivation, which inhibits the action kisspeptin neurons and, furthermore, delays the onset and progression of puberty in girls.

Sixty years of clinical experience and trial data have yielded almost no safety concerns with metformin (74). Moreover, the European Medicines Agency (EMA) has issued a favourable risk-benefit assessment for its use, which outlines its safety in humans. The major safety exception is that metformin causes subclinical increases in lactic acid and appears to cause lactic acidosis in extreme overdose (74). However, lactic acidosis has only been described in cases of renal, cardiac and hepatic failure or after deliberate overdose (75). On the other hand, the most common adverse effects of metformin are gastrointestinal symptoms, especially diarrhoea and nausea (74). A decrease in serum B12 vitamin levels has also been associated with long-term metformin treatment, but it does not seem to be clinically relevant (76).

JUSTIFICATION

For the last decades, contemporary societies have experienced an increase in incidence and prevalence of advanced puberty, especially in girls. The early sexual development of these girls, compared to the rest of their classmates, and the concerns about adult height are common reasons for consultation with outpatient pediatric endocrinologists.

Girls with advanced puberty are not considered to be under a pathological physical status. However, their condition could potentially lead to several not negligible consequences in the short and long term, not only affecting the psychosocial sphere, but also involving their physical health and well-being.

Metformin treatment during four years in rapidly maturing girls with precocious pubarche has shown favourable results in terms of delaying pubertal onset (42), delaying the age of menarche and augmenting adult height (43). Additionally, metformin monotherapy has demonstrated efficacy in reducing hepato-visceral fat excess (6,41) and slowing bone maturation in low-birth-weight rapidly maturing girls (6).

Despite the fact that all these studies have demonstrated metformin's efficacy, it has not been standardized as a valid treatment for advanced puberty. Its efficacy is poor in relation to the duration of the treatment. Four years of treatment is considerably a long period of time, in which there is a potential risk of adverse effects in girls under a non-strictly pathological condition. This, in conclusion, ends up by compromising the risk-benefit ratio of metformin's monotherapy in girls with early puberty.

Another argument against validating metformin treatment is that it must be given for at least four years to obtain significant results in terms of slowing pubertal development and bone maturation. Considering that, girls should undergo the initiation of metformin's treatment before they are diagnosed of early puberty, which typically appears between the ages of 8 and 9 years old.

The aim of this project is to demonstrate the efficacy of *mini-spiomet* treatment, a combination of spironolactone-pioglitazone-metformin, in slowing bone maturation and delaying pubertal development in mismatch girls with advanced puberty. This therapy is designed to achieve these results by targeting three different metabolic pathways that together will reduce ectopic fat. Since it is a triple therapy, it can be inferred that the effect will be greater than that of metformin monotherapy. Therefore, it should be administered for a shorter period of time to achieve results, in this case for two years.

The study design will be a placebo-controlled clinical trial, and the main reason why the trial will be compared to placebo rather than compared to metformin is because there is currently no validated standard treatment for advanced puberty. Therefore, the decision not to treat patients is considered an appropriate medical act in clinical practice.

HYPOTHESIS

MAIN HYPOTHESIS

Two years of *mini-spiomet* treatment slows down the rapid bone maturation in "mismatch" girls with early puberty.

SECONDARY HYPOTHESES

1. The administration of *mini-spiomet* delays the progression of puberty in "mismatch" girls with advanced puberty.
2. *Mini-spiomet* treatment improves endocrine and metabolic markers in "mismatch" girls with early puberty.
3. *Mini-spiomet* therapy reduces hepatic and visceral fat in "mismatch" girls with early puberty.
4. Girls treated with *mini-spiomet* have smaller uterine and ovarian volumes than girls treated with placebo, which is consistent with a delay in pubertal development.

OBJECTIVES

MAIN OBJECTIVE

The main purpose of this project is to assess if a low-dose combination of three generics (spironolactone-pioglitazone-metformin) can slow down the accelerated bone maturation (as evaluated by Δ bone age (BA) / Δ chronological age (CA) ratio over 2 years) in "mismatch" girls with early puberty.

SECONDARY OBJECTIVES

1. To determine if *mini-spiomet* treatment can slow down the rapid tempo of pubertal development, as measured by the progression of the Tanner breast stage, in "mismatch" girls with early puberty.

2. To assess whether girls treated with *mini-spiomet* have more normal levels and improved endocrine-metabolic markers, as compared to girls who received placebo.
3. To assess if *mini-spiomet* therapy in “mismatch” girls with advanced puberty can reduce the ectopic hepato-visceral fat significantly more than placebo, as assessed by abdominal MRI.
4. To determine if the uterine and ovarian volumes, assessed by pelvic ultrasound, of girls treated with *mini-spiomet* have experienced less growth (consistent with a delay in pubertal development) than the uterine and ovarian volumes from those girls treated with placebo.

METHODOLOGY

STUDY DESIGN

This project is designed as a multicentric, randomized, placebo-controlled, double-blinded and 2-year duration clinical trial. It seeks to prove for the first time the efficacy of *mini-spiomet* on decelerating bone and pubertal maturation.

The study aims to test the hypothesis that the administration of *mini-spiomet* (spironolactone 25 mg/day, pioglitazone 3.75 mg/day, metformin 425 mg/day) causes a reduction of ectopic adiposity and consequently slows down bone maturation and pubertal development compared to placebo in mismatch girls diagnosed with advanced puberty. In addition, the study whishes to demonstrate that in these girls, *mini-spiomet* therapy improves endocrine and metabolic markers.

Two groups of patients will be treated for two years (one with *mini-spiomet* and the other one with placebo) and will be followed every 6 months with follow-up visits.

Study type	Interventional: Clinical Trial, Prospective
Health care centre	Multicentre (2 hospitals)
Allocation	Randomised
Control type	Placebo
Intervention Model	Parallel assignment
Masking	Double blind (patient, investigator)
Primary purpose	Compare and demonstrate superiority of *mini-spiomet*

***Table 2**. Study design*

SETTING

This clinical trial will be a multicentre study which involves two tertiary hospitals:

- Hospital Universitari Dr. Josep Trueta (Girona) (HJT)
- Hospital Sant Joan de Déu (Barcelona) (HSJD)

Both centres are referral hospitals that provide an appropriate setting to conduct study visits and a research institute with a laboratory with the proper equipment to perform the study.

A Trial Steering Committee (TSC) will be created by principal investigators in order to ensure and enhance communication between centres.

STUDY POPULATION

The study population are Spanish Caucasian "mismatch" girls between 8 and 9 years old diagnosed of early puberty.

We define "mismatch" as the combination of the following two:

- History of birth weight between the 3rd and the 33rd percentile.
- BMI at the present between the 66th and the 97th percentile.

INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria

1. Age at study start between 8 and 9 years
2. Birth weight for gestational age between the 3rd and the 33rd percentile
3. BMI for chronological age between the 66th and the 97th percentile
4. Breast 2 Tanner stage starting between the ages of 8 and 9 years
5. Caucasian ethnicity
6. History of full-term pregnancy: 37 weeks ≤ gestational age < 42 weeks
7. Height at first visit between the 3rd percentile and the 97th percentile

Exclusion criteria

1. Excessive delay or advance of bone age (more than 2 years for chronological age)
2. Tanner stage of breast development greater than 2
3. Twin pregnancy
4. Obesity at first visit (BMI above 97th percentile)
5. Evidence for a pathological cause of the rapid maturation (i.e., congenital adrenal hyperplasia due to 21-hydroxylase deficiency)
6. Known genetic abnormality or chronic conditions, including cardiovascular, neurological, immunological, metabolic, renal, endocrine, digestive, respiratory or oncological diseases
7. Chronic use of medications, among others: anticoagulants, anti-inflammatories, oral hypoglycemic agents, antiandrogens, estrogens, progestins, glucocorticoids, digoxin. Only the use of paracetamol before or during the course of the study will be accepted
8. Acute infections or intake of antibiotics or anti-inflammatory medications in the last 14 days
9. Any disease that, in the opinion of the investigator, compromises the inclusion of the subject in the clinical trial

JUSTIFICATION OF INCLUSION CRITERIA

- **Age between 8 and 9 years old.** Early or advanced puberty is a normal variant of the onset of pubertal development. In girls, it is defined as the start of puberty between the ages of 8 and 9. This protocol will therefore include girls who experience puberty onset within this age range.

- **Birthweight (BW) for gestational between the 3rd percentile and the 33rd percentile.** The main objective of this project is to study the effect of *mini-spiomet* on advanced puberty in those girls with an excess of central ectopic fat. This excess of fat results from a mismatch between a birth weight below the mean (under 50th percentile) and postnatal weight gain resulting in a BMI over the mean (above 50th percentile). Girls with history of being small for gestational age (SGA) are not included because it has already been seen that they typically experience a postnatal catch-up growth which may lead to ectopic fat accumulation and rapid transition to puberty (77). This study, instead, aims to investigate a more standard population so that a larger number of girls with advanced puberty could be included. For this reason girls under the concept of mismatch will be included, which is a broader concept that encompasses more subjects.

- **BMI for chronological age between the 66th percentile and the 97th percentile**. Girls with a BMI above the mean (50th percentile) will be included to comply with the mismatch sequence concept. Girls with obesity (above 97th percentile) will not be included in the study as it aims to examine a more standard population and including them would mean introducing more variability into the study population, making it more susceptible to confounding. Additionally, the prevalence of obesity among girls is low, which would make it difficult to find enough subjects.

- **Breast 2 Tanner stage starting between the ages of 8 and 9 years.** Girls must show a Tanner stage 2 for breast development, indicating early pubertal

development, when they are between 8 and 9 years old to be classified as having early puberty.

- **Caucasian ethnicity**. Depending on the ethnicity the age of puberty onset can vary. Some studies (78,79) have concluded that African or black girls have an earlier pubertal timing compared to Caucasian girls.

- **Full term pregnancy (37 ≤ gestational age < 42 weeks).** Premature girls are not included because, as mentioned, the aim is to examine a more normal population of girls and therefore the extremes of normality should not be included. Otherwise, the study would be subjected to a greater possibility of confounding variables that could bias the results obtained.

- **Height at first visit between 3rd percentile and 97th percentile.** Only girls with a height between the 3rd and the 97th percentile will be included, to avoid introducing excessive variability into the study population and thus confounding.

WITHDRAWAL CRITERIA

Subjects may be withdrawn from the study because of the following reasons:

- Patient is not willing to comply with the protocol and instructions provided by the investigators.
- Patient who has poor treatment adherence rate (below 80%).
- For medical reasons, as assessed by the investigators: adverse events or sudden intercurrent relevant disease or condition that could affect the intervention effect.
- If they decline consent to continue in the study.

Girls withdrawn from the trial will not be replaced.

SAMPLE SIZE

Considering the results of studies with metformin in girls with a history of low-BW and rapid postnatal catch-up weight (6), it is expected to observe a minimum difference of 0.2 in the progression of bone maturation, assessed by the mean ratio of Δ BA over Δ CA in a year. As our study will be performed during two years, we wish to detect a difference of at least 0.4 between the two groups.

So, accepting an alpha risk of 0.05 and a beta risk of 0.2 in a two-sided test, 31 subjects are needed in the first group (mini-spiomet) and 31 in the second (placebo) to detect as statistically significant a difference greater than or equal to 0.4 units in the ratio of Δ BA over Δ CA in 2 years. The common standard deviation is assumed to be 0.5, and a drop-out rate of 20% has been estimated.

For randomization purposes, the sample will be split in three strata (chronological age, birth weight and BMI) in order to avoid having differences in the distribution of these variables at baseline between groups. Therefore, they will be needed 32 subjects in each study arm, which yields a sample size of 64 patients.

The following website has been used to calculate the sample size:

https://apisal.es/Investigacion/Recursos/granmo.html

ENROLLMENT AND PATIENT SELECTION

We will perform a consecutive non-probabilistic sampling. Our sample will be taken from girls who are referred by the general pediatrician to the pediatric endocrinologist due to the advancement of puberty. Eligible girls for participation in our study must be between 8 and 9 years old, and with a "mismatch" sequence of reduced prenatal weight gain and augmented postnatal weight gain followed by early progressive puberty.

In the first visit with each prospective candidate and their parents or legal tutors, the investigator will provide detailed information about the study (**annex 5**) explaining the possible *mini-spiomet* adverse reactions and also the right to withdraw from the study at any time. If they agree with the protocol procedure, a request to sign a written informed consent will be made (**annex 6**).

In order to accomplish the inclusion and exclusion criteria and especially ensure there are no contraindications to *mini-spiomet* treatment, a complete clinical history and physical examination will be performed during a second visit 4 weeks before the start of the study. At this visit, a baseline blood test will be carried out to rule out any abnormalities that could hinder participation in the study. The parameters to be assessed will be: circulating concentrations of 17-hydroxyprogesterone (17-OHP) in order to rule out congenital adrenal hyperplasia due to 21-hydroxylase deficiency or abnormal thyroid, kidney and liver function. Any alteration of these parameters will be exclusion criteria. Additionally, the investigator will order for safety markers and endocrine-metabolic laboratory parameters (see ***Table 3***. *Variables*) so that their results can be analysed in the following visit. Also an abdominal MRI scan, a left hand X-Ray and a pelvic ultrasound will be scheduled for the next appointment to evaluate abdominal fat distribution, bone age progression and ovarian and uterine volumes, respectively.

A week before the onset of the study, a third visit will be scheduled. During this visit, laboratory results will be checked (see ***Flow Chart***), and clinical variables such as height, weight, BMI, waist and hip circumference, Tanner breast stage, dietary habits and physical activity will be evaluated.

A total of 64 girls are needed to enable the development of the trial. Although the sample size needed is not excessively big, our inclusion and exclusion criteria are quite strict. Besides, due to the fact that our patients are divided into three strata, the rate of inclusion may be slower for the last participants. As a result, it is expected that it will take 12 months to recruit all the subjects. In case the sample is not attained, the recruitment time can be extended.

RANDOMISATION

Participants will be assigned a registration number used to identify each of them throughout the study. They will be 1:1 randomised using block randomisation into *mini-spiomet* and placebo groups. The randomisation will be made according to a randomisation list generated per centre by using the programme Blockrand: Randomisation for Block Random Clinical Trial R package, version 1.5; Published: 2020; Author: Gdreg Snow. In this list, patients will be subdivided into strata and then permuted block randomisation will be used for each stratum. There will be a total of 4 blocks of 8 subjects each, with the blocks balanced by chronological age, birth weight, BMI and treatment. This will ensure the comparability of treatment groups by main covariables:

- **Chronological age (CA)**: disproportion of younger and older patients between the two study groups could cause differences in mean bone age (80) and puberty onset, which could affect the study results. So, in order to avoid this disproportion a first group with age between 8 and 8.4 years and a second group with age between 8.5 and 9 years will be created.

- **Birth weight (BW)**: postnatal rapid catch-up growth in girls born small for gestational age (SGA) is a predisposing factor for a higher BMI, early puberty and accelerated skeletal maturation (77). Our study excludes girls who were small for gestational age at birth, but it includes girls who had birth weight for gestational age below the mean, specifically between the 3rd and the 33rd percentile. However, grouping them under this interval does not imply that they are affected

in the same way by this variable. The accumulation of ectopic adipose tissue increases with the degree of mismatch between reduced prenatal weight and postnatal weight gain. This, in turn, has a greater effect on the acceleration of bone and pubertal maturation. Therefore, two groups will be created. The first group will include girls with history of BW between the 3rd percentile and the 15th percentile, and the second group with BW between the 15th percentile and the 33rd percentile.

- **BMI**: a higher BMI could lead to more collection of adipose tissue. This drives to higher androgen conversion into estrogens and consequently faster bone maturation (81,82). Furthermore, an increase in BMI could cause not only higher fat mass, but also serum leptins that may advance puberty onset (80). For this reason, two groups will be created, the first group with BMI between the 66th and 85th percentile, and the second one with BMI between the 85th and the 97th percentile.

VARIABLES AND INSTRUMENTATION

***Table 3**. Variables and instrumentation*

VARIABLES			TYPE	INSTRUMENTATION	UNITS
Independent		Mini-spiomet or placebo	DcQV	Randomization list (Blockrand program)	mg/day
Dependent	Imaging variable	Bone age	CQV	BoneXpert	Years & SDS
		Hepatic and abdominal fat	CQV	MRI	%
		Uterine and ovarian volumes	CQV	Pelvic ultrasound examination	ml
	Endocrine and metabolic variables	Fasting glucose	CQV	Glucose oxidase method	mg/dl
		Fasting insulin	CQV	Immuno-chemiluminescence	µU/ml
		HOMA-IR	CQV		-
		IGF-I	CQV	Immuno-chemiluminescence	µg/L
		HMW-adiponectin	CQV	ELISA (Millipore, St Louis, MO)	mg/L
		usCRP	CQV	Immuno-chemiluminescence	mg/L
		Total cholesterol	CQV	Molecular absorption spectrometry	mg/dl
		LDL-cholesterol			
		HDL-cholesterol			
		Triglycerides			
		LH, FSH	CQV	Immuno-chemiluminescence	IU/l
		Estradiol	CQV		pg/ml
		Testosterone	CQV	Liquid chromatography-tandem mass spectrometry	ng/dl
		Androstenedione			
		FAI		Formula: testosterone x 100/SHBG	-
	Clinical variables	Pubertal stage	NQV	Breast Tanner stages	scale
		Height	CQV	Harpenden Stadiometer	cm
		Weight	CQV	Calibrated scale	kg
		BMI	CQV	Body weight divided by the square of the body height.	kg/m^2
		Waist circumference	CQV	Measuring tape	cm
		Hip circumference	CQV	Measuring tape	cm
Covariables		Chronological age	CQV	Clinical history	years

	BMI	CQV	Body weight divided by the square of the body height.	kg/m^2
	Birth weight	CQV	Clinical history	g & SD
	Physical activity	NQV	Physical Activity Questionnaire for Older Children (PAQ-C) (see **annex 3**)	
	Dietary habits	NQV	Kidmed questionnaire (see **annex 4**)	
	Hospital centre	NQV	HC1 (HJT) HC2 (HSJD)	
Safety	Blood count	CQV	Flow cytometry and colorimetric assay	-
	GOT, GPT and GGT	CQV	Molecular absorption spectrometry	lU/l
	Urea, Creatinine			mg/dl
	Electrolyte panel (sodium, potassium)			mEq/l
	Vitamin B12	CQV	Immuno-chemiluminescence	pg/ml
	Folic acid			ng/ml
	Adverse effects	DcQV	(See **annex 2 and 8**)	
*****DcQV **= dichotomous qualitative variable. ***CQV **= continuous quantitative variable. ***NQV **= nominal qualitative variable**				

All measures will be performed by the same observer at each centre (the respective pediatric endocrinologists) in order to avoid interobserver variability. Laboratory samples from participating centres will be analysed at each laboratory of each centre by the same analyst. Radiological images from left hand X-Ray and abdominal MRI will be obtained at both centres by the same radiological technicians respectively. The pelvic ultrasound images will be obtained by the same radiologist at each centre. Then, all radiological images will be sent to a single centre (Hospital Dr. Josep Trueta) so that they are all assessed by the same radiologist. It is important to highlight that the analysis of bone age from the left-hand X-ray will be automated using a programme called BoneXpert. It will be the same radiologist the person in charge to collect the data obtained from the BoneXpert program. In addition, to not overestimate Tanner breast stage, sometimes confused with adipose tissue, a service of ultrasound to examine breast will be hired.

INTERVENTION

STUDY TREATMENT

EXPERIMENTAL DRUG: MINI SPIOMET

The selected experimental drug will be *mini-spiomet*. It is a half-dose version of SPIOMET, which is a fixed-dose combination of spironolactone-pioglitazone-metformin that reverts PCOS phenotype by reducing hepato-visceral fat excess in "mismatch" adolescents (45–48) through targeting three metabolic pathways: spironolactone, raises brown adipose tissue activity; pioglitazone, raises adiponectin and insulin sensitivity; and metformin, raises AMPK activity as well as insulin sensitivity . SPIOMET tablets have been manufactured by pharmaceutical company Reig Jofre (Sant Joan Despí, Barcelona, Spain) (83) according to the same formula tested previously in a phase 1 clinical trial (84). This SPIOMET tablet contains active pharmaceutical ingredients which are spironolactone (50 mg), pioglitazone (7.5 mg) and metformin (850 mg); and their excipients are povidone k-30, microcrystalline cellulose, croscarmellose sodium, polyglycol 4000 PS, magnesium stearate and purified water. Patients will swallow half of the SPIOMET tablet once a day at dinner time for 24 months. Therefore, the daily *mini-spiomet* dose will contain: 25 mg spironolactone, 3.75 mg pioglitazone and 425 mg metformin.

CONTROL DRUG: PLACEBO

The control group will receive treatment with placebo. Placebo tablets will only contain the aforementioned excipients. Girls will swallow a similar tablet at the same time and for the same duration.

The tablets containing SPIOMET and placebo will have a centre line to make it easier to split the tablet into two halves just before taking it. They will also be the same size, shape and colour so that they are indistinguishable from each other to ensure the double-blind nature of the trial.

TREATMENT DURATION

Previous studies have shown that metformin administration leads to an increase in postmenarcheal height in girls with precocious pubarche (43). This increase in height is a reliable indicator of delayed bone maturation. Thus, greater height is achieved through slower bone maturation. These studies suggest that longer treatment periods are needed to achieve better height outcomes (85). In addition, there are other studies conducted on rapidly maturing girls with early puberty suggest that a duration of 3-4 years of metformin monotherapy is required to reduce hepato-visceral adiposity (39–41) and consequently slow down bone maturation (6) and delay the onset of puberty (43).

In our trial a triple drug combination therapy is used, with each drug targeting a different pathway, so that collectively are capable to reduce ectopic fat. This approach is proving to be more effective than using metformin alone. It is therefore logical that our study will be conducted over a two-year period to achieve significant changes in bone maturation.

DRUG CHARACTERISTICS

(See **annex 2**)

SAFETY

We will assess safety of the treatment by two different methods:

- **First method**. The pediatric endocrinologist involved in the trial will note and collect on a sheet (see **annex 8**) different signs and symptoms derived from treatment (adverse effects) that could occur to some girls. The appearance of signs and/or symptoms of hypoglycemia (dizziness, sweating, confusion, among others), persistent skin rash, abdominal pain, persistent nausea and/or vomiting, headaches, sinusitis or severe bacterial infections will be a reason to discontinue the treatment. In all those cases, hepatic and renal function will be periodically monitored until the disappearance of the symptoms or normalization of altered parameters.

- **Second method**. Serial laboratory tests of some safety parameters which can be altered by experimental treatment will be performed:
    - **Hepatic profile** (AST, ALT, GGT)→ if a progressive increase in transaminases levels in detected, treatment must be discontinued.
    - **Renal profile** (creatinine, urea)→ spironolactone and metformin are eliminated by kidneys. If a progressive increase of creatinine or urea is detected, treatment must be discontinued.
    - **Electrolyte panel** (sodium and potassium)→ it has been described that spironolactone may cause hyperkalemia. So, if there is an increase of potassium levels, treatment must be discontinued.
    - **Fasting glucose**→ a possible side effect of pioglitazone is hypoglycemia. if glucose levels are below 65 mg/dl treatment must be discontinued.
    - **B12 vitamin**→ it has been observed that long-term treatment with metformin can decrease B12 vitamin levels. It can be solved with treatment discontinuation.
    - **Folic acid**→ Although none of the active components of the triple therapy appear to affect folic acid levels, they will be taken into account as they are related to vitamin B12 levels, particularly in causing megaloblastic anaemia.

COMPLIANCE

Adherence to treatment will be assessed by interviewing all families/patients at each clinical visit and by counting the number of tablets remaining at the pharmacy dispensing the study medication.

Furthermore, patients will be contacted every 3 months, either in person within a clinical visit or by phone, in order to maintain a close contact with them as to ensure treatment compliance and reduce the risk of loss to follow-up. It will also allow to monitor the appearance of any possible adverse events.

SUBJECTS WITHDRAWAL

The patient can stop the treatment and quit the study at any time. If they choose to leave the trial or experience an adverse event resulting in withdrawal from the study, we will invite the family to an end-of-study visit. This visit will be followed by disclosure of treatment allocation.

Withdrawn participants will not be replaced. If necessary, missing data will be filled with the data from previous visits. The statistical method used to handle missing data will be an intention-to-treat analysis including all participants randomized to either placebo or *mini-spiomet*, ignoring non-compliance and withdrawal.

However, each case of abandonment or loss will be evaluated individually justifying the reasons for trial withdrawal. If necessary, the adverse effects will be reported to the public health authorities. Girls who decide to leave the study will continue to receive standard care.

CONCOMITANT TREATMENTS ALLOWED AND INCOMPATIBLE WITH THE STUDY

Participating girls in this trial cannot participate in another trial at the same time.

The only concomitant treatment accepted during the course of the trial is the use of paracetamol.

Concomitant treatments considered incompatible are: anticoagulants, anti-inflammatories in general, including non-steroidal anti-inflammatory drugs and selective cyclooxygenase II inhibitors, other oral hypoglycemic drugs, anti-androgens, estrogens, progestogens, digoxin, potassium-sparing diuretics, potassium supplements, oral contraceptives, iodinated contrast agents, angiotensin-converting enzyme inhibitors, angiotensin II receptor antagonists, and diuretics, particularly loop diuretics.

MASKING

The study will be double-blinded. That means neither the patient and nor the clinical investigator will have knowledge on treatment allocation until the study concludes. It may only be disclosed if the patient withdraws from the study.

Pharmacists of each centre will be the sole entity responsible for treatment masking and delivering the allocated intervention according to the randomisation list.

Statistically involved investigators, who will have no contact with the patient or with the clinical investigator, will be unblinded once experimental treatment and its data collection have been completed, so that data analysis is enabled.

PATIENT MASKING: PLACEBO

Control group will receive placebo. Placebo pills will be made of the same ingredients as SPIOMET pills except for the three active pharmaceutical ingredients. Both tablets will look the same so that they are indistinguishable.

INVESTIGATOR MASKING

If regular follow-up measurements were taken for endocrine and metabolic parameters such as fasting insulin or lipid profile (among others), it would become evident which subjects received treatment and which not. Therefore, blood samples will be stored (at -80ºC) at the Pediatric Endocrinology lab (IDIBGI) and at the Metabolic Endocrinology lab (IRSJD), in order to be analysed at the end of the study.

Parameters to be analysed will be fasting glucose, fasting insulin, HOMA-IR index, IGF-I, HMW-adiponectin, ultra-sensitive C-reactive protein, serum lipids, SHBG, LH, FSH, testosterone, androstenedione, estradiol and FAI (free androgen index).

DATA COLLECTION

The intervention visits will take place during the routine follow-up visits for early-puberty children appointed every 6 months. In this way, fewer dropouts are expected, as no added new visits will be appointed so that patients' routine is not affected by the trial.

At each visit, the follow-up measurements will be:

***Table 4**. Follow-up measurements*

Auxological (every 6 months)	***Laboratory tests (every 6 months)***	***Other parameters (yearly)***
- Height - Weight - BMI - Waist circumference - Hip circumference - Waist/hip circumference ratio - Pubertal stage (Tanner scale)	- Fasting glucose - Fasting insulin - HOMA-IR - IGF-I - HMW-adiponectin - usCRP - Triglycerides, total cholesterol, LDL and HDL - SHBG - LH, FSH, testosterone, androstenedione, estradiol and FAI - Complete blood count - AST, ALT and GGT - Creatinine and urea - Electrolyte panel - B12 vitamin and folic acid	- Bone age (X-ray) - Abdominal fat partition and hepatic fat (MRI) - Uterine and ovarian volumes (pelvic ultrasound) - Dietary habits (Kidmed questionnaire, see **annex 4**) - Physical activity (see **annex 3**)

The same principal investigator at each centre (HJT and HSJD), the pediatric endocrinologist, will assess the clinical variables every 6 months until completion of two years of follow-up.

Follow-up measurements should not be performed if the patient presents acute symptomatology (such as fever, suggesting an infection for example). Any acute infectious or inflammatory process causes an increase in stress hormones, such as cortisol, which, in turn, increase insulin production. Due to this, some of the laboratory parameters may be altered and consequently, not reliable for statistical analysis of the study. If this situation occurs, the follow-up appointment will be rescheduled within two weeks to ensure reliable measurements are taken and prevent any missing data.

The same clinical visit day, a venous peripheral blood sample in fasting state will be drawn in the early morning at each clinical centre. All blood samples will be obtained between 8.00 a.m. and 10.00 a.m. in order to avoid variability produced by circadian hormones.

Clinical measurements will be taken by the same principal investigator (the pediatrician endocrinologist) during the follow-up visits. All the data will be systematically collected in a standard template (**annex 7**).

- **Height** will be measured with a Harpenden stadiometer. The girl must wear no shoes and nor pigtails or head accessories. She will be positioned with their back and ankles against the wall while maintaining a neutral head position. Feet and legs must be straight and remain together. To avoid any measurement errors that may cause information bias, three measurements will be taken, and the average of two similar results will be used for the study.
- **Weight** will be measured with the girl only wearing underwear on a calibrated scale.
- **BMI** will be calculated as weight (in kg) divided by the square of height (in meters).
- **Waist circumference** will be measured while the girl is standing and by using a measuring tape. The girl needs to be only wearing underwear. The measurement must be done midway between the lowest rib and the iliac crest.
- **Hip circumference** will be measured while the girls is standing and by using a measuring tape. The girl needs to be only wearing underwear. The measurement

must be done at the largest circumference around the buttocks, and being careful that the tape is not pulled too tight or loose. That could result in measurement mistakes that might lead to information bias.

- **Waist/hip circumference ratio** will be calculated as waist measurement (in cm) divided by hip measurement (in cm).
- **Pubertal stage** will be assessed with Breast Tanner Stages of puberty (**annex 1**). Clinical sign of breast development might not be easily distinguished from fat adipose tissue in overweighted girls (10). When there are doubts whether the breast corresponds to breast development or adipomastia, an ultrasound will be performed to differentiate mammary glands from adipose tissue.
- **Physical activity** will be assessed by the Physical Activity Questionnarie for Older Children (PAQ-C) (86) (see **annex 3**) that girls will complete during each follow-up visits at the corresponding centre.
- **Dietary habits** will be assessed by the Kidmed questionnaire (87) (see **annex 4**) that girls will complete during each follow-up visits at the corresponding centre.
- **Acceptability of the tablet** will be evaluated by using a simple questionnaire with numerical rating scales and the integrated 100 mm visual analog scale (VAS) or facial hedonic scale.

Metabolic and endocrine variables analysis will be analysed at the corresponding laboratories of HJT and HSJD (except for some parameters that will be analysed centrally, it is specified in each case):

- **Fasting glucose** will be measured by the glucose oxidase method.
- **Fasting insulin, IGF-I, usCRP** and **SHBG** will be analyzed by immuno-chemiluminescence.
- **Fasting insulin sensitivity** will be estimated from fasting insulin and glucose levels using de homeostasis model assessment for insulin resistance (**HOMA-IR**) formula:

  HOMA-IR = (fasting insulin, mU/L) x (fasting glucose, mg/dL) / 405

- **Triglycerides, total cholesterol, LDL-cholesterol,** and **HDL-cholesterol** will be assayed by molecular absorption spectrometry.

- **HMW-adiponectin** will be analysed by ELISA (Millipore, St Louis, MO).
- **LH, FSH** and **estradiol** will be measured by immune-chemiluminescence.
- Circulating **testosterone** and **androstenedione** concentrations will be centrally assessed by liquid chromatography-tandem mass spectrometry.
- **Free Androgen Index (FAI)** will be calculated with the following formula:

FAI = testosterone (nmol/L) x 100/SHBG (nmol/L)

Imaging studies will be performed annually, at the treatment start, after 1 year on treatment, and after 2 years from the onset of the study.. The measurements to be assessed will be:

- **Uterine and ovarian measures** will be assessed with a high-resolution ultrasonography by the same observer at each clinical centre (HST and HSJD). Both observers will be blinded to treatment assignment, well-trained, and familiar with pre-standardized criteria to accurately characterize the findings. Repeated measurements will be conducted to minimize intra-operator variability. The ultrasound machine should be properly calibrated to ensure measurements are as accurate as possible.

  A 5-MHz parallel scanner will be used to obtain the uterine and ovarian images. The most important preliminary to the examination is making sure that the patient bladder's is full. The fuller the bladder, the better the images. The examination will be performed in supine position (27).

  To determine the volume of the uterus two sections (the transversal and the longitudinal one) through the corresponding axes of the organ are necessary to be measured. Then, the uterus volume (V) is calculated according to the ellipsoid formula (27):

$$V = 4/3 \cdot \pi \cdot a \cdot b \cdot c$$

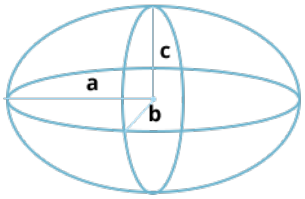


***Image 1**. From internet* (88)

  It is not easy to distinguish the ovaries from surrounding structures in a child, so it may be helpful to use the uterine body as the guiding structure. From the longitudinal axis of the uterus, the scanner is tilted laterally until the ovary

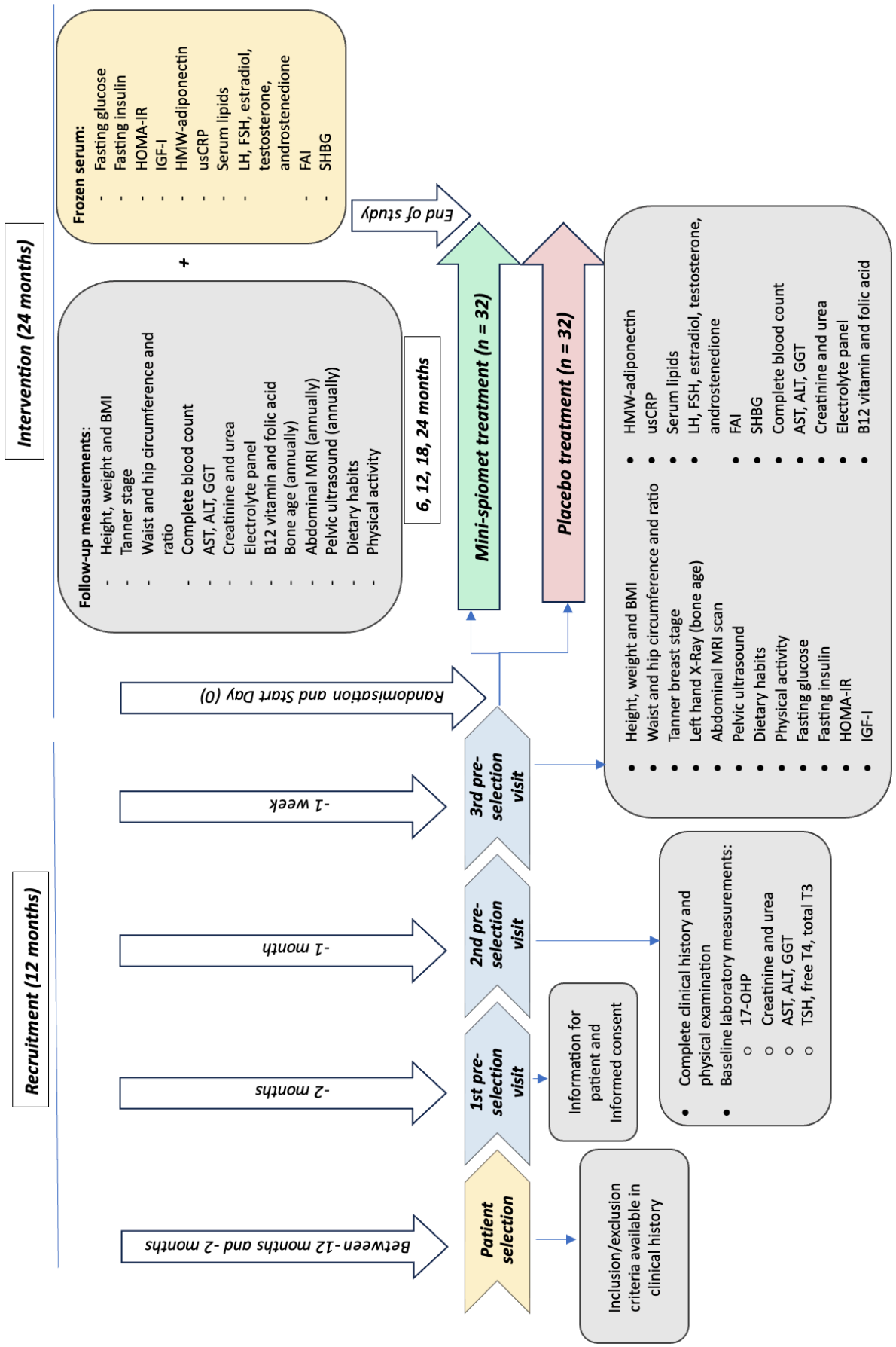
appears on the screen. Sometimes, it is also advisable to locate the ovary from the contralateral side by pointing the scanner diagonally through the bladder. The standard sections are transversal and longitudinal parallel to the axes of the organ. Ovarian volume is calculated from the measurements of the ovary's three dimensions (27).

Once the ultrasound images are acquired, they will be sent to a central reader (principal radiologist of HJT), also blinded to treatment allocation, who will process and interpret the images from both study centres.

- **Abdominal fat partitioning and hepatic fat** will be analyzed by the same MRI scan [multi slice 1.5 Tesla scan (Signa LX Echo Speed Plus Excite, General Electric Healthcare, Milwaukee, WI)] at both clinical sites (HJT and HSJD). Scans will be performed by the same operator (radiologist technician) at each centre, blinded to the treatment allocation. Then, images will be sent to the central radiologist reader.

- **Bone age** will be determined on the basis of a left-hand X-Ray performed by the same operator (radiologist technician) at each centre (HJT and HSJD). X-Ray image will be evaluated with the BoneXpert programme [version 3; February 2021 (Visiana, Denmark)]. This method will yield bone ages by Greulich and Pyle and by Tanner-Whitehouse 2 (TW2) (6). As Greulich and Pyle and TW2 methods produce different values for bone age which are significant in clinical practice, it is suggested that only one method should be used when performing serial measurements on an individual patient. The TW2 method has shown to be more reproducible than Greulich and Pyle method, so preferably only the TW2 method should be used for bone age assessment (89). Bone age maturation will be annualized by calculating the ratio between the increase of bone age (BA) over the increase in chronological age (CA) (ΔBA / ΔCA). Although bone age images will be interpreted in an automated way by BoneXpert programme, they will also be sent to the central radiologist reader so that the findings can be supervised.

FLOW CHART


Recruitment (12 months)
Intervention (24 months)
Between -12 months and -2 months
-2 months
-1 month
-1 week
Randomisation and Start Day (0)
6, 12, 18, 24 months
End of study
Patient selection
1st pre-selection visit
2nd pre-selection visit
3rd pre-selection visit
Inclusion/exclusion criteria available in clinical history
Information for patient and Informed consent
Complete clinical history and physical examination
Baseline laboratory measurements:
17-OHP
Creatinine and urea
AST, ALT, GGT
TSH, free T4, total T3
Mini-spiomet treatment (n = 32)
Placebo treatment (n = 32)
Height, weight and BMI
Waist and hip circumference and ratio
Tanner breast stage
Left hand X-Ray (bone age)
Abdominal MRI scan
Pelvic ultrasound
Dietary habits
Physical activity
Fasting glucose
Fasting insulin
HOMA-IR
IGF-I
HMW-adiponectin
usCRP
Serum lipids
LH, FSH, estradiol, testosterone, androstenedione
FAI
SHBG
Complete blood count
AST, ALT, GGT
Creatinine and urea
Electrolyte panel
B12 vitamin and folic acid
Follow-up measurements:
Height, weight and BMI
Tanner stage
Waist and hip circumference and ratio
Complete blood count
AST, ALT, GGT
Creatinine and urea
Electrolyte panel
B12 vitamin and folic acid
Bone age (annually)
Abdominal MRI (annually)
Pelvic ultrasound (annually)
Dietary habits
Physical activity
+
Frozen serum:
Fasting glucose
Fasting insulin
HOMA-IR
IGF-I
HMW-adiponectin
usCRP
Serum lipids
LH, FSH, estradiol, testosterone, androstenedione
FAI
SHBG

STATISTICAL ANALYSIS

Statistical analysis will be performed with the SPSS program, version 23.0 (SPSS, Chicago, Illinois, USA).

UNIVARIATE DESCRIPTIVE ANALYSIS

Mini-spiomet will be statistically considered a dichotomous qualitative variable (*Mini-spiomet* / Placebo). Tanner stage will be considered a quantitative discrete variable. Adverse effects will be considered a qualitative nominal variable, as well as the hospital centre, dietary habits, physical activity and tablet acceptability. The rest of variables will be treated as continuous quantitative variables. Results for categorical variables will be described as frequencies (n) and percentages (%). Quantitative variables assuming a normal distribution will be expressed as mean $\pm$ standard deviation, and those for variables without a normal distribution will be expressed as median and interquartile range.

BIVARIATE ANALYSIS

Results for main variables will be compared in order to ensure that comparable treatment groups were generated through the randomisation process. T-Student test will be used to compare continuous quantitative variables if normal distribution can be assumed, and U-Mann-Whitney test will be applied in case of discrete and continuous quantitative variables in which normal distribution cannot be assumed. Chi-square test will be used to compare categorical variables.

MULTIVARIATE ANALYSIS

Linear mixed models (LMM) will be applied in order to perform the analysis of response to treatment for endpoint quantitative variables (bone age, ectopic fat, metabolic and endocrine markers, and uterine and ovarian volumes) between *mini-spiomet* treatment and control group. Poisson regression will be applied to analyse Tanner stage response between each treatment group. To compare categorical variables (such as adverse effects) logistic

regression will be applied. All these multivariate analyses will be performed adjusting for covariables to avoid confusion caused by the effect of other variables on the effect of the treatment. These covariables are: chronological age, birth weight, BMI, dietary habits, physical activity and hospital centre.

An intention-to-treat analysis will be applied in case of:

- Losses to follow-up.
- Protocol violations.
- Withdrawals from the study.
- Non-compliance.
- Refusal of the allocated treatment.
- Other deviations from the standard protocol.

Imputation of missing values for endpoint variables will be performed using the latest observed values for each variable and subject.

A p value <0.05 will be considered statistically significant.

ETHICAL AND LEGAL CONSIDERATIONS

This clinical trial will be conducted in accordance with the medical ethics requirements defined on the World Medical Declaration of Helsinki involving "Ethical Principles for Medical Research Involving Human Subjects" (last update October 2013) (**annex 9**), and the Principles of Biomedical Ethics from Beauchamp and Childress from 1970 and reviewed in 2009:

- **Autonomy**: the study will respect the values and personal choices of all participants. Before being included in the trial, all girls and their parents will be informed properly about the trial and given an information sheet (**annex 5**). As all participants will be under 18 years old, written informed consent (**annex 6**) will be provided to their parents or legal tutors and must be signed before taking part in the project. They will also be informed that they have the right to decline participation in the study and may withdraw at any time without consequence.
- **Non-maleficence**: patients taking part in the trial will not be subjected to malicious intent. Those who may be affected by any of the interventions will be excluded.
- **Beneficence**: acting to benefit others is a moral obligation. The study complies with this principle by implementing a novel therapy aimed at preventing and reducing the accelerated bone maturation and the rapid pubertal progression in girls with advanced puberty who currently have no other treatment options.
- **Justice**: in order to uphold the principle of equity, the study will ensure an equitable distribution of the benefits of well-being and avoid any discrimination in access to health resources or against any group of people.

The field trial development will also comply with the regulatory framework for clinical trials: "*Real Decreto Legislativo 1090/2015, de 4 de diciembre por el que se regulan los ensayos clínicos con medicamentos, los Comités de Ética de la investigación con medicamentos y Registro Español de Estudios Clínicos*", "*Real Decreto Legistaivo 1/2015, de 24 de julio, por el que se aprueba el texto refundido de la Ley de garantías y uso racional de los medicamentos y productos sanitarios*", "*Ley 14/2007 de 3 de julio, de Investigación Biomédica*", and "*Reglamento (UE) nº536/2014 del Parlamento Europeo y del Consejo, de 16 de abril de 2014, sobre los ensayos clínicos de medicamentos de uso humano*".

The study will ensure patients anonymity by assigning them a code number in the database and only the research team will have access to it. All data collected for the trial will only be used for research purposes and will always be treated preserving the confidentiality of the patients according to "*Ley Orgánica 3/2018, de 5 de diciembre de Protección de Datos Personales y garantía de derechos digitales (LOPDGDD*)", and "*Reglamento (UE) 2016/679 del Parlamento y Consejo Europeo, de 27 de abril relativo a la protección de personas físicas en cuanto al tratamiento de datos personales y a la libre circulación de estos datos*".

The trial will be submitted to the "Comitè d'Ètica d'Investigació Clínica (CEIC)" of Hospital Josep Trueta of Girona, as it is the study coordinating centre, in order to be reviewed and approved before any further procedure. As a final step, the protocol must be approved by "Asociación Española de Medicamentos y Productos Sanitarios (AEMPS)" before final approval of the clinical trial.

This trial has an insurance to take the responsibility towards its members if any adverse event is suffered because of our intervention.

The main goal of this research is to develop generalizable knowledge to improve human health and quality of life. Investigators must agree to publish all data and results with total transparency, including unfavourable data or events, and every investigator will have to declare no conflicts of interest.

LIMITATIONS AND STRENGTHS

STRENGHTS

- This study is the first clinical trial in the literature using half-dose version of SPIOMET to delay bone maturation and pubertal development. The proposed treatment, a low-dose combination of generics (*mini-spiomet*) targets the pathophysiological roots of early puberty. This has the potential to reduce short-term psychosocial comorbidities such as embarrassment, as well long-term comorbidities like type 2 diabetes or metabolic syndrome.

- Spironolactone, pioglitazone and metformin are inexpensive drugs with a good safety profile use in children, which have few and transient side effects, and the majority being non-relevant.

- As this is a multicentre study it has more potential to generalize its results to a larger population of girls with early puberty.

- The design of the study is double-blinded, that means both patients and investigators are unaware of who is receiving the actual treatment and who is receiving placebo. This helps minimize bias in the interpretation of results, as neither the girls nor the investigators can be influenced by knowledge of the treatment assignments.

- The trial is randomized and placebo-controlled. The use of randomization for the treatment allocation and a placebo group allows for a baseline comparison to assess the true effects of the treatment. Any observed differences between the treatment group and the placebo group can be attributed to the treatment itself rather than external factors, which enhances the internal validity of the study.

- The assessment of the main outcome variable, bone maturation, is carried out by an automated method (the program BoneXpert) thus avoiding subjectivity, intrapersonal variability, and error of the human eye.

- The longitudinal assessment of abdominal fat partitioning using MRI offers several advantages that contribute to a more comprehensive understanding of the intervention's impact. It provides insights into the temporal patterns of response, enables examination of individual variability, and captures dynamic effects understanding whether changes occur rapidly or gradually which can be important for interpreting the intervention's efficacy.

LIMITATIONS

- BMI is the one of the main covariates in the study because children's weight and height are constantly changing, and small changes in BMI can affect bone age maturation. To avoid this confounding effect, we have stratified the sample into two BMI groups, even though randomisation usually distributes variables equally in both groups. Furthermore, all participants will be informed of the importance of maintaining a stable weight throughout the study. The same stratification has been performed for those other covariates: chronological age and birth weight.

- In a multicentre study, there may be variability between main investigators and selected personnel when collecting data, particularly with the assessment of clinical variables. To minimize this interobserver variability, all personnel involved in data collection will receive the same training to perform examinations and will follow common procedures for data collection. Medical images, obtained through MRI and ultrasound, will be acquired by two different radiologists at each centre (HJT and HSJD) following a standardized protocol. These images will then be sent to the coordinating centre, HJT, for being interpreted by the same radiologist

- The breast development (assessed by Tanner stages) does not necessarily mean the true onset of puberty in every case, and it could be overestimated as it could also

be due to the presence of adipose tissue, adipomastia, especially in overweight girls. In order to avoid overestimating Tanner breast stage, a service of ultrasound to make breast examination to the girls will be hired.

- Other clinical variables, particularly the height can vary due to girls position, if she rises more or less the head or arches de back, among others. This could made vary the BMI results calculation causing an information bias for the study results. To solve that, measurement will be realized three times and if the results are not the same, the average between the two more similar ones will be used for the study.

- It is a prospective study which lasts 2 years, so there is a risk of drop-out or non-compliance of the treatment. This has been taken into account when calculating the sample size.

- Regarding the budget, as this is a clinical trial, it is a high-cost study. However, it we want to answer our hypotheses this is the best study design which should be followed.

- One of the main limitations is the lack of ethnic variability in the study population. This means it will be required to prove the results in other populations.

- Although it is a double-blind clinical trial, side effects can impair the unblinding process and produce bias. One investigator will assess the side effects and will hide it to the rest of investigators.

- The fact that this study is compared to placebo and not compared to metformin could be considered a limitation. The three main arguments for designing a placebo-controlled trial rather than a comparison with metformin are:

    - Firstly, note that this is the first time proving *mini-spiomet's* efficacy on bone maturation. Therefore, it is necessary to first demonstrate statistically significant differences in efficacy from placebo.

- Secondly, finding statistically significant differences between the experimental *mini-spiomet* treatment and metformin monotherapy would require too large a sample of patients for the study to be feasible.
- Furthermore, if such a large sample of girls with advanced puberty (n 300 or 400 patients) were to be achieved, they would be offered an experimental treatment that has not been shown to be effective against placebo in treating their condition.

WORKING PLAN

PARTICIPATING CENTRES

The clinical trial will be held in two clinical centres: Hospital Josep Trueta (HJT) which includes Institut d'Investigació Biomèdica (IDIBGI) (Girona), and Hospital Sant Joan de Déu (HSJD) which includes Institut de Reserca Sant Joan de Déu (IRSJD) (Esplugues, Barcelona).

RESEARCH TEAM

- **Trial Steering Committee** (**TSC**): is the responsible of trial and it will be constituted by the principal investigator of each institution (HJT and HSJD) and the research coordinators. They will meet once per semester to coordinate and supervise all the tasks. The **principal investigators** will be ultimately responsible for the overall research and the design, administration and conduct of the research protocol, the publication of results and the writing of the extracted conclusions. The **research coordinators** will be responsible for the supervisions of the project regarding compliance with regulations and protocols.
- **Coordinating centre**: Hospital Josep Trueta-IDIBGI. 972419623
- **Co-Investigators**: for each hospital participating in the study there will be one person in charge in charge to properly coordinate each task from each department involved:
    - Nurses, who will perform blood extractions.
    - Laboratory analysts, who will determine laboratory parameters.
    - Pharmacists, who will dispense medication according to the randomization and will check the compliance of treatment by counting the number of tablets returned.
    - Radiological technicians, who will be in charge of acquiring X-Ray and MRI images.
    - Radiologists:
        - Brest ultrasound radiologist (to rule out adipomastia).
        - Pelvic ultrasound radiologist (to assess uterine and ovarian volumes).
        - The primary radiologist responsible for interpreting all acquired images.

- **Data manager**: will be responsible to store all the information and research data of the project in an electronic database. At the end of the project, an SPSS file will be created to perform the statistical analysis of the results.
- **Statistic specialists**: will be in charge to perform the statistical analysis of the study.

STUDY STAGES

The research team will carry out the tasks of coordination, interpretation and dissemination of the results. The sequence of the activities is detailed below:

0. **STUDY DESIGN** (November 2023 – January 2024)
   - **1st meeting** (November 2023): main objectives, hypotheses and methodology were established.
   - **Protocol elaboration** (November 2023 – February 2024): bibliographic research and protocol elaboration with detailed definition of study variables and theoretical methodology of data collection have been carried out.

   Led by: the TSC

1. **INITIAL COORDINATION** (January 2024 – February 2024)

   The first in person meeting will take place with all the selected investigators in the Hospital Josep Trueta. The principal investigators will present the project design, the execution plan with all different phases of the study and will review the roles of each participant. In this meeting, will take place the training of the pediatricians to perform patients' selection and evaluation, minimizing interobserver variability. The rest of co-investigators will be taught how to collect and report their data in the study database. All the investigators will also evaluate the protocol and will be asked to identify problems and provide proposal for changes or improvement in design. In addition, the meeting will be useful to solve all the questions and check that the protocol has been understood and will be followed according to what's been established.

   Led by: all the research team

2. **ETHICAL EVALUATION** (February 2024 – April 2024)

According to all the proposals from the different investigators, a definitive protocol will be elaborated. It will be submitted to the Clinical Research Ethical Committee (CEIC, *Comitè Ètic d'Investigació Clínica*) at Hospital Josep Trueta de Girona, and also to the AEMPS (*Agencia Española de Medicamentos y Productos Sanitarios*) for its approval. During this phase an insurance will be contracted as well. The time request in this stage may vary according to the time taken by the CEIC and AEMPS to approve the project. Any modification of the protocol will be done to achieve the CEIC's and AEMPS's required conditions.

Led by: the TSC

3. PARTICIPANT'S RECRUITMENT AND DATA COLLECTION

- **Recruitment** (May 2024 – April 2025). A non-probabilistic consecutive sampling will be used, based on inclusion and exclusion criteria. All the preselection visits with their respective tests will be performed to help in the process. Once seen the patient is adequate to enter in the study, will be given information about the study and treatment. Parents or legal tutors must sign the informed consent if they and their child agree to participate. The planned inclusion period is of 12 months, so a member in charge of patient flow analysis will evaluate the recruitment time in order to prove that the rate of inclusion is met.
- **Randomisation, masking and allocation**. Participants meeting the inclusion criteria and none of the exclusion criteria, after signing the informed consent, will be given an identification random number. Patients will have to present this identification number to the hospital pharmacy, and there, the pharmacist will perform the randomised enrolment and allocation to one of the study groups, masking it to the patient.
- **Follow-up and data collection** (May 2024 – April 2027). Every 6 months, a follow-up visit will be scheduled, and all the complementary examinations and tests as described in "Data collection" will be performed. Serum samples of the patients will be frozen. Critical parameters that can unmask the treatment arm will be assessed at the end of the trial. Measured variables will be electronically recorded in a shared database by the involved two centres. To ensure the quality and homogeneity of

data collection according to the protocol, results registered in the database will be screened by the data manager for possible errors during data collection.

Led by: all the research team

4. STATISTICAL ANALYSIS, RESULTS DISCUSSION AND CONCLUSIONS

The statistical analysis will be performed by a subcontracted statistician.

- **Statistical analysis** (May 2027 – August 2027). At the end of the study, appropriate statistical tests will be applied for all collected data. As previously described descriptive and bivariate analysis will be performed followed by the multivariate analysis.
- **Results discussion and conclusions** (September 2027– October 2027). The outcomes of the trial will be interpreted and conclusions will be derived therof, written and discussed. The TSC will be involved.

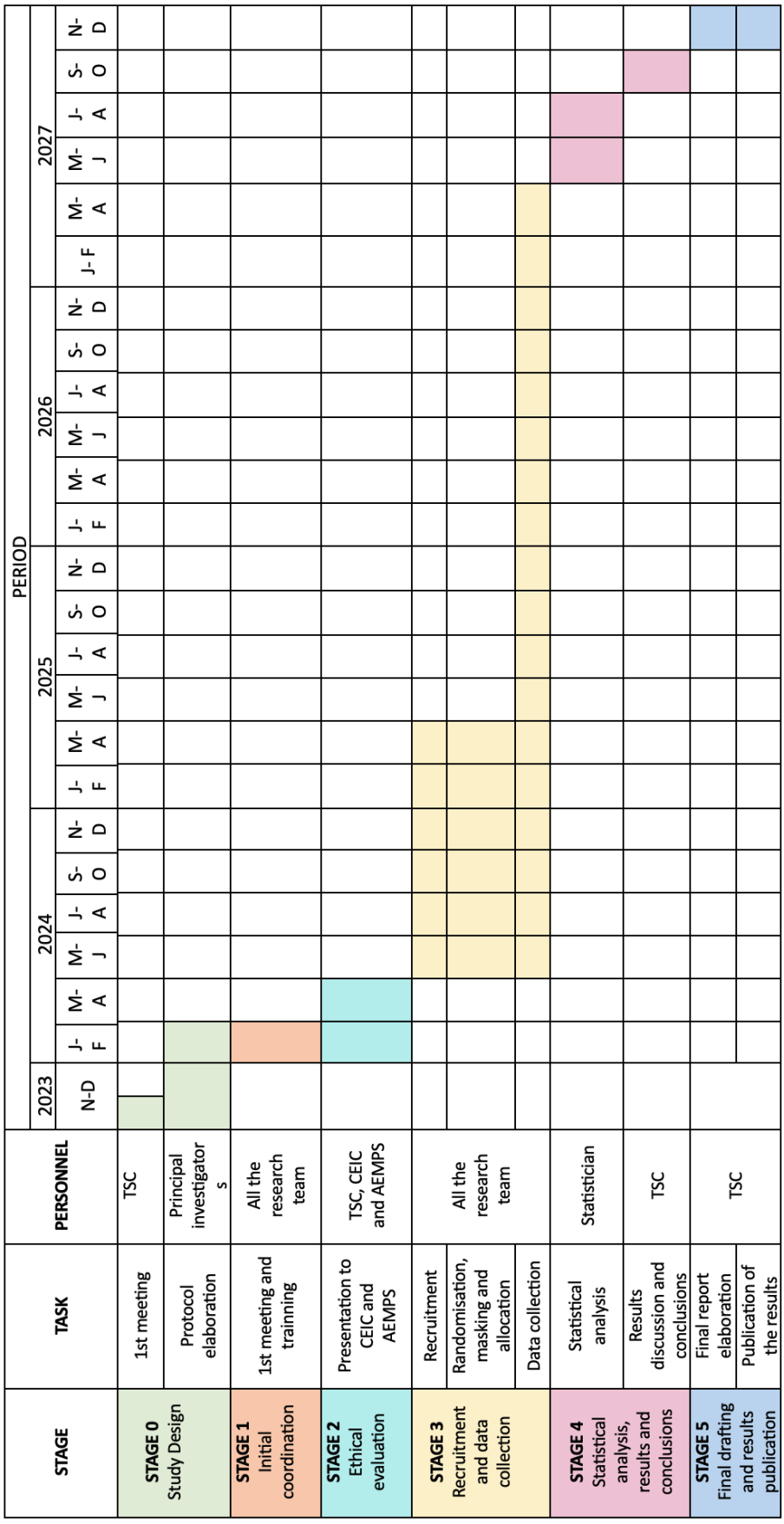
Led by: the statistician and the TSC

5. **FINAL DRAFTING AND RESULTS PUBLICATION** (November 2027 – December 2027)

- **Final report elaboration**. A report with the final results and conclusions will be written, attaching the graphics and tables needed to draft the final article.
- **Publication of the results**. Whether results support or not the initial hypotheses will not affect their publication. Dissemination strategy consists on an open access to online publication in journals such as “The Journal of Pediatrics” and “The Journal of Clinical Endocrinology and Metabolism” as well as study results presentation in conferences from *Sociedad Española de Endocrinologia Pediatrica* and European Society for Pediatric Endocrinology.

Led by: the TSC

CHRONOGRAM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J-A
S-O
N-D
STAGE
TASK
PERSONNEL
STAGE 0 Study Design
1st meeting
TSC
Protocol elaboration
Principal investigators
STAGE 1 Initial coordination
1st meeting and trainning
All the research team
STAGE 2 Ethical evaluation
Presentation to CEIC and AEMPS
TSC, CEIC and AEMPS
STAGE 3 Recruitment and data collection
Recruitment
Randomisation, masking and allocation
Data collection
All the research team
STAGE 4 Statistical analysis, results and conclusions
Statistical analysis
Statistician
Results discussion and conclusions
TSC
STAGE 5 Final drafting and results publication
Final report elaboration
Publication of the results
TSC

BUDGET

***Table 5**. Budget*

1. STAFF COSTS				
	Cost	**Nº persons**	**Time**	**Total**
Statistician	35€/h	1	80h	2800 €
Data manager	30€/h	1	60h	1800 €
				4600 €
2. IMPLEMENTATION COSTS				
	Cost	**Nº patients**	**Quantity**	**Total**
Inventory material costs	0	64	1	0
Services and disposable items costs				
- Laboratory parameters				
○ Complete Blood Count (serum)	0	64	5	0
○ Fasting glucose (serum)	1,27 €/u	64	5	406,4 €
○ Fasting insulin (serum)	6,98 €/u	64	5	2233,6 €
○ IGF-I (serum)	10,35 €/u	64	5	3312 €
○ HOMA-IR index	-	64	5	-
○ HMW-adiponectin (serum)	10,63 €/u	64	5	3401,6 €
○ Ultrasensitive C-reactive protein	10,43 €/u	64	5	3337,6 €
○ Lipid profile (serum)	2,66 € /u	64	5	851,2 €
○ LH	0	64	5	0
○ FSH	0	64	5	0
○ Testosterone	0	64	5	0
○ Androstenedione	0	64	5	0
○ Estradiol	0	64	5	0
○ FAI index	-	64	5	-
○ SHBG	5,86 €/u	64	5	1875,2 €
○ AST, ALT, GGT (serum)	7,84 €/u	64	6	3010,6, €
○ Creatinine (serum)	1,27 €/u	64	6	487,7 €
○ Urea (serum)	6,98 €/u	64	6	2680,3 €
○ Electrolyte panel (sodium, potassium)	2,02 €/u	64	5	646,4 €
○ B12 vitamin (serum)	2,07 €/u	64	5	662,4 €
○ Folate (serum)	2,33 €/u	64	5	745,6 €
○ 17-OHP	2,11 €/u	64	1	135 €
○ TSH	2,02 €/u	64	1	129,3 €
○ Free T4	1,98 €/u	64	1	126,7 €
○ Total T3	1,93 €/u	64	1	123,5 €
- Left hand X-Ray	0	64	3	0
- Abdominal MRI	100 €/u	64	3	19200 €
- Pelvic ultrasound	0	64	3	0
Drug purchase				
- *Mini-spiomet*	4,56 €/box*	32	8	1167,4 €
- Placebo	7,35 €/box	32	8	1881,6 €
Liability insurance	-	-	1	20000 €

AEMPS authorization expenses	-	-	1	1500 €
				67914,1 €
3. DISSEMINATION OF THE RESULTS				
	Cost	**Quantity**		**Total**
Congress of the Spanish Society of Pediatric Endocrinology	800 €/p	2		1600 €
Congress of the European Society of Pediatric Endocrinology	1600 €/p	2		3200 €
Publication fees	-	-		3000 €
Software and bibliography	-	-		1000 €
				8800 €
4. SUBCONTRACTING OF PROFESSIONAL SERVICES				
Contract Research Organization (CRO) (ADKNOMA)	-	-		25000 €
Contracting pharmacy services				
- SPIOMET production	-	-		11000 €
Contracting of radiology services				
- Echography service	25 €/ subject	2		3200 €
- Central radiologist reader	60 €/ subject	1		3840 €
				43040 €
5. INDIRECT AND ADDED COSTS				2350 €
			TOTAL BUDGET	**126704,1 €**

*1 box = 50 tablets // 1 SPIOMET tablet = 2 *mini-spiomet* tablets

RESOURCES ALREADY AVAILABLE

- The two hospitals involved in the trial provide pediatric services with clinical offices that serve as the physical framework for including and following up with patients.
- In each pediatric endocrinologist's office, the necessary instruments to measure clinical variables are available: Harpenden Stadiometer (to measure height), calibrated scale (to measure weight) and measuring tape (to measure hip and waist circumferences).
- The research team personnel will not receive additional compensation for their participation in the project, as their involvement is part of their work duties.
- Both centres have their respective laboratories for analytical processing of samples and nurses to collect blood samples. Some laboratory parameters will be free cost

for the trial because they are inherent to the basal condition of the patient and therefore paid by the hospital. These already included parameters are: complete blood count, LH, FSH, testosterone, androstenedione and estradiol.

- Both hospitals have a radiology service. The left-hand X-ray images will not be budgeted as fungible as they are already done routinely in girls with advanced puberty for annually monitoring of the bone age.
- No budget will be needed for CEIC as Hospital Dr. Josep Trueta, as the coordinating centre, has their own one.

REQUESTED RESOURCES

Implementation costs

- The trial budget will include a number of laboratory parameters, which are intended to provide information for the testing of our hypotheses and the achievement of our trial objectives, as well as the safety parameters. The cost of all these laboratory parameters is summarized in ***Table 5***.

Subcontracting of personal services

- Both centres have their own pharmacy department, but the production costs of the experimental treatment (SPIOMET tablets) will be included in the budget as it is a is a three-drug combination produced and dispensed by the hospital whose prescription indications are still being tested in experimental studies. The pharmaceutical company in charge of manufacturing SPIOMET tablets will be Reig Jofre (Sant Joan Despí, Barcelona, Spain).
- During the trial, three radiologists will be hired. Two of them will be assigned to the extra ultrasound service and will be distributed to one hospital each. Their purpose is to conduct a breast ultrasound to rule out adipomastia and prevent overestimation of the Tanner breast stage. The role of the third radiologist recruited will be to read and interpret all medical images (X-ray, MRI and pelvic ultrasound) collected during the trial. It should be recalled that the X-ray images will be analysed using an automated programme called BoneXpert.

- A Contract Research Organisation (CRO) named ADKNOMA Health Research SL will be hired to provide assurance of quality for our study. The CRO is responsible for overseeing strict adherence to the protocol to ensure proper patient selection and data collection. A minimum of 4 monitoring visits are expected to be performed during the study.

The rest of included costs are summaried in ***Table 5***.

HEALTH IMPACT

As mentioned above, there has been a global trend over the recent decades towards an earlier age of pubertal onset, particularly in girls. At the same time, there has been an increase in childhood overweight and obesity in modern societies which may have contributed to this advance in the onset of puberty. As a result, early puberty has become one of the main reasons for referral to pediatric endocrinologists.

Early puberty may have several negative health consequences for the girls who experience it. Not only in terms of psychosocial maladjustment, but also in terms of physical well-being in the adulthood, such as ending up in a full-blown phenotype of adolescent polycystic ovarian syndrome (PCOS), developing metabolic syndrome and insulin resistance leading to higher cardiovascular risk and type 2 diabetes, increasing blood pressure and increasing the risk for some cancer such as breast or endometrial cancer.

Not all the girls with an advanced pubertal development will experience all of the consequences mentioned above. It is known that some of these girls will progress slowly through puberty and will have longer a duration of pubertal growth spurt with a normal time of menarche. However, there will be others who may develop poorly and progress rapidly through puberty.

There is currently no effective treatment for girls with early puberty. GnRH analogues have not shown favourable results in delaying the onset of puberty in these girls, and metformin's monotherapy has shown low efficacy, considering that girls have to undergo four years of treatment with the possibility of experiencing some of its side effects. For this reason, there is no validated standard treatment in girls with early puberty.

The proposed novel treatment with a low-dose combination of three generics (*mini-spiomet*) that work togther to reduce ectopic fat through different pathways, is the first to target the pathophysiological roots of the condition. It therefore has the potential to reduce short- and long-term comorbidities, thereby reducing the financial burden on healthcare systems.

BIBLIOGRAPHY

1. Mª José Martínez-Adeo Ollero, Godoy Molina E. Pubertad precoz y variantes de la normalidad. Protoc Diagn Ter Pediatr [Internet]. 2019 [cited 2024 Jan 10];1:239–52. Available from: https://www.aeped.es/sites/default/files/documentos/15_pubertadp.pdf

2. Eckert-Lind C, Busch AS, Petersen JH, Biro FM, Butler G, Bräuner EV, et al. Worldwide Secular Trends in Age at Pubertal Onset Assessed by Breast Development Among Girls: A Systematic Review and Meta-analysis. JAMA Pediatr [Internet]. 2020 Apr 6 [cited 2024 Jan 10];174(4):1–11. Available from: https://jamanetwork.com/journals/jamapediatrics/fullarticle/2760573

3. Worthman CM, Trang K. Dynamics of body time, social time and life history at adolescence. Nature [Internet]. 2018 Feb [cited 2024 Jan 10];554(7693):451–7. Available from: https://www.nature.com/articles/nature25750

4. O'Keeffe LM, Frysz M, Bell JA, Howe LD, Fraser A. Puberty timing and adiposity change across childhood and adolescence: disentangling cause and consequence. Hum Reprod [Internet]. 2020 [cited 2024 Jan 10];35(12):1–11. Available from: https://pubmed.ncbi.nlm.nih.gov/33242326/

5. Fan HY, Huang YT, Hsieh RH, Chao JCJ, Tung YC, Lee YL, et al. Birthweight, time-varying adiposity growth and early menarche in girls: A Mendelian randomisation and mediation analysis. Obes Res Clin Pract [Internet]. 2018 Sep [cited 2024 Jan 10];12(5):445–51. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1871403X18301030

6. de Zegher F, García Beltrán C, López-Bermejo A, Ibáñez L. Metformin for Rapidly Maturing Girls with Central Adiposity: Less Liver Fat and Slower Bone Maturation. Horm Res Paediatr [Internet]. 2018 [cited 2023 Nov 30];89(2):136–40. Available from: https://www.karger.com/Article/FullText/479369

7. Navarro VM, nueva E a sitio externo E enlace se abrirá en una ventana. Metabolic regulation of kisspeptin — the link between energy balance and reproduction. Nat Rev Endocrinol [Internet]. 2020 [cited 2023 Nov 22];16(8):407–20. Available from: https://www.proquest.com/docview/2422263341/abstract/F9B15B6D6B2640F6PQ/1

8. Pralong FP. Insulin and NPY pathways and the control of GnRH function and puberty onset. Mol Cell Endocrinol [Internet]. 2010 Aug [cited 2024 Jan 10];324(1–2):82–6. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0303720710000559

9. Frisch RE, Revelle R. Height and Weight at Menarche and a Hypothesis of Critical Body Weights and Adolescent Events. Sci New Ser [Internet]. 1970;169(3943):397–9. Available from: http://www.jstor.org/stable/1729591

10. Reinehr T, Roth CL. Is there a causal relationship between obesity and puberty? Lancet Child Adolesc Health [Internet]. 2019 Jan [cited 2024 Jan 10];3(1):44–54. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2352464218303067

11. Huang A, Reinehr T, Roth CL. Connections between obesity and puberty. Curr Opin Endocr Metab Res [Internet]. 2020 Oct [cited 2024 Jan 10];14:160–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S2451965020300892

12. Grumbach MM. The Neuroendocrinology of Human Puberty Revisited. Horm Res Paediatr [Internet]. 2002 [cited 2024 Jan 11];57(Suppl. 2):2–14. Available from: https://www.karger.com/Article/FullText/58094

13. Mathew H, Castracane VD, Mantzoros C. Adipose tissue and reproductive health. Metabolism [Internet]. 2018 Sep [cited 2024 Jan 11];86:18–32. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0026049517303098

14. Avendaño MS, Vazquez MJ, Tena-Sempere M. Disentangling puberty: novel neuroendocrine pathways and mechanisms for the control of mammalian puberty. Hum Reprod Update [Internet]. 2017 Nov 1 [cited 2024 Jan 10];23(6):737–63. Available from: https://academic.oup.com/humupd/article/23/6/737/4097589

15. Cortés ME, Carrera B, Rioseco H, Pablo Del Río J, Vigil P. The Role of Kisspeptin in the Onset of Puberty and in the Ovulatory Mechanism: A Mini-review. J Pediatr Adolesc Gynecol [Internet]. 2015 Oct [cited 2024 Jan 11];28(5):286–91. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1083318814003556

16. Heras V, Castellano JM, Fernandois D, Velasco I, Rodríguez-Vazquez E, Roa J, et al. Central Ceramide Signaling Mediates Obesity-Induced Precocious Puberty. Cell Metab [Internet]. 2020 Dec [cited 2024 Jan 15];32(6):951–66. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413120305349

17. Franssen D, Barroso A, Ruiz-Pino F, Vázquez MJ, García-Galiano D, Castellano JM, et al. AMP-activated protein kinase (AMPK) signaling in GnRH neurons links energy status and reproduction. Metabolism [Internet]. 2021 Feb [cited 2024 Jan 15];115:1–15. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0026049520303243

18. Tam CS, De Zegher F, Garnett SP, Baur LA, Cowell CT. Opposing Influences of Prenatal and Postnatal Growth on the Timing of Menarche. J Clin Endocrinol Metab [Internet]. 2006 Nov 1 [cited 2024 Jan 14];91(11):4369–73. Available from: https://academic.oup.com/jcem/article/91/11/4369/2656537

19. De Zegher F, Malpique R, Garcia-Beltran C, Ibáñez L. Towards a simple marker of hepato-visceral adiposity and insulin resistance: The Z-score change from weight-at-birth to BMI-in-childhood. Pediatr Obes [Internet]. 2019 Oct [cited 2024 Jan 15];14(10):1–3. Available from: https://onlinelibrary.wiley.com/doi/10.1111/ijpo.12533

20. De Zegher F, Reinehr T, Malpique R, Darendeliler F, López-Bermejo A, Ibáñez L. Reduced Prenatal Weight Gain and/or Augmented Postnatal Weight Gain Precedes Polycystic Ovary Syndrome in Adolescent Girls. Obesity [Internet]. 2017 Sep [cited 2024 Jan 14];25(9):1486–9. Available from: https://onlinelibrary.wiley.com/doi/10.1002/oby.21935

21. De Zegher F, López-Bermejo A, Ibáñez L. Central Obesity, Faster Maturation, and 'PCOS' in Girls. Trends Endocrinol Metab [Internet]. 2018 Dec [cited 2024 Jan 12];29(12):815–8. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1043276018301668

22. Ong KK, Potau N, Petry CJ, Jones R, Ness AR, Honour JW, et al. Opposing Influences of Prenatal and Postnatal Weight Gain on Adrenarche in Normal Boys and Girls. J Clin Endocrinol Metab [Internet]. 2004 Jun [cited 2024 Jan 14];89(6):2647–51. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2003-031848

23. Ibáñez L, Lopez-Bermejo A, Díaz M, Suárez L, De Zegher F. Low-Birth Weight Children Develop Lower Sex Hormone Binding Globulin and Higher Dehydroepiandrosterone Sulfate Levels and Aggravate their Visceral Adiposity and Hypoadiponectinemia between Six and Eight Years of Age. J Clin Endocrinol Metab [Internet]. 2009 Oct 1 [cited 2024 Jan 14];94(10):3696–9. Available from: https://academic.oup.com/jcem/article/94/10/3696/2596616

24. Holland WL, Miller RA, Wang ZV, Sun K, Barth BM, Bui HH, et al. Receptor-mediated activation of ceramidase activity initiates the pleiotropic actions of adiponectin. Nat Med [Internet]. 2011 Jan [cited 2024 Jan 15];17(1):55–63. Available from: https://www.nature.com/articles/nm.2277

25. Diamanti-Kandarakis E, Dunaif A. Insulin Resistance and the Polycystic Ovary Syndrome Revisited: An Update on Mechanisms and Implications. Endocr Rev [Internet]. 2012 Dec 1 [cited 2024 Jan 15];33(6):981–1030. Available from: https://academic.oup.com/edrv/article/33/6/981/2354926

26. Holm K, Mosfeldt Laursen E, Brocks V, Müller J. Pubertal maturation of the internal genitalia: an ultrasound evaluation of 166 healthy girls. Ultrasound Obstet Gynecol [Internet]. 1995 Sep [cited 2024 Jan 19];6(3):175–81. Available from: https://obgyn.onlinelibrary.wiley.com/doi/10.1046/j.1469-0705.1995.06030175.x

27. Ranke MB, editor. Sonographic Measurements of Endocrine Tissue. In: Diagnostics of endocrine function in children and adolescents. 3rd ed. Basel Freiburg: Karger; 2003. p. 56–62.

28. Shim KS. Pubertal growth and epiphyseal fusion. Ann Pediatr Endocrinol Metab [Internet]. 2015 [cited 2024 Jan 18];20(1):8–12. Available from: http://e-apem.org/journal/view.php?doi=10.6065/apem.2015.20.1.8

29. Even L, Andersson B, Kriström B, Albertsson-Wikland K, Hochberg Z. Role of growth hormone in enchondroplasia and chondral osteogenesis: evaluation by X-ray of the hand. Pediatr Res [Internet]. 2014 Jul [cited 2024 Jan 18];76(1):109–14. Available from: https://www.nature.com/articles/pr201456

30. Weise M, De-Levi S, Barnes KM, Gafni RI, Abad V, Baron J. Effects of estrogen on growth plate senescence and epiphyseal fusion. Proc Natl Acad Sci [Internet]. 2001 Jun 5 [cited 2024 Jan 15];98(12):6871–6. Available from: https://pnas.org/doi/full/10.1073/pnas.121180498

31. Xu L, Wang Q, Wang Q, Lyytikäinen A, Mikkola T, Völgyi E, et al. Concerted actions of insulin-like growth factor 1, testosterone, and estradiol on peripubertal bone growth: A 7-year longitudinal study. J Bone Miner Res [Internet]. 2011 Sep [cited 2024 Jan 18];26(9):2204–11. Available from: https://asbmr.onlinelibrary.wiley.com/doi/10.1002/jbmr.422

32. Ong YY, Sadananthan SA, Aris IM, Tint MT, Yuan WL, Huang JY, et al. Mismatch between poor fetal growth and rapid postnatal weight gain in the first 2 years of life is associated with higher blood pressure and insulin resistance without increased adiposity in childhood: the GUSTO cohort study. Int J Epidemiol [Internet]. 2020 Oct 1 [cited 2024 Jan 15];49(5):1591–603. Available from: https://academic.oup.com/ije/article/49/5/1591/5897702

33. Petry CJ, Ong KK, Dunger DB. Age at menarche and the future risk of gestational diabetes: a systematic review and dose response meta-analysis. Acta Diabetol [Internet]. 2018 Dec [cited 2024 Jan 15];55(12):1209–19. Available from: http://link.springer.com/10.1007/s00592-018-1214-z

34. Cheng TS, Day FR, Lakshman R, Ong KK. Association of puberty timing with type 2 diabetes: A systematic review and meta-analysis. Ma RCW, editor. PLOS Med [Internet]. 2020 Jan 6 [cited 2024 Jan 15];17(1):e1003017. Available from: https://dx.plos.org/10.1371/journal.pmed.1003017

35. The LifeLines Cohort Study, The InterAct Consortium, kConFab/AOCS Investigators, Endometrial Cancer Association Consortium, Ovarian Cancer Association Consortium, PRACTICAL consortium, et al. Genomic analyses identify hundreds of variants associated with age at menarche and support a role for puberty timing in cancer risk. Nat Genet [Internet]. 2017 Jun [cited 2024 Jan 15];49(6):834–41. Available from: https://www.nature.com/articles/ng.3841

36. Mrug S, Elliott MN, Davies S, Tortolero SR, Cuccaro P, Schuster MA. Early Puberty, Negative Peer Influence, and Problem Behaviors in Adolescent Girls. Pediatrics [Internet]. 2014 Jan 1 [cited 2024 Jan 15];133(1):7–14. Available from: https://publications.aap.org/pediatrics/article/133/1/7/68324/Early-Puberty-Negative-Peer-Influence-and-Problem

37. Carel JC. Precocious puberty and statural growth. Hum Reprod Update [Internet]. 2004 Mar 1 [cited 2024 Jan 15];10(2):135–47. Available from: https://academic.oup.com/humupd/article-lookup/doi/10.1093/humupd/dmh012

38. Bouvattier C, Coste J, Rodrigue D, Teinturier C, Carel JC, Chaussain JL, et al. Lack of Effect of GnRH Agonists on Final Height in Girls with Advanced Puberty: A Randomized Long-Term Pilot Study. J Clin Endocrinol Metab [Internet]. 1999 [cited 2024 Jan 15];84(10):3575–8. Available from: https://pubmed.ncbi.nlm.nih.gov/10522998/

39. Ibáñez L, López-Bermejo A, Díaz M, Marcos MV, De Zegher F. Metformin Treatment for Four Years to Reduce Total and Visceral Fat in Low Birth Weight Girls with Precocious Pubarche. J Clin Endocrinol Metab [Internet]. 2008 May 1 [cited 2023 Dec 11];93(5):1841–5. Available from: https://academic.oup.com/jcem/article/93/5/1841/2598890

40. Ibáñez L, Lopez-Bermejo A, Diaz M, Marcos MV, De Zegher F. Pubertal Metformin Therapy to Reduce Total, Visceral, and Hepatic Adiposity. J Pediatr [Internet]. 2010 Jan [cited 2023 Dec 11];156(1):98–103. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022347609006453

41. Díaz M, Bassols J, López-Bermejo A, De Zegher F, Ibáñez L. Metformin treatment to reduce central adiposity after prenatal growth restraint: a placebo-controlled pilot study in prepubertal children: Early metformin intervention in SGA children. Pediatr Diabetes [Internet]. 2015 Nov [cited 2023 Dec 11];16(7):538–45. Available from: https://onlinelibrary.wiley.com/doi/10.1111/pedi.12220

42. Ibáñez L, Ong K, Valls C, Marcos MV, Dunger DB, De Zegher F. Metformin Treatment to Prevent Early Puberty in Girls with Precocious Pubarche. J Clin Endocrinol Metab [Internet]. 2006 Aug [cited 2024 Jan 16];91(8):2888–91. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2006-0336

43. Ibáñez L, Lopez-Bermejo A, Diaz M, Marcos MV, De Zegher F. Early metformin therapy to delay menarche and augment height in girls with precocious pubarche. Fertil Steril [Internet]. 2011 Feb [cited 2023 Dec 11];95(2):727–30. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0015028210024660

44. Ibáñez L, López-Bermejo A, Díaz M, Marcos MV, De Zegher F. Early Metformin Therapy (Age 8–12 Years) in Girls with Precocious Pubarche to Reduce Hirsutism, Androgen Excess, and Oligomenorrhea in Adolescence. J Clin Endocrinol Metab [Internet]. 2011 Aug [cited 2024 Jan 16];96(8):1262–7. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2011-0555

45. Ibáñez L, Díaz M, García-Beltrán C, Malpique R, Garde E, López-Bermejo A, et al. Toward a Treatment Normalizing Ovulation Rate in Adolescent Girls With Polycystic Ovary Syndrome. J Endocr Soc [Internet]. 2020 May 1 [cited 2023 Dec 8];4(5):1–9. Available from: https://academic.oup.com/jes/article/doi/10.1210/jendso/bvaa032/5805163

46. De Zegher F, Ibáñez L. Letter to the Editor: Tackling NAFLD in Adolescent Polycystic Ovary Syndrome: Reducing Liver Fat to Mimic Weight Loss. Hepatology [Internet]. 2021 Apr [cited 2023 Dec 11];73(4):1623–4. Available from: https://journals.lww.com/10.1002/hep.31592

47. De Zegher F, Diaz M, Ibañez L. From adolescent PCOS to adult MAFLD: opposing effects of randomised interventions. BMJ Open Gastroenterol [Internet]. 2021 May [cited 2023 Dec 11];8(1):1–3. Available from: https://bmjopengastro.bmj.com/lookup/doi/10.1136/bmjgast-2020-000574

48. Ibáñez L, Del Río L, Díaz M, Sebastiani G, Pozo ÓJ, López-Bermejo A, et al. Normalizing Ovulation Rate by Preferential Reduction of Hepato-Visceral Fat in Adolescent Girls With Polycystic Ovary Syndrome. J Adolesc Health [Internet]. 2017 Oct [cited 2023 Dec 8];61(4):446–53. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1054139X17302021

49. Fallo F, Veglio F, Bertello C, Sonino N, Della Mea P, Ermani M, et al. Prevalence and Characteristics of the Metabolic Syndrome in Primary Aldosteronism. J Clin Endocrinol Metab [Internet]. 2006 Feb [cited 2024 Jan 18];91(2):454–9. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2005-1733

50. Buglioni A, Cannone V, Sangaralingham SJ, Heublein DM, Scott CG, Bailey KR, et al. Aldosterone Predicts Cardiovascular, Renal, and Metabolic Disease in the General Community: A 4-Year Follow-Up. J Am Heart Assoc [Internet]. 2015 Dec [cited 2024 Jan 18];4(12):1–14. Available from: https://www.ahajournals.org/doi/10.1161/JAHA.115.002505

51. Bochud M, Nussberger J, Bovet P, Maillard MR, Elston RC, Paccaud F, et al. Plasma Aldosterone Is Independently Associated With the Metabolic Syndrome. Hypertension [Internet]. 2006 Aug [cited 2024 Jan 18];48(2):239–45. Available from: https://www.ahajournals.org/doi/10.1161/01.HYP.0000231338.41548.fc

52. Thuzar M, Ho KKY. Mechanisms in Endocrinology: Brown adipose tissue in humans: regulation and metabolic significance. Eur J Endocrinol [Internet]. 2016 Jul [cited 2024 Jan 18];175(1):R11–25. Available from: https://academic.oup.com/ejendo/article/175/1/R11/6654921

53. Thuzar M, Law WP, Dimeski G, Stowasser M, Ho KKY. Mineralocorticoid antagonism enhances brown adipose tissue function in humans: A randomized placebo-controlled cross-over study. Diabetes Obes Metab [Internet]. 2019 Mar [cited 2023 Dec 8];21(3):509–16. Available from: https://dom-pubs.pericles-prod.literatumonline.com/doi/10.1111/dom.13539

54. García-Beltran C, Cereijo R, Quesada-López T, Malpique R, López-Bermejo A, De Zegher F, et al. Reduced circulating levels of chemokine CXCL14 in adolescent girls with polycystic ovary syndrome: normalization after insulin sensitization. BMJ Open Diabetes Res Care [Internet].

2020 Feb [cited 2023 Nov 30];8(1):1–7. Available from: https://drc.bmj.com/lookup/doi/10.1136/bmjdrc-2019-001035

55. Ganie MA, Khurana ML, Nisar S, Shah PA, Shah ZA, Kulshrestha B, et al. Improved Efficacy of Low-Dose Spironolactone and Metformin Combination Than Either Drug Alone in the Management of Women With Polycystic Ovary Syndrome (PCOS): A Six-Month, Open-Label Randomized Study. J Clin Endocrinol Metab [Internet]. 2013 Sep [cited 2023 Dec 8];98(9):3599–607. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2013-1040

56. Chiquette E, Ramirez G, DeFronzo R. A Meta-analysis Comparing the Effect of Thiazolidinediones on Cardiovascular Risk Factors. Arch Intern Med [Internet]. 2004 [cited 2024 Jan 18];164:2097–104. Available from: https://pubmed.ncbi.nlm.nih.gov/15505122/

57. Inzucchi SE, Bergenstal RM, Buse JB, Diamant M, Ferrannini E, Nauck M, et al. Management of hyperglycaemia in type 2 diabetes, 2015: a patient-centred approach. Update to a Position Statement of the American Diabetes Association and the European Association for the Study of Diabetes. Diabetologia [Internet]. 2015 Mar [cited 2024 Jan 18];58(3):429–42. Available from: http://link.springer.com/10.1007/s00125-014-3460-0

58. Palmer SC, Mavridis D, Nicolucci A, Johnson DW, Tonelli M, Craig JC, et al. Comparison of Clinical Outcomes and Adverse Events Associated With Glucose-Lowering Drugs in Patients With Type 2 Diabetes: A Meta-analysis. JAMA [Internet]. 2016 Jul 19 [cited 2024 Jan 18];316(3):313–24. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2016.9400

59. Manolopoulos KN, Karpe F, Frayn KN. Gluteofemoral body fat as a determinant of metabolic health. Int J Obes [Internet]. 2010 Jun [cited 2024 Jan 18];34(6):949–59. Available from: https://www.nature.com/articles/ijo2009286

60. Karpe F, Pinnick KE. Biology of upper-body and lower-body adipose tissue—link to whole-body phenotypes. Nat Rev Endocrinol [Internet]. 2015 Feb [cited 2024 Jan 18];11(2):90–100. Available from: https://www.nature.com/articles/nrendo.2014.185

61. Karastergiou K, Smith SR, Greenberg AS, Fried SK. Sex differences in human adipose tissues – the biology of pear shape. Biol Sex Differ [Internet]. 2012 Dec [cited 2024 Jan 18];3(1):1–12. Available from: https://bsd.biomedcentral.com/articles/10.1186/2042-6410-3-13

62. Tchernof A, Després JP. Pathophysiology of Human Visceral Obesity: An Update. Physiol Rev [Internet]. 2013 Jan [cited 2024 Jan 18];93(1):359–404. Available from: https://www.physiology.org/doi/10.1152/physrev.00033.2011

63. Huang T, Qi Q, Zheng Y, Ley SH, Manson JE, Hu FB, et al. Genetic Predisposition to Central Obesity and Risk of Type 2 Diabetes: Two Independent Cohort Studies. Diabetes Care [Internet]. 2015 Jul 1 [cited 2024 Jan 18];38(7):1306–11. Available from: https://diabetesjournals.org/care/article/38/7/1306/31082/Genetic-Predisposition-to-Central-Obesity-and-Risk

64. Snijder MB, Visser M, Dekker JM, Goodpaster BH, Harris TB, Kritchevsky SB, et al. Low subcutaneous thigh fat is a risk factor for unfavourable glucose and lipid levels, independently of high abdominal fat. The Health ABC Study. Diabetologia [Internet]. 2005 Feb [cited 2024 Jan 18];48(2):301–8. Available from: http://link.springer.com/10.1007/s00125-004-1637-7

65. Canoy D, Boekholdt SM, Wareham N, Luben R, Welch A, Bingham S, et al. Body Fat Distribution and Risk of Coronary Heart Disease in Men and Women in the European Prospective Investigation Into Cancer and Nutrition in Norfolk Cohort. Circulation [Internet]. 2024 Jan 18;116:2933–43. Available from: https://pubmed.ncbi.nlm.nih.gov/18071080/

66. Tchkonia T, Thomou T, Zhu Y, Karagiannides I, Pothoulakis C, Jensen MD, et al. Mechanisms and Metabolic Implications of Regional Differences among Fat Depots. Cell Metab [Internet]. 2013 May [cited 2024 Jan 18];17(5):644–56. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1550413113001113

67. Saltiel AR, Olefsky JM. Thiazolidinediones in the Treatment of Insulin Resistance and Type II Diabetes. Diabetes [Internet]. 1996;45(12):1661–9. Available from: https://pubmed.ncbi.nlm.nih.gov/8922349/

68. Kodama N, Tahara N, Tahara A, Honda A, Nitta Y, Mizoguchi M, et al. Effects of Pioglitazone on Visceral Fat Metabolic Activity in Impaired Glucose Tolerance or Type 2 Diabetes Mellitus. J Clin Endocrinol Metab [Internet]. 2013 Nov [cited 2024 Jan 18];98(11):4438–45. Available from: https://academic.oup.com/jcem/article-lookup/doi/10.1210/jc.2013-2920

69. White U, Fitch MD, Beyl RA, Hellerstein MK, Ravussin E. Adipose depot-specific effects of 16 weeks of pioglitazone on in vivo adipogenesis in women with obesity: a randomised controlled trial. Diabetologia [Internet]. 2021 Jan [cited 2024 Jan 17];64(1):159–67. Available from: https://link.springer.com/10.1007/s00125-020-05281-7

70. Ibáñez L, López-Bermejo A, Díaz M, Enríquez G, Valls C, De Zegher F. Pioglitazone (7·5 mg/day) added to flutamide–metformin in women with androgen excess: additional increments of visfatin and high molecular weight adiponectin. Clin Endocrinol (Oxf) [Internet]. 2008 Feb [cited 2024 Jan 17];68(2):317–20. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-2265.2007.03137.x

71. Capano L. A pilot dose finding study of pioglitazone in autistic children. Mol Autism [Internet]. 2018 [cited 2024 Jan 18];1–14. Available from: https://pubmed.ncbi.nlm.nih.gov/30498564/

72. Reitman ML, Schadt EE. Pharmacogenetics of metformin response: a step in the path toward personalized medicine. J Clin Invest [Internet]. 2007 May 1 [cited 2024 Jan 17];117(5):1226–9. Available from: http://www.jci.org/cgi/doi/10.1172/JCI32133

73. Foretz M, Hébrard S, Leclerc J, Zarrinpashneh E, Soty M, Mithieux G, et al. Metformin inhibits hepatic gluconeogenesis in mice independently of the LKB1/AMPK pathway via a decrease in hepatic energy state. J Clin Invest [Internet]. 2010 Jul 1 [cited 2024 Jan 17];120(7):2355–69. Available from: http://www.jci.org/articles/view/40671

74. Flory J, Lipska K. Metformin in 2019. JAMA [Internet]. 2019 May 21 [cited 2024 Jan 19];321(19):1–2. Available from: http://jama.jamanetwork.com/article.aspx?doi=10.1001/jama.2019.3805

75. Pasquel FJ, Hinedi Z, Umpierrez GE, Klein R, Adigweme A, Coralli R, et al. Metformin-Associated Lactic Acidosis. Am J Med Sci [Internet]. 2015 Mar [cited 2024 Jan 17];349(3):263–7. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0002962915300999

76. Liu Q, Li S, Quan H, Li J. Vitamin B12 Status in Metformin Treated Patients: Systematic Review. Pietropaolo M, editor. PLoS ONE [Internet]. 2014 Jun 24 [cited 2024 Jan 17];9(6):1–6. Available from: https://dx.plos.org/10.1371/journal.pone.0100379

77. Durda-Masny M, Stróżewska W, Szwed A. Catch-Up Growth as a Risk Factor for Rapid Weight Gain, Earlier Menarche and Earlier Pubertal Growth Spurt in Girls Born Small for Gestational Age (SGA)—A Longitudinal Study. Int J Environ Res Public Health [Internet]. 2022 Dec 14 [cited 2023 Dec 30];19(24):1–13. Available from: https://www.mdpi.com/1660-4601/19/24/16808

78. Chumlea WC, Schubert CM, Roche AF, Kulin HE, Lee PA, Himes JH, et al. Age at Menarche and Racial Comparisons in US Girls. Pediatrics [Internet]. 2003 Jan 1 [cited 2024 Jan 20];111(1):110–3. Available from: https://publications.aap.org/pediatrics/article/111/1/110/28486/Age-at-Menarche-and-Racial-Comparisons-in-US-Girls

79. Kaplowitz PB, Slora EJ, Wasserman RC, Pedlow SE, Herman-Giddens ME. Earlier Onset of Puberty in Girls: Relation to Increased Body Mass Index and Race. Pediatrics [Internet]. 2001 Aug 1 [cited 2024 Jan 20];108(2):347–53. Available from: https://publications.aap.org/pediatrics/article/108/2/347/63908/Earlier-Onset-of-Puberty-in-Girls-Relation-to

80. Klein KO, Newfield RS, Hassink SG. Bone maturation along the spectrum from normal weight to obesity: a complex interplay of sex, growth factors and weight gain. J Pediatr Endocrinol Metab [Internet]. 2016 Jan 1 [cited 2023 Dec 30];29(3):1–8. Available from: https://www.degruyter.com/document/doi/10.1515/jpem-2015-0234/html

81. Grumbach MM. Estrogen, Bone, Growth and Sex: A Sea Change in Conventional Wisdom: Department of Pediatrics, University of California, San Francisco, USA. J Pediatr Endocrinol Metab [Internet]. 2000 Dec 1 [cited 2023 Dec 30];13(s2):1439–56. Available from: https://www.degruyter.com/document/doi/10.1515/jpem-2000-s619/html

82. Lee H, Shim Y, Jeong H, Kwon E, Hwang J. The Association between Bone Age Advancement and Insulin Resistance in Prepubertal Obese Children. Exp Clin Endocrinol Diabetes [Internet]. 2015 Nov 24 [cited 2023 Dec 30];123(10):604–7. Available from: http://www.thieme-connect.de/DOI/DOI?10.1055/s-0035-1559795

83. Garcia-Beltran C, Malpique R, Andersen MS, Bas F, Bassols J, Darendeliler F, et al. SPIOMET4HEALTH—efficacy, tolerability and safety of lifestyle intervention plus a fixed dose combination of spironolactone, pioglitazone and metformin (SPIOMET) for adolescent girls and young women with polycystic ovary syndrome: study protocol for a multicentre, randomised, double-blind, placebo-controlled, four-arm, parallel-group, phase II clinical trial. Trials [Internet]. 2023 Sep 15 [cited 2023 Dec 11];24(1):1–25. Available from: https://trialsjournal.biomedcentral.com/articles/10.1186/s13063-023-07593-6

84. Somani N, Turvy D. Hirsutism: An Evidence-Based Treatment Update. Am J Clin Dermatol [Internet]. 2014 Jul [cited 2023 Dec 11];15(3):247–66. Available from: http://link.springer.com/10.1007/s40257-014-0078-4

85. Kuzik N, Myette-Côté É, Carson V, Slater L, Boulé NG. Evaluating the Effects of Metformin Use on Height in Children and Adolescents: A Meta-analysis of Randomized Clinical Trials. JAMA Pediatr [Internet]. 2015 Nov 1 [cited 2023 Dec 8];169(11):1–8. Available from: http://archpedi.jamanetwork.com/article.aspx?doi=10.1001/jamapediatrics.2015.2186

86. Crocker PRE, Bailey DA, Faulkner RA, Kowalski KC, McGRATH R. Measuring general levels of physical activity: preliminary evidence for the Physical Activity Questionnaire for Older Children. Med Sci Sports Exerc [Internet]. 1997 Oct [cited 2024 Jan 27];29(10):1344. Available from: https://journals.lww.com/acsm-msse/fulltext/1997/10000/measuring_general_levels_of_physical_activity_.11.aspx

87. www.nutricionprecision.com. Cuestionario de adhesión a la Dieta Mediterránea en niños y adolescentes [Internet]. [cited 2024 Jan 27]. Available from: https://www.nutricionprecision.com/kidmed-2019-actualizacion-del-cuestionario-kidmed-36.html

88. Surface Area of an Ellipsoid Formula [Internet]. [cited 2024 Jan 26]. Available from: https://www.easycalculation.com/formulas/ellisoid-surface-area-formul.html

89. Bull RK, Edwards PD, Kemp PM, Fry S, Hughes IA. Bone age assessment: a large scale comparison of the Greulich and Pyle, and Tanner and Whitehouse (TW2) methods. Arch Dis Child [Internet]. 1999 Aug 1 [cited 2024 Jan 5];81(2):172–3. Available from: https://adc.bmj.com/lookup/doi/10.1136/adc.81.2.172

90. Boswell H. Normal Pubertal Physiology in Females. In: Dietrich JE, editor. Female puberty: a comprehensive guide for clinicians. New York: Springer; 2014. p. 7–30.

91. :: CIMA ::. PROSPECTO ESPIRONOLACTONA ALTER 25 mg COMPRIMIDOS RECUBIERTOS EFG [Internet]. [cited 2024 Jan 27]. Available from: https://cima.aemps.es/cima/dochtml/p/63835/Prospecto_63835.html

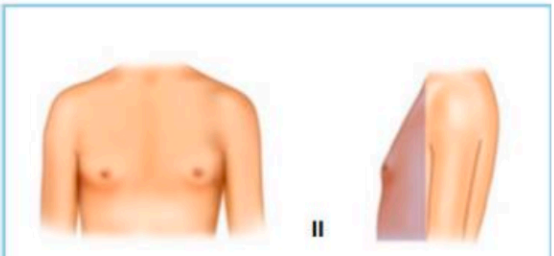
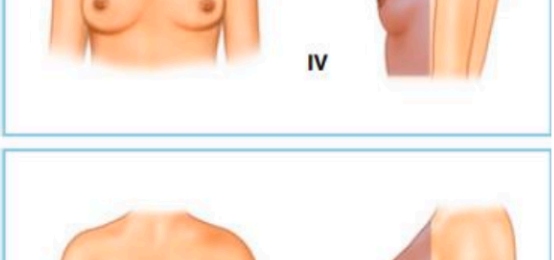
92. :: CIMA ::. PROSPECTO PIOGLITAZONA NORMON 30 mg COMPRIMIDOS EFG [Internet]. [cited 2024 Jan 27]. Available from: https://cima.aemps.es/cima/dochtml/p/75153/P_75153.html

93. :: CIMA ::. FICHA TECNICA METFORMINA TEVA 850 mg COMPRIMIDOS RECUBIERTOS CON PELICULA EFG [Internet]. [cited 2023 Dec 13]. Available from: https://cima.aemps.es/cima/dochtml/ft/71399/FichaTecnica_71399.html

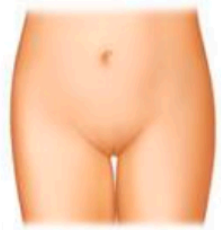
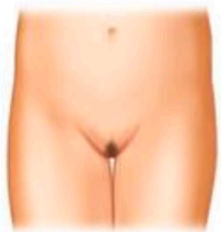
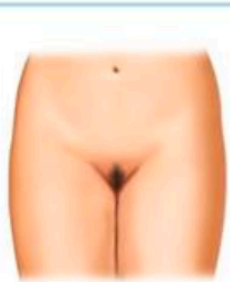
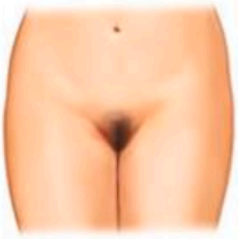
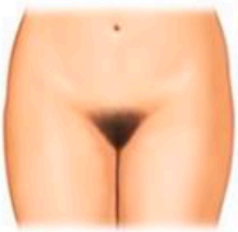
94. Matos-Duarte M, Martínez-de-Haro V, Sanz-Arribas I, Andrade AGP, Chagas MH. Estudio longitudinal de la flexibilidad funcional en mayores físicamente activos / Longitudinal study of Functional Flexibility in Olfer Physically Active. Rev Int Med Cienc Act Física Deporte [Internet]. 2017 [cited 2024 Jan 27];65(2017). Available from: https://revistas.uam.es/rimcafd/article/view/7362

ANNEXES

ANNEX 1

Tanner Stage1	Preadolescent	Only papilla is elevated	
Tanner Stage 2	Breast budding	Enlargement and widening of the areola and mound-like elevation of the breast and papilla	
Tanner Stage 3		Further enlargement of breast and areola with NO separation of contours	
Tanner Stage 4		Projection of the areola and papilla to form secondary mound above the level of the breast and further enlargement	
Tanner Stage 5	Adult Breast	Projection of the papilla only, as the areola recesses to the mature contour of the breast	

Tanner stages of female pubertal breast development. (90)

Tanner Stage1	Preadolescent	No discernable difference between vellus hair on the mons and anterior abdominal wall, no pubic hair	 I
Tanner Stage 2		Appearance of few, sparse, lightly pigmented hairs, with minimal curl on the labia	 II
Tanner Stage 3		Hair becomes darker, coarser and begins to spread over the junction of the labia	 III
Tanner Stage 4		Adult hair type emergens, covers mons pubis, but does not extend to the thighs	 IV
Tanner Stage 5	Adult hair pattern	Adult hair type in the classic female pattern	 V

Tanner stages of female pubertal pubic hair development. (90)

ANNEX 2

· SPIRONOLACTONE (91)

Contraindications

- Hypersensitivity to spironolactone or any of the excipients.
- Kidney failure (estimated glomerular filtrate <30 ml/min), acute or progressive nephropathy.
- Hyponatremia.
- Hyperkalemia (serum potassium >5 mmol/l) at the start of the treatment.
- Concomitant use of potassium-sparing diuretics (including eplerenone) or potassium supplements, or double blocking renin-angiotensin-aldosterone axis with combination of angiotensin-converting enzyme inhibitor (ACEi) and angiotensin II receptor antagonist (AIIRA).

Interactions

- Ciclosporin: concomitant use of spironolactone and ciclosporin is not recommended as they both increase serum potassium which can have severe and potentially mortal consequences.
- Heparin, low molecular weight heparin: they concomitant use with spironolactone can cause hyperkalemia. There also has been observed an increase of diuresis with concomitant use of heparin and spironolactone.
- Non-steroidal anti-inflammatories: acetylsalicylic acid and indomethacin can attenuate diuretic action of spironolactone due to intrarenal prostaglandins synthesis inhibition. Hyperkalemia has been observed with concomitant use of indomethacin and potassium-sparing diuretics.
- Anticoagulants: spironolactone reduces their anticoagulant effect.
- Noradrenaline: spironolactone reduces their vasoconstrictive effects.
- Antihypertensives: spironolactone can increase their effect. Antihypertensives drugs doses can be reduced to half when spironolactone is added to the treatment.
- Lithium: diuretic drugs reduce renal depuration of lithium which adds a high risk of lithium toxicity
- Digoxin: there has been demonstrated spironolactone increases half-life of digoxin which can generate and increase of digoxin serum levels and consequently digitalis toxicity.
- Alcohol, barbiturates and opiates: orthostatic hypotension can be potentiated with their concomitant use with spironolactone.

- Cholestyramine: metabolic acidosis has been communicated usually associated to hyperkalemia in patients who receive spironolactone in combination with cholestyramine.
- Ammonium chloride: hyperchloremic metabolic acidosis has been communicated usually associated to hyperkalemia in patients who receive spironolactone in combination with ammonium chloride (for example in liquorice)
- Cortisone: spironolactone interferes in fluorometric Mattingly method for plasmatic cortisone levels determination.
- Trimethoprim/sulfamethoxazole (cotrimoxazole): their concomitant use with spironolactone can cause clinically significant hyperkalemia.
- Abiraterone: is not recommended its use in combination with spironolactone as it can increase prostate-specific antigen in patients with prostatic cancer treated with abiraterone.

Adverse effects

Frequencies of adverse reactions are defined as:

Very common (≥1/10)

- Hyperkalemia in patients with severe kidney failure who are receiving concomitant treatment with potassium supplements, ACEi, AIIRA or diabetic patients.
- Headache, fatigue, somnolence
- Indigestion, diarrhea
- Men: decreased libido, erectile dysfunction, impotence, gynecomastia
- Women: breast disorders, mastalgia, menstrual disorders, deeper voice (irreversible in many cases)

Common (≥1/100 to <1/10)

- Hyponatremia (specially during intensive treatment with thiazide diuretics)
- Debility, lethargy in cirrhotic patients, paresthesia
- Nausea and vomiting
- Discomfort
- Women: decreased libido, amenorrhea, postmenopausal hemorrhage, changes in vaginal secretions

Uncommon (≥1/1000 to <1/100)

- Acidosis in patients with hepatic problems
- Confusion

- Cutaneous eruption, urticaria, erythema, chloasma, itch, exanthema
- Muscle spasms
- High levels of creatinine in serum

Rare (≥1/10.000 to <1/1000)

- Thrombocytopenia, eosinophilia and leucopenia (including agranulocytosis)
- Eczema (allergic reaction type 1), hypersensitity
- Dehydration, porphyria, increased plasmatic and urine levels of nitrogen, hyperuricemia (which can cause gout in predisposed patients)

Very rare (<1/10.000)

- Breast cancer
- Vasculitis
- Gastric inflammation, gastric ulcer, gastrointestinal hemorrhage, cramp
- Hepatitis
- Alopecia, hypertrichosis, annular centrifugal erythema
- Systemic lupus erythematosus (SLE), osteomalacia
- Acute renal failure

Unknown frequency

- Minor androgenic effects including hirsutism.
- Hyperchloremic metabolic acidosis (probably with hyperkalemia) in decompensated hepatic cirrhosis, even though normal renal function.
- Dizziness, ataxia
- Minor hypotension
- Stevens-Johnson syndrome, toxic epidermal necrolysis, pemphigoid, drug reaction with eosinophilia and systemic symptoms.

<u>Overdose</u>

Overdose symptoms usually are nausea and vomiting and less frequently confusion, drowsiness, rash and diarrhea. Infertility could be produced if spironolactone is administered at high doses (450 mg/day).

Hyperkalemia symptoms (paresthesia, muscle debility…) usually are not associated with acute overdose. Its treatment consists of reducing potassium ingest, administering diuretics, intravenous glucose with insulin and ionic exchange resins orally.

·PIOGLITAZONE (92)

Contraindications

- Pioglitazone allergy or to any of the excipients.
- Cardiac failure present or suffered in the past.
- Hepatic disease.
- Diabetic ketoacidosis.
- Bladder cancer present or suffered in the past.
- Hematuria not controlled by any doctor.

Interactions

Pioglitazone has not pharmacokinetic interactions, for that reason, other medication can be taken at the same time as pioglitazone.

Adverse effects

Frequencies of adverse reactions are defined as:

Very common (≥1/10)

- Hypoglycemia

Common (≥1/100 to <1/10)

- Cardiac failure in patients in treatment with pioglitazone in combination with insulin. Symptoms are edema, dyspnea or rapid weight gain.
- Edema.
- Bone fractures in women.
- Respiratory infection.
- View disorders.
- Weight gain.
- Numbness.
- Headache, dizziness.
- Joint and back pain.
- Flatulence.

- Slight decrease in the number of erythrocytes.

Uncommon (≥1/1000 to <1/100)

- Bladder cancer. Symptoms are hematuria, pain with urination or urgency to void.
- Sinusitis
- Insomnia, fatigue.
- Sweating.
- Increased appetite.

Unknown frequency

- Bone fractures in men.
- Blurred vision.
- Allergic reactions.
- Hepatic enzymes increase.

Overdose

In cases of accidental overdose, serum glucose levels may fall below normal, but this can be resolved by the ingestion of sugar. In any case, you must contact the Toxicology Information Service on 91 562 04 20.

·METFORMIN (93)

Contraindications

- Hypersensitivity to metformin or any of the excipients.
- Diabetic ketoacidosis, diabetic precoma or any type of metabolic acidosis.
- Acute renal failure (estimated glomerular filtrate <30 ml/min)
- Acute disorders which can alter renal function: dehydration, severe infection, shock.
- Acute or chronic disease that could produce hypoxia such as heart failure, respiratory failure, recent myocardium infarct or shock.
- Liver failure, acute alcoholic intoxication or alcoholism.

Interactions

The main interactions metformin has been:

- Alcohol: acute alcoholic intoxication is associated with increasing risk of lactic acidosis, especially in case of fasting or malnutrition and liver failure.

- Iodine contrast: intravascular administration of iodine contrast can produce nephropathy producing blood metformin accumulation and higher risk of lactic acidosis. For this reason, metformin has to be discontinued and not restarted until 48h.
- Non-steroidal anti-inflammatories (including selective cyclooxygenase II inhibitors), ACEi, AIIRA and diuretics (especially the loop ones): they increase lactic acidosis risk as they decrease renal function.
- Drugs with intrinsic hyperglycaemic activity (glucocorticoids, beta2-agonists and diuretics): Closer blood glucose controls are required, especially at the beginning of treatment. If necessary, metformin posology should be adjusted during therapy with the other drug and after it is discontinued.

Adverse effects

Frequencies of adverse reactions are defined as:

Very common (≥1/10)

- Gastrointestinal disorders such as nausea, vomiting, diarrhea, abdominal pain and lost of appetite. These disorders tend to appear at the beginning of treatment and in most cases, they are solved spontaneously. Gastrointestinal tolerance can be achieved by increasing metformin dose slowly.

Common (≥1/100 to <1/10)

- Nervous system disorders especially taste disorder.

Uncommon (≥1/1000 to <1/100)

Rare (≥1/10.000 to <1/1000)

Very rare (<1/10.000)

- Skin reactions such as erythema, urticaria and itching.
- Lactic acidosis. Their clinical manifestations are acidotic dyspnea, abdominal pain, muscle cramps, asthenia, hypothermia and coma. In case there is presence of these symptoms, metformin must be discontinued and medic attendance should be searched immediately.
- Megaloblastic anemia. Extended use of metformin in time can produce reduction of B12 vitamin levels.

- Hepatobiliary disorders. Hepatitis or liver function disorders have been described. They disappear when metformin is discontinued.

However, it is important to notify suspected adverse reactions after its approval. It allows a continuous supervision of benefit/risk relation of the drug. Possible new adverse reactions should be notified through Pharmacovigilance Spanish System for Medical Products for Humane Use:

Overdose

Metformin treatment should be discontinued if lactic acidosis symptoms appear. It is a medical urgency which must be treated in the hospital by hemodialysis.

ANNEX 3

Adapted from: (94)

Cuestionario de actividad física para niños (PAQ-C)

Quiero conocer cuál es tu nivel de actividad física en los últimos 7 días. Esto incluye todas aquellas actividades como deportes, gimnasia o danza que te hacen sudar o sentirte cansado, o juegos que hagan que se acelere tu respiración como jugar chapadas, saltar la soga, correr, trepar y otras.

Recuerda:

- No hay preguntas buenas o malas. Esto **NO** es un examen
- Contesta las preguntas de la forma más honesta y sincera posible. Esto es **MUY IMPORTANTE**.

1. Actividad Física en tu tiempo libre: ¿Has hecho alguna de estas actividades en los **últimos 7 días**? Si tu respuesta es sí: ¿cuántas veces lo has hecho? (Marca un solo recuadro por actividad)

Actividad	No	1-2	3-4	5-6	7 o MÁS
Saltar la soga					
Juegos (Ejem: Chapadas, las escondidas)					
Montar en bicicleta					
Caminata o paseo a pie					
Salir a correr al parque					
Natación					
Bailar/danza					
Gimnasia					
Fútbol					
Vóley					

Básquet					
Atletismo					
Artes Marciales					
Otros (menciona cuál):					

2. **En los últimos 7 días**, durante las clases de educación física, ¿cuántas veces estuviste muy activo durante las clases jugando intensamente, corriendo, saltando, haciendo lanzamientos? (Marca solo una respuesta)

   ___ No hice/hago educación física

   ___ Casi nunca

   ___ Algunas veces

   ___ A menudo

   ___ Siempre

3. **En los últimos 7 días** ¿qué hiciste durante el recreo? (Marca solo una respuesta)

   ___ Estar sentado (hablar, leer, trabajo de clase)

   ___ Estar o pasear por los alrededores

   ___ Correr o jugar un poco

   ___ Correr y jugar bastante

   ___ Correr y jugar intensamente todo el tiempo

4. **En los últimos 7 días** ¿qué hiciste normalmente antes y después de comer? (Marca solo una respuesta)

   ___ Estar sentado (hablar, leer, trabajo de clase)

   ___ Estar o pasear por los alrededores

   ___ Correr o jugar un poco

   ___ Correr y jugar bastante

   ___ Correr y jugar intensamente todo el tiempo

5. **En los últimos 7 días**, inmediatamente después del colegio, ¿cuántos días jugaste, hiciste deporte o bailes en los que estuvieras muy activo? (Marca solo una respuesta)

___ Ninguna
___ 1 vez en la última semana
___ 2-3 veces en la última semana
___ 4 veces en la última semana
___ 5 veces o más en la última semana

6. **En los últimos 7 días**, ¿cuántos días entre las 6 p.m y 10 p.m jugaste, bailaste o hiciste deportes en los que estuvieras muy activo? (Marca solo una respuesta)

___ Ninguna
___ 1 vez en la última semana
___ 2-3 veces en la última semana
___ 4 veces en la última semana

7. **El último fin de semana**, ¿cuántas veces jugaste, bailaste o hiciste deportes en los que estuvieras muy activo? (Marca solo una respuesta)

___ Ninguna
___ 1 vez en la última semana
___ 2-3 veces en la última semana
___ 4 veces en la última semana
___ 5 veces o más en la última semana

8. ¿Cuál de las siguientes frases describen mejor **tu última semana**? Lee las cinco alternativas antes de decidir cuál te describe mejor. (Marca solo una respuesta)

___ Todo o la mayoría de mi tiempo libre lo dediqué a actividades que suponen poco esfuerzo físico o no hice

___ Algunas veces (1 o 2 veces) hice actividad física en mi tiempo libre (por ejemplo: hacer deportes, correr, nadar, montar en bicicleta, hace aeróbicos)

___ A menudo (3-4 veces a la semana) hice actividad física en mi tiempo libre

___ Frecuentemente (5-6 veces en la última semana) hice actividad física en mi tiempo libre

___ Muy frecuentemente (7 o más veces en la última semana) hice actividad física en mi tiempo libre

9. Señala con qué frecuencia hiciste actividad física para **cada día de la semana** (como hacer deporte, jugar, bailar o cualquier otra actividad)

	Frecuencia				
Días de la semana	Ninguna	Poca	Regular	Frecuente	Muy frecuente
Lunes					
Martes					
Miércoles					
Jueves					
Viernes					
Sábado					
Domingo					

10. ¿Estuviste enfermo(a) esta última semana o algo impidió que hicieras normalmente actividades físicas?

Sí ☐ No ☐

Si la respuesta es sí, cuál fue el motivo:

ANNEX 4

From: (87)

KIDMED 2019 (1)

Item	Pregunta	Criterio	P*
1	Toma una fruta todos los días.	+1	
2	Toma una segunda fruta todos los días.	+1	
3	Toma verduras frescas o cocinadas regularmente todos los días.	+1	
4	Toma verduras frescas o cocinadas más de una vez al día.	+1	
5	Toma pescado regularmente (al menos 2-3 veces/semana).	+1	
6	Acude a un restaurante de comida rápida (v.g. hamburguesería) una o más veces a la semana.	-1	
7	Toma legumbres mas de una vez a la semana.	+1	
8	Toma pasta integral o arroz integral casi a diario (5 o más veces a la semana).	+1	
9	Desayuna un cereal integral o derivado integral (v.g. pan integral).	+1	
10	Toma frutos secos regularmente (al menos 2-3 veces/semana).	+1	
11	En casa se utiliza aceite de oliva.	+1	
12	No desayuna a diario.	-1	
13	Desayuna un lácteo (yogur, leche…).	+1	
14	Desayuna bollería industrial (galletas, pastas, cruasán…).	-1	
15	Toma 2 yogures y/o queso (40g) todos los días.	+1	
16	Toma dulces y golosinas varias veces al día.	-1	

*P: Puntuación.

Interpretación: Rango puntuación: 0-12.

Puntuación ≥8: Calidad dietética óptima.

Puntuación 4-7: Calidad dietética intermedia. Se necesita implementar mejoras para mejorar la adhesión a la MedDiet.

Puntuación ≤3: Calidad dietética muy baja.

ANNEX 5

FULL D'INFORMACIÓ AL PACIENT PER A PACIENTS MENORS DE 18 ANYS
(A ENTREGAR A MARE/PARE/TUTOR LEGAL DE LA PACIENT)

EFECTES DEL TRACTAMENT AMB SPIOMET A MEITAT DE DOSIS EN NENES AMB PUBERTAT AVANÇADA I ACCELERACIÓ DE LA MADURACIÓ ÒSSIA: ESTUDI MULTICÈNTRIC, ALEATORITZAT I CONTROLAT AMB PLACEBO (miniSPIOMET)

Promotor: Servei de Pediatria. Hospital Universitari Dr. Josep Trueta, Girona.
Codi de l'estudi: miniSPIOMET

Benvolguts, benvolgudes:

Els demanem la seva autorització per la participació de la seva filla en el present estudi.

Abans de decidir si autoritza o no la participació de la seva filla en aquest estudi, és important que entengui per què es realitza i què comporta. Si us plau, prengui tot el temps que sigui necessari per llegir atentament la següent informació i consulti qualsevol dubte amb el metge o equip mèdic que realitza l'estudi.

Quin és l'objectiu d'aquest estudi?
Durant els primers anys de vida, és important que existeixi un equilibri entre el guany de pes prenatal i l'augment de pes postnatal per tal de prevenir l'aparició d'alteracions metabòliques. Es coneix que un desajust entre el pes prenatal (baix) i un major guany de pes postnatal (elevat) pot predisposar a l'acumulació de greix ectòpic (al voltant dels òrgans) i a l'aparició de resistència a la insulina. Aquests processos poden iniciar un desenvolupament puberal precoç (pubàrquia precoç abans dels 8 anys i pubertat avançada entre els 8-9 anys) que pot esdevenir en un major risc per a la Síndrome d'Ovari Poliquístic (SOPQ) a l'adolescència.

Estudis previs han demostrat que el tractament amb metformina en nenes amb pubàrquia precoç i pubertat avançada redueix els nivells d'insulina en sang, redueix el greix ectòpic i desaccelera la progressió de la pubertat. Altres estudis en adolescents amb SOPQ han mostrat que la combinació de metformina+pioglitazona+espironolactona regula els cicles menstruals, normalitza els nivells hormonals i disminueix el greix ectòpic amb més eficàcia que la monoteràpia amb metformina.

L'objectiu principal de l'estudi és comprovar si el tractament amb mig comprimit de spiomet (combinació dels tres medicaments genèrics descrits: metformina+pioglitazona+espironolactona) durant 1 any, en comparació amb un placebo, pot reduir el greix ectòpic (greix visceral i hepàtic), normalitzar els valors hormonals i desaccelerar la maduració sexual en nenes amb pubertat avançada.

Quin és el fàrmac utilitzat en aquest estudi?
El tractament farmacològic consistirà en mig comprimit de spiomet (que conté: 25 mg de espironolactona, 3.75 mg de pioglitazona i 475 mg de metformina). La pacient prendrà el fàrmac diàriament durant 2 anys. L'altra meitat prendran placebo, un comprimit exactament igual en aparença al medicament objecte d'estudi però que no conté aquest medicament, sinó tan sols l'excipient. L'assignació del comprimit amb medicació o amb placebo serà aleatòria i cega (ni vostès com a tutors legals ni els investigadors de l'estudi coneixeran aquesta assignació fins a la finalització

de l'estudi). El motiu és evitar que el fet de conèixer quin comprimit rep la filla pugui afectar els resultats de l'estudi.

Per què proposem la participació de la seva filla en aquest estudi?
S'ofereix participar en un assaig clínic amb spiomet o placebo a totes aquelles nenes de 8-9 anys d'edat amb pubertat avançada que presenten factors de risc (haver tingut un baix pes al naixement i presentar en aquest moment un índex de massa corporal elevat).

La participació de la seva filla en aquest estudi és obligatòria? Pot vostè canviar d'idea?
Vostè és lliure de decidir si accepta o no que la seva filla participi en aquest estudi. La participació és totalment voluntària. Encara que inicialment acceptés participar, en qualsevol moment i sense necessitat d'especificar el motiu vostè pot demanar als responsables de l'estudi l'eliminació de totes les dades i mostres recollides que es trobin emmagatzemades sense que això repercuteixi en la seva atenció mèdica. Sigui quina sigui la seva decisió, no afectarà a la relació de la seva filla amb el metge de l'estudi ni tampoc amb l'atenció mèdica que rep actualment o podrà rebre en un futur.

En què consisteix la meva participació i la de la meva filla?
La seva participació a l'estudi comporta que la seva filla haurà de prendre mig comprimit del fàrmac spiomet o placebo (segons assignació aleatòria) diàriament, a l'hora de sopar, durant 1 any. A més, es realitzarà una visita mèdica completa amb anàlisis de sang i recollida de qüestionaris abans, durant i al final del tractament (0, 6, 12, 18 i 24 mesos) amb una durada aproximada de 30-40 minuts.
Addicionalment, es realitzarà també una prova per determinar l'edat òssia de la seva filla (0, 1 i 2 anys) d'una durada aproximada de 15 minuts; una ressonància magnètica (MRI) per determinar la distribució de greix ectòpic amb una durada aproximada de 20 minuts, i també una ecografia pèlvica per determinar les mesures i volums dels ovaris i úter amb una durada aproximada de 10 min (0, 1 any i 2 anys).

Quines dades es recolliran per a l'estudi?
L'estudi recollirà dades sobre l'avançament de l'edat òssia abans i després del tractament (0-2 any). Addicionalment, es recolliran dades antropomètriques (pes, talla, IMC, perímetre de cintura), estadi puberal (Tanner), dades metabòliques (glucosa, insulina, IGF-1, adiponectina d'alt pes molecular, proteïna C-reactiva, SHBG, andrògens, estrògens, LH, FSH, perfil lipídic), dades relacionades amb l'estat de salut i/o possibles efectes secundaris [funció hepàtica (ALT, AST, GGT), renal (urea, creatinina), glòbuls vermells, glòbuls blancs, vitamina B12, àcid fòlic, ionograma)] i dades sobre els hàbits alimentàries (dieta) i activitat física abans, durant, i després del tractament (0, 6, 12, 18 i 24 mesos). Les dades d'imatge (greix visceral i greix hepàtic mitjançant MRI, edat òssia mitjançant radiografia de la mà esquerra, i volum d'úter i ovaris mitjançant l'ecografia pèlvica) (0, 1 i 2 anys).

Quines mostres es recolliran per a l'estudi?
Es realitzaran 5 extraccions de sang al llarg de l'estudi (0, 6, 12, 18 i 24 mesos) i el volum màxim de cada extracció serà de 23 ml. La sang obtinguda s'enviarà al laboratori clínic de l'hospital per a la realització de l'analíltica general i una mostra es congelarà per als estudis analítics específics que s'analitzaran en dos centres [IMIM (Hospital del Mar) i Hospital San Joan de Deu]. Se li demanarà el consentiment perquè l'excedent d'aquestes mostres es guardi en una col.lecció privada que està registrada a l'Institut Carlos III amb codi de registre C0007072 per possibles futurs estudis en aquesta línea de recerca. Com que és una col·lecció privada registrada, les mostres només es faran servir en la línia actual de recerca i no es podran cedir a tercers.

Totes les extraccions es realitzaran en un ambient tranquil, després d'haver aplicat crema anestèsica a la pell (crema EMLA, d'ús habitual en pediatria) i amb personal experimentat que minimitzaran les molèsties per a la seva filla.

Quins són els possibles riscs i efectes secundaris associats al fàrmac *spiomet*?
El fàrmac spiomet no té possibles riscs o efectes secundaris greus. No obstant, la pacient ha de posar-se en contacte amb el seu metge en el cas d'observar qualsevol reacció adversa o inesperada. La pacient serà seguida amb visites mèdiques al llarg de l'estudi, i en el cas de detectar nivells de glucosa menors de 65 mg/dL, increment progressiu de creatinina, urea, transaminasa o nivells de potassi, es pararà el tractament i es deixarà de prendre el fàrmac. Addicionalment, l'aparició de símptomes d'hipoglicèmia, l'aparició d'erupció cutània persistent, dolor abdominal, vòmits o nàusees persistents, mal de cap, sinusitis o infeccions bacterianes severes també serà motiu per a parar el tractament i deixar de prendre el fàrmac. En aquest últim cas, la funció renal i hepàtica seria monitoritzada periòdicament fins a la desaparició dels símptomes i fins a la normalització dels paràmetres alterats.

Quines són els possibles avantatges relacionats amb la participació en aquest estudi?
El beneficis esperats del tractament en cas que la seva filla rebi spiomet són: la desacceleració de la pubertat, la desacceleració del tancament dels cartílags del creixement i la millora del perfil metabòlic. A més a més, potencialment també es reduiran les comorbiditats associades a aquesta maduració avançada (problemes de conducta o d'ajustament psicosocial, risc de desenvolupar diabetis tipus 2 o diabetis gestacional, i risc de càncer de mama i d'endometri).
En cas que la seva filla rebi placebo, no s'esperen beneficis directes per l'absència d'agents farmacològics al comprimit, però és possible que hi hagi beneficis indirectes per la participació a l'assaig clínic, que pot fer que la seva filla modifiqui lleugerament els hàbits de vida perquè siguin més saludables.

Com s'utilitzaran les dades mèdiques, les mostres biològiques i les dades personals de la meva filla?
Tota la informació recollida en aquest estudi serà codificada i introduïda en una base de dades computeritzada (en un ordinador) per al seu anàlisi amb la finalitat de dur a terme l'estudi referit en aquest document. Les dades recollides es conservaran durant el temps que puguin ser útils per a dur a terme les activitats d'investigació especificades.
Els resultats d'aquest estudi s'utilitzaran per a la seva presentació en congressos mèdics o la publicació en revistes científiques, però mai contindràn el nom o altres dades personals identificatives que permetin identificar les persones que han participat.
Les dades podran ser cedides únicament per a ser utilitzades en investigacions futures del mateix àmbit pel qual s'han recollit, així com quan constitueixi una obligació legal o sigui necessari per salvaguardar l'interès vital. L'ús comercial d'aquestes dades està estrictament prohibit.

Les mostres biològiques obtingudes seran etiquetades amb un codi per mantenir la confidencialitat del participant en l'estudi i es guardaran a l'Institut d'Investigació Biomèdica de Girona (IDIBGI) com a col.lecció privada. Un cop finalitzada la investigació, és possible que hi hagi mostres sobrants. En relació a aquestes, se li ofereixen les següents opcions:

A. L'emmagatzematge de l'excedent de la mostra a la col·lecció privada del grup d'investigació per la seva utilització en projectes futurs.
B. La destrucció de la mostra sobrant.

El tractament de les seves dades personals es legitima mitjançant la signatura d'aquest consentiment exprés i per escrit.

L'hospital universitari de Girona Dr. Josep Trueta serà el responsable del tractament de les seves dades personals. Vostè podrà exercir els seus drets d'accés, rectificació, supressió, oposició, limitació i portabilitat, així com obtenir informació addicional sobre l'ús de les seves dades, dirigint-se a:

Hospital universitari de Girona Dr. Josep Trueta
Avinguda de França s/n - 17007 Girona

Podrà també presentar reclamació davant l'Autoritat Catalana de Protecció de Dades (APDCAT).
Totes les dades de caràcter personal i informació recollida o generada en l'estudi quedaran protegits d'acord amb la legislació vigent sobre protecció de dades de caràcter personal (Reglament UE 2016/679, de 27 d'abril de 2016, relatiu a la protecció de les persones físiques respecte al tractament de dades personals i a la lliure circulació d'aquestes dades (RGPD) i la Llei Orgànica 3/2018, de 5 de desembre, de Protecció de Dades Personals i garantia dels drets digitals (LOPDGDD), publicada al BOE del 6 de desembre de 2018).
Ningú, excepte el seu metge i el personal directament relacionat amb aquest estudi, podrà conèixer la seva identitat. Únicament les autoritats sanitàries podran tenir accés a les seccions rellevants de l'estudi, si així ho sol·licitessin.

Qui ha revisat i aprovat aquest estudi?
L'estudi ha rebut el dictamen favorable del Comitè d'Ètica de la Investigació Mèdica (CEIM) de Girona així com l'aprovació de l'Agencia Espanyola del Medicament i Productes Sanitaris (AEMPS).

Informació de contacte de l'estudi:
Si vostè té algun dubte, necessita algun aclariment o la seva filla experimenta un canvi en el seu estat de salut o una reacció insòlita durant la seva participació, es pot posar en contacte amb:

Hospital Dr. Josep Trueta de Girona
Av de França s/n
17007 Girona, SPAIN
+34 972 940 200 (ext. 2810)

Informació del personal mèdic i investigador de l'estudi:
Institut d'Investigació Biomèdica de Girona (IDIBGI)
Edifici M2, Parc Hospitalari Martí i Julià
C/ Dr Castany s/n
17190 Salt, SPAIN

Li agraïm el temps dedicat a llegir aquest full d'informació.

Vostè rebrà una còpia firmada del full d'informació i d'aquest consentiment informat.

ANNEX 6

CONSENTIMENT INFORMAT PER A PACIENTS MENORS DE 18 ANYS
(A ENTREGAR A MARE/PARE/TUTOR LEGAL DE LA PACIENT)

Promotor: Servei de Pediatria. Hospital Universitari Dr. Josep Trueta, Girona.
Codi de l'estudi: miniSPIOMET

Jo, ... amb DNI núm.....
(nom i cognoms del familiar,representant legalo persona vinculada de fet)
en qualitat de familiar/representat legal o de parentesc de la pacient .....
.....i amb correu electrònic de contacte .....
(nom i cognoms de la pacient)

DECLARO

Que el/la professional .....
(nom i cognoms de la persona que proporciona la informació)
m'ha facilitat la informació oral i escrita sobre l'estudi **"Efectes del tractament amb spiomet a meitat de dosis en nenes amb pubertat avançada i acceleració de la maduració òssia: estudi multicèntric, aleatoritzat i controlat amb placebo".**

He llegit i comprès el full d'informació que se m'ha entregat en còpia.
He pogut fer preguntes sobre l'estudi i s'han respost de manera satisfactòria, aclarint els meus dubtes.
He rebut suficient informació sobre l'estudi i comprenc les indicacions i riscos d'aquest procés.
Comprenc que la participació es voluntària, i que puc retirar-me de l'estudi quan vulgui, sense donar explicacions, i sense que això repercuteixi en l'assistència mèdica.

En conseqüència,

Dono la meva conformitat per a que la meva filla participi en aquest estudi.

☐ Sí ☐ No

Dono la meva conformitat per a que es puguin usar, i si fos el cas cedir a tercers, les dades recollides en aquest estudi per a investigacions futures compreses dins el mateix àmbit.

☐ Sí ☐ No

Dono la meva conformitat per a que es puguin usar les mostres biològiques recollides en aquest estudi. Si hi hagués excedent de la mostra, afirmo conèixer les opcions de destinació en finalitzar el projecte de recerca. I en aquest sentit:

☐ Permeto que les mostres siguin guardades en la col·lecció privada del grup d'investigació i utilitzades en investigacions futures.

☐ Sol·licito la destrucció de la mostra excedent.

Firma de la mare/del pare:

☐ Firmen els dos progenitors

Si només firma un:
☐ Confirmo que l'altre progenitor no s'oposa a la participació de la nostra filla a l'estudi
☐ El firmant és l'únic tutor legal

Data: __ __ / __ __ / __ __

Firma del/la investigador/a:

Data: __ __ / __ __/ _

ANNEX 7

Centre:

☐ Hospital de Girona Dr. Josep Trueta (HJT)
☐ Hospital Sant Joan de Déu (HSJD)

QUADERN DE RECOLLIDA DE DADES

EFECTOS DEL TRATAMIENTO CON SPIOMET A MITAD DE DOSIS EN NIÑAS CON PUBERTAD ADELANTADA Y ACELERACIÓN DE LA MADURACIÓN ÓSEA: ESTUDIO MULTICÉNTRICO, ALEATORIZADO Y CONTROLADO CON PLACEBO

Criterios de inclusión

1) Edad al inicio del estudio: 8,0 ≤ edad ≤ 9,3 años
2) Peso al nacer para la edad gestacional (PN-EG) en tercil inferior:
   −1,96 (percentil 3) ≤ PN-EG Z-score ≤ −0,44 (percentil 33)
3) Índice de masa corporal para la edad cronológica en la 1ª visita en tercil superior:
   +0,44 (percentil 66) ≤ IMC Z-score ≤ +1,96 (percentil 97)
4) Pubertad adelantada progresiva [desarrollo mamario bilateral (estadio 2 de Tanner)] de inicio entre los 7,7 y los 9,0 años, con un mínimo de 4 meses de progresión
5) Etnia blanca
6) Embarazo a término: 37 ≤ edad gestacional < 42 semanas
7) Talla en la 1ª visita: percentil 3 ≤ altura ≤ percentil 97
8) Consentimiento informado por escrito de progenitores o representante legal.

Criterios de exclusión

1) Retraso o adelanto excesivo de la edad ósea (más de 2 años para la edad cronológica).
   De cara al cribado, se aceptará una radiografía de edad ósea realizada dentro de los 3 meses anteriores. En este caso, se deberá realizar una nueva radiografía de edad ósea dentro de la semana anterior o siguiente al inicio del tratamiento
2) Estadio de Tanner de desarrollo mamario superior a 2
3) Embarazo gemelar
4) Obesidad en la 1ª visita (Z-score del IMC por encima de +1,96 para la edad cronológica)
5) Evidencia de una causa patológica de maduración rápida (entre otros: hiperplasia suprarrenal congénita debido a deficiencia de 21-hidroxilasa)
6) Anomalía genética conocida o condiciones crónicas, incluidas enfermedades cardiovasculares, neurológicas, inmunológicas, metabólicas, renales, endocrinas, digestivas, respiratorias u oncológicas
7) Uso crónico de medicamentos, entre otros: anticoagulantes, antiinflamatorios, hipoglucemiantes orales, antiandrógenos, estrógenos, progestágenos, glucocorticoides, digoxina. Solo se aceptará el uso de paracetamol antes o durante el curso del estudio
8) Infecciones agudas o ingesta de antibióticos o antiinflamatorios en los últimos 14 días.
   Este criterio se aplica únicamente a las extracciones de sangre. Las extracciones de sangre deben aplazarse 14 días después de que el paciente deje de tener síntomas y deje de tomar alguno de estos medicamentos.
9) Historia previa de hipersensibilidad a cualquiera de los fármacos utilizados en el ensayo clínico, o bien a sus excipientes.
10) Cualquier enfermedad que, a criterio del investigador, comprometa la inclusión del sujeto en el ensayo clínico.

VISITA PRESELECCIÓ

- Es mira la medicació concomitant o prèvia ☐
- S'entrega i s'explica la fulla d'informació de l'estudi i **es recullen els consentiments informats*** **(x2)** – *pare/mare/tutor legal han d'omplir-los amb la seva lletra ☐
- Es revisen criteris inclusió/exclusió i s'indica a la història clínica:
  - Medicació concomitant actual i prèvia ☐
  - Antecedents mèdics ☐
  - Dades clíniques al naixement ☐
  - Dades clíniques actuals ☐
  - Edat a l'inici de la telarquia i pubarquia (anys i mesos) ☐
  - Proves que es demanen/proves pendents ☐

Data d'avui _____

Data de la signatura del CI _____

Medicació actual/prèvia _____

Antecedents mèdics _____

Etnia blanca Sí / No

Dades clíniques al naixement

Pes (kg)		Pes SDS	
Longitud (cm)		Longitud SDS	
Edat gestacional (setmanes i dies)		Embaràs gemel·lar (bessons)	SÍ / NO
Reproducció assistida?	SÍ / NO	Tipus de reprod. Assistida	

**El que està en gris es pot omplir posteriorment*

Dades clíniques actuals

Edat actual (anys i mesos)	____a ____m

Pes (kg)		Pes SDS	
Talla (cm)		Talla SDS	
IMC		IMC SDS	
Circumferència cintura (cm)		Circumferència maluc (cm)	
Relació CC		Tanner (mames)	

Altres dades de la pubertat

Edat a l'inici de la telarquia (anys i mesos)	____a ____m

Edat òssia

**és vàlida qualsevol radiografia dels 3 mesos previs*

Data	_____ /_____ /_____
Elegible per a l'assaig? **Observacions**	SÍ / NO

Analítica

Data	_____ /_____ /_____
Elegible per a l'assaig? **Observacions**	SÍ / NO

Si compleix criteris:

- ✓ **ALEATORITZAR**

VISITA 1: BASAL

Data d'avui: ____ /____ /____

- Es comprova que es compleixen els criteris d'inclusió □ NO □ SÍ
- Es comprova que no existeixi cap criteri de exclusió: □ NO □ SÍ
- Es dispensa la medicació, segons aleatorització: □ NO □ SÍ

  S'indica que cal prendre, a diari, mig comprimit amb el sopar. Cal tallar el comprimit amb un ganivet afilat i guardar l'altra meitat dins l'envàs. La medicació no pot superar els 25ºC.
- Es demana història mèdica de seguiment/medicació: □ NO □ SÍ
- Es contesta el qüestionari Kidmed: □ NO □ SÍ
- Es contesta el qüestionari activitat física PAQ-C □ NO □ SÍ
- Es fa radiografia per determinar edat òssia: □ NO □ SÍ
- Es fa analítica i es recullen mostres Biobanc: □ NO □ SÍ

Dades clíniques basals

Edat actual (anys i mesos)	____a ____m	Edat òssia (anys i mesos)	____a ____m

Pes (kg)		Pes SDS	
Talla (cm)		Talla SDS	
IMC		IMC SDS	
Circumferència cintura (cm)		Circumferència cintura SDS	
Circumferència maluc (cm)		Relació CC	
Tanner (mames)			

Analítica basal

Data	**Tubs per analítica (LAB CLINIC)**	**Tubs per processar (BIOBANC)**
____ / ____ / ____	2 tubs de sèrum (5 mL) 1 tub de EDTA (4 mL)	1 tub de sèrum (5 mL) 1 tub de EDTA (4 mL)

Incidències: _____ _____

Observacions _____

_____

VISITA 1: BASAL

RM abdominal

Data RM	Greix total	Greix subcutani	Greix visceral	Greix intrahepàtic (%)
___ / ___ / ___				

Observacions _____

_____

Ecografia pèlvica

Data RM	Volum úter	Volum ovari D	Volum ovari E
___ / ___ / ___			

Observacions _____

_____

<u>VISITA 2</u>: 3 mesos ± 15 dies (telefònica)

Data d'avui: ____ /____ /____

Esdeveniments clínics adversos: □ NO □ SI, especificar:

Observacions _____

_____

VISITA 3: 6 mesos ± 15 dies

Data d'avui: ____ /____ /____

- Es dispensa la medicació, segons aleatorització: □ NO □ SÍ
- Es demanen esdeveniments clínics adversos (EA): □ NO □ SÍ

  En cas afirmatiu, especifica:

- Es demana medicació concomitant: □ NO □ SÍ
- Es contesta el qüestionari Kidmed: □ NO □ SÍ
- Es contesta el qüestionari d'activitat física PAQ-C □ NO □ SÍ
- Es contesta qüestionari d'acceptabilitat de la pastilla: □ NO □ SÍ
- Es fa analítica i es recullen mostres Biobanc: □ NO □ SÍ

Dades clíniques als 6 mesos

Edat actual (anys i mesos)	____a ____m

Pes (kg)		Pes SDS	
Talla (cm)		Talla SDS	
IMC		IMC SDS	
Circumferència cintura (cm)		Circumferència cintura SDS	
Circumferència maluc (cm)		Relació CC	
Tanner (mames)			

Analítica basal als 6 mesos

Data	**Tubs per analítica (LAB CLINIC)**	**Tubs per processar (BIOBANC)**
____ / ____ / ____	2 tubs de sèrum (5 mL) 1 tub de EDTA (4 mL)	1 tub de sèrum (5 mL) 1 tub de EDTA (4 mL)

Incidències: _____ _____

Observacions _____

_____

VISITA 3: 6 mesos ± 15 dies

Medicació retornada del kit dels 0-6 mesos

Pastilles retornades	Quantitat
Sí / No Data: ____ / ____ / ____	

Observacions _____

_____

<u>VISITA 4</u>: 9 mesos ± 15 dies (telefònica)

Data d'avui: ____ /____ /____

Esdeveniments clínics adversos: □ NO □ SI, especificar:

Observacions _____

_____

VISITA 5: 12 mesos ± 7 dies

Data d'avui: ____ /____ /____

- Es demanen esdeveniments clínics adversos (EA): □ NO □ SÍ

  En cas afirmatiu, especifica:

- Es demana medicació concomitant: □ NO □ SÍ
- Es contesta el qüestionari Kidmed: □ NO □ SÍ
- Es contesta el qüestionari d'activitat física PAQ-C □ NO □ SÍ
- Es contesta qüestionari d'acceptabilitat de la pastilla: □ NO □ SÍ
- Es fa analítica i es recullen mostres Biobanc: □ NO □ SÍ
- Es fa radiografia per determinar edat òssia □ NO □ SÍ

Dades clíniques als 12 mesos

Edat actual (anys i mesos)	____a ____m	Edat òssia (anys i mesos)	____a ____m

Pes (kg)		Pes SDS	
Talla (cm)		Talla SDS	
IMC		IMC SDS	
Circumferència cintura (cm)		Circumferència cintura SDS	
Circumferència cadera (cm)		Relació CC	
Tanner (mames)			

Analítica als 12 mesos

Data	**Tubs per analítica (LAB CLINIC)**	**Tubs per processar (BIOBANC)**
____ / ____ / ____	2 tubs de sèrum (5 mL) 1 tub de EDTA (4 mL)	1 tub de sèrum (5 mL) 1 tub de EDTA (4 mL)

Incidències: _____ _____

Observacions _____

_____

VISITA 5: 12 mesos ± 7 dies

RM abdominal als 12 mesos

Data RM	Greix total	Greix subcutani	Greix visceral	Greix intrahepàtic (%)
___ / ___ / ___				

Observacions _____

_____

Ecografia pèlvica als 12 mesos

Data RM	Volum úter	Volum ovari D	Volum ovari E
___ / ___ / ___			

Observacions _____

_____

Medicació retornada del kit dels 6-12 mesos

Pastilles retornades	Quantitat
Sí / No Data: ____ / ____ / ____	

Observacions _____

_____

VISITA 6: 18 mesos ± 15 dies

Data d'avui: ____ /____ /____

- Es dispensa la medicació, segons aleatorització: □ NO □ SÍ
- Es demanen esdeveniments clínics adversos (EA): □ NO □ SÍ

  En cas afirmatiu, especifica:

- Es demana medicació concomitant: □ NO □ SÍ
- Es contesta el qüestionari Kidmed: □ NO □ SÍ
- Es contesta el qüestionari d'activitat física PAQ-C □ NO □ SÍ
- Es contesta qüestionari d'acceptabilitat de la pastilla: □ NO □ SÍ
- Es fa analítica i es recullen mostres Biobanc: □ NO □ SÍ

Dades clíniques als 18 mesos

Edat actual (anys i mesos)	____a ____m

Pes (kg)		Pes SDS	
Talla (cm)		Talla SDS	
IMC		IMC SDS	
Circumferència cintura (cm)		Circumferència cintura SDS	
Circumferència cadera (cm)		Relació CC	
Tanner (mames)			

Analítica basal als 18 mesos

Data	**Tubs per analítica (LAB CLINIC)**	**Tubs per processar (BIOBANC)**
____ / ____ / ____	2 tubs de sèrum (5 mL) 1 tub de EDTA (4 mL)	1 tub de sèrum (5 mL) 1 tub de EDTA (4 mL)

Incidències: _____ _____

Observacions _____

_____

VISITA 3: 18 mesos ± 15 dies

Medicació retornada del kit dels 12-18 mesos

Pastilles retornades	Quantitat
Sí / No Data: ____ / ____ / ____	

Observacions _____

_____

VISITA 7: 24 mesos ± 15 dies

Data d'avui: ____ /____ /____

- Es demanen esdeveniments clínics adversos (EA): □ NO □ SÍ

  En cas afirmatiu, especifica:

- Es demana medicació concomitant: □ NO □ SÍ
- Es contesta el qüestionari Kidmed: □ NO □ SÍ
- Es contesta el qüestionari d'activitat física PAQ-C □ NO □ SÍ
- Es contesta qüestionari d'acceptabilitat de la pastilla: □ NO □ SÍ
- Es fa analítica i es recullen mostres Biobanc: □ NO □ SÍ
- Es fa radiografia per determinar edat òssia □ NO □ SÍ

Dades clíniques als 24 mesos

Edat actual (anys i mesos)	____a ____m	Edat òssia (anys i mesos)	____a ____m

Pes (kg)		Pes SDS	
Talla (cm)		Talla SDS	
IMC		IMC SDS	
Circumferència cintura (cm)		Circumferència cintura SDS	
Circumferència cadera (cm)		Relació CC	
Tanner (mames)			

Analítica als 24 mesos

Data	**Tubs per analítica (LAB CLINIC)**	**Tubs per processar (BIOBANC)**
____ / ____ / ____	2 tubs de sèrum (5 mL) 1 tub de EDTA (4 mL)	1 tub de sèrum (5 mL) 1 tub de EDTA (4 mL)

Incidències: _____ _____

Observacions _____

_____

VISITA 5: 24 mesos ± 15 dies

RM abdominal als 24 mesos

Data RM	Greix total	Greix subcutani	Greix visceral	Greix intrahepàtic (%)
___ / ___ / ___				

Observacions _____

_____

Ecografia pèlvica als 24 mesos

Data RM	Volum úter	Volum ovari D	Volum ovari E
___ / ___ / ___			

Observacions _____

_____

Medicació retornada del kit dels 18-24 mesos

Pastilles retornades	Quantitat
Sí / No Data: _____ / _____ / _____	

Observacions _____

_____

ANNEX 8

Hospital Dr. Josep Trueta Av. França s/n 17007 Girona	HOJA DE RECOGIDA DE EFECTOS ADVERSOS	

Código paciente	Fecha
/__/__/__/__/__/__/	/__/__/ /__/__/ /__/__/__/__/ Día Mes Año

Responsable de la recogida de datos:

Firma:

Nombre: _____

Apellidos:

_____

Señale con una cruz si la paciente ha sufrido alguno de los siguientes signos/síntomas, relacionados con posibles efectos adversos debidos al tratamiento en estudio metformina:

SIGNO / SINTOMA	
Náuseas	
Vómitos	
Diarreas	
Dolor abdominal	
Pérdida de apetito	
Alteración del sabor	
Eritema	
Prurito	
Urticaria	
Disnea	
Hipotermia	
Coma	
Otros	

Descripción detallada del evento:

_____
_____
_____
_____
_____
_____
_____
_____

ANNEX 9

DECLARACIÓN DE HELSINKI DE LA AMM – PRINCIPIOS ÉTICOS PARA LAS INVESTIGACIONES MÉDICAS EN SERES HUMANOS

Adoptada por la
18a Asamblea Médica Mundial, Helsinki, Finlandia, junio 1964
y enmendada por la
29a Asamblea Médica Mundial, Tokio, Japón, octubre 1975
35a Asamblea Médica Mundial, Venecia, Italia, octubre 1983
41a Asamblea Médica Mundial, Hong Kong, septiembre 1989
48a Asamblea General Somerset West, Sudáfrica, octubre 1996
52a Asamblea General, Edimburgo, Escocia, octubre 2000
Nota de Clari□cación, agregada por la Asamblea General de la AMM, Washington 2002 Nota de Clari□cación, agregada por la Asamblea General de la AMM, Tokio 2004
59a Asamblea General, Seúl, Corea, octubre 2008
64a Asamblea General, Fortaleza, Brasil, octubre 2013

Introducción

1. La Asociación Médica Mundial (AMM) ha promulgado la Declaración de Helsinki como una propuesta de principios éticos para investigación médica en seres humanos, incluida la investigación del material humano y de información identi□cables.

La Declaración debe ser considerada como un todo y un párrafo debe ser aplicado con consideración de todos los otros párrafos pertinentes.

2. Conforme al mandato de la AMM, la Declaración está destinada principalmente a los médicos. La AMM insta a otros involucrados en la investigación médica en seres humanos a adoptar estos principios.

Principios generales

3. La Declaración de Ginebra de la Asociación Médica Mundial vincula al médico con la fórmula «velar solícitamente y ante todo por la salud de mi paciente", y el Código Internacional de Etica Médica a□rma que: «El médico debe considerar lo mejor para el paciente cuando preste atención médica".

4. El deber del médico es promover y velar por la salud, bienestar y derechos de los pacientes, incluidos los que participan en investigación médica. Los conocimientos y la conciencia del médico han de subordinarse al cumplimiento de ese deber.

5. El progreso de la medicina se basa en la investigación que, en último término, debe incluir estudios en seres humanos.

6. El propósito principal de la investigación médica en seres humanos es comprender las causas, evolución y efectos de las enfermedades y mejorar las intervenciones preventivas, diagnósticas y terapéuticas (métodos, procedimientos y tratamientos). Incluso, las mejores intervenciones probadas deben ser evaluadas continuamente a través de la investigación para que sean seguras, e□caces, efectivas, accesibles y de calidad.

7. La investigación médica está sujeta a normas éticas que sirven para promover y asegurar el respeto a todos los seres humanos y para proteger su salud y sus derechos individuales.

8. Aunque el objetivo principal de la investigación médica es generar nuevos conocimientos, este objetivo nunca debe tener primacía sobre los derechos y los intereses de la persona que participa en la investigación.

9. En la investigación médica, es deber del médico proteger la vida, la salud, la dignidad, la integridad, el derecho a la autodeterminación, la intimidad y la con□dencialidad de la información personal de las personas que participan en investigación. La responsabilidad de la protección de las personas que toman parte en la investigación debe recaer siempre en un médico u otro profesional de la salud y nunca en los participantes en la investigación, aunque hayan otorgado su consentimiento.

10. Los médicos deben considerar las normas y estándares éticos, legales y jurídicos para la investigación en seres humanos en sus propios países, al igual que las normas y estándares internacionales vigentes. No se debe permitir que un requisito ético, legal o jurídico nacional o internacional disminuya o elimine cualquiera medida de protección para las personas que participan en la investigación establecida en esta Declaración.

11. La investigación médica debe realizarse de manera que reduzca al mínimo el posible daño al medio

11. La investigación médica debe realizarse de manera que reduzca al mínimo el posible daño al medio ambiente.

12. La investigación médica en seres humanos debe ser llevada a cabo sólo por personas con la educación, formación y cali□caciones cientí□cas y éticas apropiadas. La investigación en pacientes o voluntarios sanos necesita la supervisión de un médico u otro profesional de la salud competente y cali□cado apropiadamente.

13. Los grupos que están subrepresentados en la investigación médica deben tener un acceso apropiado a la participación en la investigación.

14. El médico que combina la investigación médica con la atención médica debe involucrar a sus pacientes en la investigación sólo en la medida en que esto acredite un justi□cado valor potencial preventivo, diagnóstico o terapéutico y si el médico tiene buenas razones para creer que la participación en el estudio no afectará de manera adversa la salud de los pacientes que toman parte en la investigación.

15. Se debe asegurar compensación y tratamiento apropiados para las personas que son dañadas durante su participación en la investigación.

Riesgos, Costos y Bene□cios

16. En la práctica de la medicina y de la investigación médica, la mayoría de las intervenciones implican algunos riesgos y costos.

La investigación médica en seres humanos sólo debe realizarse cuando la importancia de su objetivo es mayor que el riesgo y los costos para la persona que participa en la investigación.

17. Toda investigación médica en seres humanos debe ser precedido de una cuidadosa comparación de los riesgos y los costos para las personas y los grupos que participan en la investigación, en comparación con los bene□cios previsibles para ellos y para otras personas o grupos afectados por la enfermedad que se investiga.

Se deben implementar medidas para reducir al mínimo los riesgos. Los riesgos deben ser monitoreados, evaluados y documentados continuamente por el investigador.

18. Los médicos no deben involucrarse en estudios de investigación en seres humanos a menos de que estén seguros de que los riesgos han sido adecuadamente evaluados y de que es posible hacerles frente de manera satisfactoria.

Cuando los riesgos que implican son más importantes que los bene□cios esperados o si existen pruebas concluyentes de resultados de□nitivos, los médicos deben evaluar si continúan, modi□can o suspenden inmediatamente el estudio.

Grupos y personas vulnerables

19. Algunos grupos y personas sometidas a la investigación son particularmente vulnerables y pueden tener más posibilidades de sufrir abusos o daño adicional.

Todos los grupos y personas vulnerables deben recibir protección especí□ca.

20. La investigación médica en un grupo vulnerable sólo se justi□ca si la investigación responde a las necesidades o prioridades de salud de este grupo y la investigación no puede realizarse en un grupo no vulnerable. Además, este grupo podrá bene□ciarse de los conocimientos, prácticas o intervenciones derivadas de la investigación.

Requisitos cientí□cos y protocolos de investigación

21. La investigación médica en seres humanos debe conformarse con los principios cientí□cos generalmente aceptados y debe apoyarse en un profundo conocimiento de la bibliografía cientí□ca, en otras fuentes de información pertinentes, así como en experimentos de laboratorio correctamente realizados y en animales, cuando sea oportuno. Se debe cuidar también del bienestar de los animales utilizados en los experimentos.

22. El proyecto y el método de todo estudio en seres humanos deben describirse claramente y ser justi□cados en un protocolo de investigación.

El protocolo debe hacer referencia siempre a las consideraciones éticas que fueran del caso y debe indicar cómo se han considerado los principios enunciados en esta Declaración. El protocolo debe incluir información sobre □nanciamiento, patrocinadores, a□liaciones institucionales, posibles con□ictos de interés e incentivos para las personas del estudio y la información sobre las estipulaciones para tratar o compensar a las personas que han sufrido daños como consecuencia de su participación en la investigación.

En los ensayos clínicos, el protocolo también debe describir los arreglos apropiados para las estipulaciones después del ensayo.

Comités de ética de investigación

23. El protocolo de la investigación debe enviarse, para consideración, comentario, consejo y aprobación al comité de ética de investigación pertinente antes de comenzar el estudio. Este comité debe ser transparente en su funcionamiento, debe ser independiente del investigador, del patrocinador o de cualquier otro tipo de in□uencia indebida y debe estar debidamente cali□cado. El comité debe considerar las leyes y reglamentos vigentes en el país donde se realiza la investigación, como también las normas internacionales vigentes, pero no se debe permitir que éstas disminuyan o eliminen ninguna de las protecciones para las personas que participan en la investigación establecidas en esta Declaración.

El comité tiene el derecho de controlar los ensayos en curso. El investigador tiene la obligación de proporcionar información del control al comité, en especial sobre todo incidente adverso grave. No se debe hacer ninguna enmienda en el protocolo sin la consideración y aprobación del comité. Después

que termine el estudio, los investigadores deben presentar un informe final al comité con un resumen de los resultados y conclusiones del estudio.

Privacidad y confidencialidad

24. Deben tomarse toda clase de precauciones para resguardar la intimidad de la persona que participa en la investigación y la confidencialidad de su información personal.

Consentimiento informado

25. La participación de personas capaces de dar su consentimiento informado en la investigación médica debe ser voluntaria. Aunque puede ser apropiado consultar a familiares o líderes de la comunidad, ninguna persona capaz de dar su consentimiento informado debe ser incluida en un estudio, a menos que ella acepte libremente.

26. En la investigación médica en seres humanos capaces de dar su consentimiento informado, cada participante potencial debe recibir información adecuada acerca de los objetivos, métodos, fuentes de financiamiento, posibles conflictos de intereses, afiliaciones institucionales del investigador, beneficios calculados, riesgos previsibles e incomodidades derivadas del experimento, estipulaciones post estudio y todo otro aspecto pertinente de la investigación. El participante potencial debe ser informado del derecho de participar o no en la investigación y de retirar su consentimiento en cualquier momento, sin exponerse a represalias. Se debe prestar especial atención a las necesidades específicas de información de cada participante potencial, como también a los métodos utilizados para entregar la información.

Después de asegurarse de que el individuo ha comprendido la información, el médico u otra persona calificada apropiadamente debe pedir entonces, preferiblemente por escrito, el consentimiento informado y voluntario de la persona. Si el consentimiento no se puede otorgar por escrito, el proceso para lograrlo debe ser documentado y atestiguado formalmente.

Todas las personas que participan en la investigación médica deben tener la opción de ser informadas sobre los resultados generales del estudio.

27. Al pedir el consentimiento informado para la participación en la investigación, el médico debe poner especial cuidado cuando el participante potencial está vinculado con él por una relación de dependencia o si consiente bajo presión. En una situación así, el consentimiento informado debe ser pedido por una persona calificada adecuadamente y que nada tenga que ver con aquella relación.

28. Cuando el participante potencial sea incapaz de dar su consentimiento informado, el médico debe pedir el consentimiento informado del representante legal. Estas personas no deben ser incluidas en la investigación que no tenga posibilidades de beneficio para ellas, a menos que ésta tenga como objetivo promover la salud del grupo representado por el participante potencial y esta investigación no puede realizarse en personas capaces de dar su consentimiento informado y la investigación implica sólo un riesgo y costo mínimos.

29. Si un participante potencial que toma parte en la investigación considerado incapaz de dar su consentimiento informado es capaz de dar su asentimiento a participar o no en la investigación, el médico debe pedirlo, además del consentimiento del representante legal. El desacuerdo del participante potencial debe ser respetado.

30. La investigación en individuos que no son capaces física o mentalmente de otorgar consentimiento, por ejemplo los pacientes inconscientes, se puede realizar sólo si la condición física/mental que impide otorgar el consentimiento informado es una característica necesaria del grupo investigado. En estas circunstancias, el médico debe pedir el consentimiento informado al representante legal. Si dicho representante no está disponible y si no se puede retrasar la investigación, el estudio puede llevarse a cabo sin consentimiento informado, siempre que las razones específicas para incluir a individuos con

una enfermedad que no les permite otorgar consentimiento informado hayan sido estipuladas en el protocolo de la investigación y el estudio haya sido aprobado por un comité de ética de investigación. El consentimiento para mantenerse en la investigación debe obtenerse a la brevedad posible del individuo o de un representante legal.

31. El médico debe informar cabalmente al paciente los aspectos de la atención que tienen relación con la investigación. La negativa del paciente a participar en una investigación o su decisión de retirarse nunca debe afectar de manera adversa la relación médico-paciente.

32. Para la investigación médica en que se utilice material o datos humanos identi□cables, como la investigación sobre material o datos contenidos en biobancos o depósitos similares, el médico debe pedir el consentimiento informado para la recolección, almacenamiento y reutilización. Podrá haber situaciones excepcionales en las que será imposible o impracticable obtener el consentimiento para dicha investigación. En esta situación, la investigación sólo puede ser realizada después de ser considerada y aprobada por un comité de ética de investigación.

Uso del placebo

33. Los posibles bene□cios, riesgos, costos y e□cacia de toda intervención nueva deben ser evaluados mediante su comparación con las mejores intervenciones probadas, excepto en las siguientes circunstancias:

Cuando no existe una intervención probada, el uso de un placebo, o ninguna intervención, es aceptable; o

cuando por razones metodológicas cientí□camente sólidas y convincentes, sea necesario para determinar la e□cacia y la seguridad de una intervención el uso de cualquier intervención menos e□caz que la mejor probada, el uso de un placebo o ninguna intervención.

Los pacientes que reciben cualquier intervención menos e□caz que la mejor probada, el placebo o ninguna intervención, no correrán riesgos adicionales de daño grave o irreversible como consecuencia de no recibir la mejor intervención probada.

Se debe tener muchísimo cuidado para evitar abusar de esta opción.

Estipulaciones post ensayo

34. Antes del ensayo clínico, los auspiciadores, investigadores y los gobiernos de los países an□triones deben prever el acceso post ensayo a todos los participantes que todavía necesitan una intervención que ha sido identi□cada como bene□ciosa en el ensayo. Esta información también se debe proporcionar a los participantes durante el proceso del consentimiento informado.

Inscripción y publicación de la investigación y difusión de resultados

35. Todo estudio de investigación con seres humanos debe ser inscrito en una base de datos disponible al público antes de aceptar a la primera persona.

36. Los investigadores, autores, auspiciadores, directores y editores todos tienen obligaciones éticas con respecto a la publicación y difusión de los resultados de su investigación. Los investigadores tienen el deber de tener a la disposición del público los resultados de su investigación en seres humanos y son responsables de la integridad y exactitud de sus informes. Todas las partes deben aceptar las normas éticas de entrega de información. Se deben publicar tanto los resultados negativos e inconclusos como los positivos o de lo contrario deben estar a la disposición del público. En la publicación se debe citar la fuente de □nanciamiento, a□liaciones institucionales y con□ictos de intereses. Los informes sobre

investigaciones que no se ciñan a los principios descritos en esta Declaración no deben ser aceptados para su publicación.

Intervenciones no probadas en la práctica clínica

37. Cuando en la atención de un paciente las intervenciones probadas no existen u otras intervenciones conocidas han resultado ine caces, el médico, después de pedir consejo de experto, con el consentimiento informado del paciente o de un representante legal autorizado, puede permitirse usar intervenciones no comprobadas, si, a su juicio, ello da alguna esperanza de salvar la vida, restituir la salud o aliviar el sufrimiento. Tales intervenciones deben ser investigadas posteriormente a n de evaluar su seguridad y e cacia. En todos los casos, esa información nueva debe ser registrada y, cuando sea oportuno, puesta a disposición del público.

**Los párrafos 26, 27, 28 y 29 han sido revisados editorialmente por el Secretariado de la AMM el 5 de mayo de 2015.*