Sedentary lifestyle and precocious puberty in girls during COVID-19 pandemic: an Italian experience

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Abstract

Objective

This retrospective study aimed to evaluate children observed for suspected precocious puberty in 5 Italian centers of Pediatric Endocrinology during the first wave of COVID-19 pandemic (March-September 2020), compared to subjects observed in the same period of the previous year.

Design

The study population (490 children) was divided according to year of observation and final diagnosis: transient thelarche (TT), non-progressive precocious puberty (NPP), central precocious puberty (CPP), or early puberty (EP).

Results

Between March and September 2020, 338 subjects were referred for suspected precocious puberty, compared to 152 subjects in the same period of 2019 (+222%). The increase was observed in girls (328 subjects in 2020 versus 140 in 2019, p<0.05), especially during the second half of the period considered (92 girls from March to May versus 236 girls from June to September); while no difference was observed in boys (10 subjects in 2020 versus 12 in 2019). The percentage of girls with confirmed CPP was higher in 2020, compared to 2019 (135/328 girls [41%] versus 37/140 [26%], p <0.01). Anthropometric and hormonal parameters in 2019 and 2020 CPP girls were not different; 2020 CPP girls showed a more prolonged use of electronic devices and a more sedentary lifestyle both before and during the pandemic, compared to the rest of the 2020 population.

Conclusions

The present findings corroborate the recently reported association between the complex lifestyle changes related to the lockdown and a higher incidence of central precocious puberty in Italian girls.
Introduction

Puberty represents an essential step of dynamic transition from childhood to adulthood leading to full reproductive capacity. The mechanisms underlying this process are not completely known, and include genetic and epigenetic factors, as well as energy balance and variation in the expression of brain neurotransmitters and neuropeptides (1-4).

A trend towards earlier puberty onset has been observed in the last decades (5-7). It has been linked to changes in body weight and obesity (8, 9), exposure to environmental endocrine disruptors (10), and stressful life events (11). Recently, it has been proposed that precocious puberty may represent the expression of an adaptive mechanism to escape from ectopic adiposity in girls with low birth weight and significant postnatal weight gain (12-15).

Precocious puberty is defined as the onset of thelarche before the age of eight years in females and of testicular enlargement before nine years in males, equivalent to 2 standard deviations (SD) below the mean age at the onset of puberty (16).

At the end of 2019, a novel coronavirus identified as SARS-CoV-2 (Severe Acute Respiratory Syndrome Coronavirus 2), causing atypical pneumonia defined as COVID-19 (coronavirus disease 2019), was isolated in China (17). It has rapidly expanded worldwide leading to a still ongoing global pandemic (https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19%2D%2D-11-march-2020. Accessed 30 September 2021).

In order to reduce transmission rate, intensive care admissions and thus hospital bed saturation, in March 2020 the Italian Government imposed a strict lockdown extended to the whole country (https://www.gazzettaufficiale.it/eli/id/2020/03/11/20A01605/sg. Accessed 30 September 2021).

Everyday life was disrupted introducing the so-called “smart-working” for many workers and “e-learning” for students, using digital platforms on electronic devices. Gatherings and any kind of outdoor sport activity were prohibited.

Since the beginning of March 2020, we have observed an increase of outpatient pediatric endocrinological consultations for suspected precocious or early puberty. In a previous preliminary
study, we reported a doubling of the consultations for suspected precocious puberty recorded in the outpatient clinic database of the Endocrinology Unit of Bambino Gesù Children’s Hospital between March and September 2020, in comparison with the same period of 2019 (246 vs 118 patients respectively), with a prevalent increase of girls (18).

No significant differences in the number of consultations were observed during the remaining months of 2020, compared to the corresponding months of 2019 (93 vs 78 subjects respectively, p=0.10) (data not published).

A previous Italian study had reported an increased incidence of newly diagnosed central precocious puberty (CPP) and a faster pubertal progression during and after lockdown (19).

The time spent on electronic devices increased during lockdown, while daily physical activity decreased with a concomitant increase of sedentary behavior (20). Finally, an increase of stress-related symptoms has been observed in children and adolescents throughout the pandemic (21, 22).

This retrospective multicenter study aimed to confirm this phenomenon and provide a plausible explanation.

**Methods**

**Subjects**

The study involved 506 subjects observed in 5 Italian tertiary centers of Pediatric Endocrinology, in the periods March-September 2020 and March-September 2019 (“Bambino Gesù” Children’s Hospital in Rome, IRCCS “Giannina Gaslini” Hospital in Genoa, University Hospital “Federico II” in Naples, Pediatric Hospital Microcitemico “Antonio Cao” in Cagliari, Pediatric Clinic of Perugia Hospital).

Data were collected retrospectively from the outpatient clinic database of each center. Girls younger than 3 years referred for idiopathic premature thelarche were not considered. Four patients affected by Silver Russell Syndrome were excluded due to their known susceptibility to develop precocious puberty, while 12 subjects were lost during follow-up. The study population (total = 490 children)
was divided into 2019 and 2020 groups and subsequently into different subgroups according to the final diagnosis: transient thelarche (TT), non-progressive precocious puberty (NPP), central precocious puberty (CPP), or early puberty (EP), when pubertal signs started between eight and nine years in girls and between nine and ten years in boys. The study design is summarized in Figure 1.

The Institutional Review Board of “Bambino Gesù” Children’s Hospital” approved the study protocol.

**Data Collection**

Age, sex, ethnicity, family history of precocious and early puberty, maternal age at menarche, history of adoption, mid-parental height (MPH), age at the onset of pubertal signs and age at the first observation were retrieved from clinical records. Height, weight, birth weight and body mass index (BMI) were expressed as standard deviation score (SDS) according to the Italian standards (23). Pubertal stage was recorded according to Marshall and Tanner’s genital stage (24), using Prader orchidometer for testicular volume (25).

The following hormone levels were recorded at baseline, when available: FSH, LH, estradiol, and testosterone. Gonadotropin Releasing Hormone (GnRH) stimulation test was performed in 270 girls (64 in 2019 and 206 in 2020) and 15 boys (6 in 2019 and 9 in 2020), by the intravenous administration of GnRH (Lutrelef; Ferring) at a dosage of 100 mcg, with FSH and LH measurement at 0, +30, +60 and +90 minutes after the injection.

A detectable basal LH level (> 0.2 IU/L) and/or a peak response of LH after GnRH infusion >5 IU/L, with or without serum estradiol levels >20 pg/mL, were considered suggestive of CPP (26, 27, 16). In the absence of these biochemical parameters, subjects with slow pubertal progression were classified as NPP. Subjects presenting with thelarche that disappeared during the diagnostic work-up were defined as affected by TT.
Peripheral precocious puberty due to non-classical congenital adrenal hyperplasia was excluded with basal and corticotropin-stimulated 17-OH-Progesterone in patients presenting with pubarche and/or adrenarche in association with thelarche.
Laboratory measurements

Estradiol, LH, and FSH were measured by chemiluminescence immunoassay (ADVIA Centaur; Siemens). Estradiol intra- and inter-assay coefficients of variation were 3.1 and 6%, respectively. The analytical measuring range for estradiol assay was 11.8 to 3000 pg/mL (43.3–11 013 pmol/L). LH intra- and inter-assay variability coefficients were 2.2 and 4.4%, respectively, with analytical measuring range 0.1 to 200.0 IU/L. FSH intra- and inter-assay variability coefficients were 2.2 and 3.7%, respectively, with analytical measuring range 0.3 to 200.0 IU/L. 17-OH-Progesterone was measured by radioimmunoassay (ICN-Pharmaceutical Inc). Mean intra- and inter-assay coefficients of variation were 8.8% and 12%, respectively. Testosterone was measured by chemiluminescence immunoassay (Centaur-Bayer provided by Siemens Medical Solutions Diagnostics), intra- and inter-assay coefficients of variation were 3.8% and 5%, respectively.

Bone age assessment

Bone age (BA), in years, was estimated from an X-ray of the left hand and wrist using the Greulich & Pyle atlas (28) and revised by expert endocrinologists. Bone age advancement was defined as the difference between BA and chronological age expressed in years.

Imaging

Pelvic ultrasound was performed to assess uterine diameters and the endometrium pattern. A uterine longitudinal diameter >34 mm was considered suggestive of estrogenic stimulation and incipient puberty. The majority of children in the CPP subgroups (22/37 in 2019 and 90/135 in 2020) underwent MRI of the hypothalamus-pituitary area (16).

Questionnaires

Lifestyle questionnaires investigating physical activity, eating habits and electronic device use at the onset of pubertal signs were administered to both the 2020 and 2019 groups. The Italian version of the “Physical Activity Questionnaire for Older Children” (PAQ-C-It) (29) was used for physical activity. A second questionnaire, analyzing the pandemic-related stress, was administered to 2020 group subjects.
Statistical analysis

Statistical analysis was performed using the SPSS Statistics software for Windows, version 22.0 (IBM Corp). Continuous quantitative variables are expressed as mean ± SD and categorical variables are expressed as frequency distribution, median and interquartile range (IQR). Comparison of continuous variables was performed using the Student t test. Categorical variables were compared by the chi-square test, while differences in distributions of ordinal data from independent samples were tested with Mann-Whitney U and Goodman and Kruskal’s gamma tests. The level of significance was set at 0.05 (2-tailed).

Results

Between March and September 2020, 338 subjects were referred for suspected precocious puberty, compared to 152 subjects observed in the same period of 2019 (+222%).

The prevalent increase was observed in girls (328 subjects in 2020 vs 140 subjects in 2019, p<0.05). In particular, the increase was more evident during the second part of the period considered; 236 girls were observed between June and September (72%) and 92 girl between March and May (28%).

No difference was observed in boys (10 subjects in 2020 vs 12 subjects in 2019). Based on these results, we decided to further analyze only the female population of each period.

Family history of precocious and early puberty was positive in 30% of the total female population, without differences between 2019 and 2020 (31% and 29%, respectively). Eight girls had been adopted (4 girls in 2019 and 4 girls in 2020), three of them belonged to the NPP subgroup and five to the CPP subgroup.

The percentage of girls with CPP was significantly higher in the 2020 group, compared to the 2019 group (135/328 vs 37/140 girls, equivalent to 41% vs 26%, p <0.01), while a relative reduction of EP was observed in 2020 compared to 2019 (10% vs 17% respectively, p<0.01). The proportion of TT and NPP was similar between 2019 and 2020. A higher number of girls was diagnosed with CPP during the second part of the 2020 time interval, but the percentage of CPP cases out of the
total number of girls observed was not different (101/236 girls between June and September [43%] vs 37/92 girls between March and May [40%], p:0.67).

The clinical characteristics of all the female subgroups are summarized in Table 1. Both 2019 and 2020 subgroups showed a trend towards a postnatal weight gain (defined as BMI SDS – birth weight SDS), without significant differences between 2019 and 2020 subgroups. The mean difference of the upward mismatch between birth weight and BMI SDS reached statistical significance in both 2019 CPP and EP subgroups (p<0.05), and in 2020 CPP subgroup (p<0.05) (Figure 2).

Anthropometric parameters and biochemical findings were similar in CPP subgroups of 2019 and 2020. Only 16% of the MRI scans (7 cases in 2019 and 11 cases in 2020 subgroups) showed minor brain abnormalities, none of them related to organic CPP.

Anthropometric parameters were similar in 2019 and 2020 TT subgroups, except for a significantly lower BMI SDS in 2020 TT subjects (-0.17±0.99 vs 0.47±1.02 respectively; p<0.01).

Height SDS was significant different in the two NPP populations (0.96 ± 1.16 in 2019 vs 0.54 ± 1.13 in 2020, p<0.05), while BMI SDS was not different (0.31±1.10 in 2019 vs 0.15±1.28 in 2020; p:0.447).

Both NPP and EP subgroups of 2020 were significantly younger at the time of the first consultation (7.19±0.85 years in 2020 NPP vs 7.49±0.88 in 2019 NPP, p<0.05; 8.64±0.44 years in 2020 EP vs 8.91±0.45 in 2019 EP, p<0.05). On the contrary, the age at the onset of pubertal signs was similar in 2019 and 2020 subgroups.

**Lifestyle Questionnaires**

Lifestyle questionnaires were administered in order to compare the different populations (2019 vs 2020), as well as the subgroups of the same year (CPP vs TT, NPP and EP patients). The same questionnaire was also used to analyze lifestyle changes during the pandemic, compared to pre-pandemic habits, in the 2020 subgroups.
As expected, the overall time spent on electronic devices was greater in the 2020 group (median 5-10 hours/week, IQR [1-5h – 10-15h] in 2019 vs 15-20 hours/week, IQR [5-10h – more than 25h] in 2020; p<0.001) (Figure 3A), although no difference was observed among the different 2020 subgroups.

All the 2020 population attending primary school reported the introduction of “e-learning” concurrently with the lockdown, with an increase of weekly device use for homework (median never, IQR [0] in 2019 vs 5-10 hours/week, IQR [never – >10 h] in 2020; p<0.001), without significant difference between the 2020 subgroups.

The same weekly time spent on electronic devices for leisure activities was reported in 2019 and 2020 groups (median 5-10 hours/week, IQR [1-5h – >10h] in 2019 versus 5-10 hours/week, IQR [never – >10h] in 2020; p=0.24).

The subgroup of 2020 CPP girls reported prolonged overall weekly use of electronic devices, already present before the pandemic, in comparison with the rest of the 2020 population (median 1-5 hours/week, IQR [never – 5-10 h] in CPP vs 1-5 hours/week, IQR [0] in other subgroups; p<0.05) (Figure 3B).

Physical activity (expressed as hours/week) was considerably lower in the 2020 group (median: no physical activity, IQR [never – 1-2 hours/week] in 2020 vs median: 1-2 hours/week, IQR [0] in 2019; p< 0.001) (Figure 4).

Weekly physical activity was significantly lower in 2020 CPP girls, compared to the other 2020 subgroups taken together (median never, IQR [0] in CPP vs never, IQR [never – 1-2 hours/week] in other subgroups p<0.05) (Figure 5A). The same result was evident comparing pre-pandemic habits of the 2020 CPP subgroup with pre-pandemic habits of the other 2020 subgroups (median 1-2 hours/week, IQR [0] in CPP vs 1-2 hours/week, IQR [never – 3-4 hours/week] in other groups, p<0.05) (Figure 5B).

Finally, 47% of the 2020 subjects reported a significant increase in the sense of hunger during the pandemic, compared to the previous year. Daily intake of leavened food was increased in 38% of
the 2020 subjects, although no difference was evident between the 2020 CPP subgroup and the other 2020 subgroups taken together. No differences in the weekly intake of meat or junk food were reported between 2019 and 2020 groups.

The second questionnaire, analyzing the pandemic-related stress in the 2020 group, showed that more than half of the 2020 subjects (59%) reported behavioral changes and a relevant increase in stress-related symptoms (63%), without any differences between CPP and the other subgroups.
Discussion

Our multicenter retrospective study revealed a relevant increase of the number of consultations for suspected precocious puberty in girls (+222%) between March and September 2020 compared to the same period of 2019. In addition, the percentage of confirmed cases with true central precocious puberty was significantly higher in the 2020 group compared to the 2019 group (41% vs 26%, respectively).

Two previous studies reported an increase in the number of new cases of central precocious puberty in girls during the COVID-19 pandemic (18, 19). Furthermore, Stagi et al. (19) described a faster pubertal progression in patients already followed for slowly progressive CPP. The authors reported an increase of BMI after lockdown in these subjects, assuming a possible causal role of weight gain on pubertal progression.

Since 1970 it has been hypothesized that a critical body weight and a critical level of adiposity may trigger pubertal development (30-33, 9). Leptin, secreted by adipocytes, acts as a permissive stimulus on GnRH pulsatile secretion (33, 34). On the contrary, adiponectin inhibits GnRH release resulting in a delayed onset of puberty. TNF-α and IL-6 inhibit the production of adiponectin stimulating puberty onset (35). Kisspeptins, released by hypothalamic kisspeptin neurons, have a stimulatory action on GnRH neurons and seem to be the gatekeepers of puberty onset (36). Recent studies demonstrated that childhood obesity causes early puberty onset by the activation of kisspeptin release and eventually GnRH pulsatile secretion, although the specific changes induced by leptin on kisspeptin neurons are not fully understood (37). Finally, epigenetic factors as sirtuins and ceramides have been implicated on the modulation of Kiss1 transcription (8, 36, 38).

In our study, BMI was not increased in 2020 patients compared to 2019 patients, with z-score BMI within +1 SDS in all 2019 and 2020 subgroups. Nevertheless, an upward mismatch was observed comparing mean birth weight and BMI SDS in both 2019 and 2020 CPP. This finding supports the proposed hypotheses that an early pubertal maturation in girls may represent an adaptive process to escape from ectopic adiposity in subjects with low birth weight and increased postnatal weight gain.
In these subjects prenatal subcutaneous adipocyte development (adipogenesis) is impaired preventing the safe postnatal subcutaneous fat accumulation when an early and fast postnatal weight gain occurs (12-14). Other authors have interpreted this mismatch as a consequence rather than a trigger of early puberty, as female puberty is characterized by an early change of body composition with body fat increase (15).

Physical activity was considerably lower or nearly absent in the 2020 group. Interestingly, 2020 CPP girls were more sedentary both before and during the pandemic compared to 2019 CPP subjects. Poor physical activity may have exerted a negative influence on body composition decreasing muscle mass and increasing ectopic fat deposition, without a clear BMI increase. Fat accumulation has been related to high inflammatory cytokine levels and low adiponectin levels leading to an early onset and fast progression of puberty (35).

While intense physical activity has been associated with delayed puberty, less data is available on the relationship between moderate physical activity or sedentary lifestyle and early puberty (39). Beyond the purely physical exercise, several studies demonstrated a positive association between physical activity and psychological well-being in children and adolescents. On the other hand, a sedentary lifestyle was related to both increased depression and lower life satisfaction and happiness. It has been suggested that promoting physical activity and decreasing sedentary behavior might protect mental health in children and adolescents (40). Early studies (41, 42) suggested that psychological stress itself (due to insecure bonds with parents or parental conflicts) may modify pubertal timing. A recent study reported that anxiety and other internalizing symptoms in prepubertal girls are associated with early pubertal onset, independently from maternal anxiety, BMI, ethnicity and maternal education (11).

A few recent studies (43-45) investigated the impact of COVID-19 and quarantine on mental health of children and adolescents, reporting a significant increase in behavioral and emotional disorders as a consequence of school closures. Post-traumatic stress disorder has been described in 30% of children during quarantine or social isolation (45).
In our study more than half of the 2020 population reported behavioral changes and a relevant increase in stress-related symptoms. We could assume that a dysregulation of stress-induced brain neurotransmitters underlies the increase of new cases of precocious puberty in girls, related to the COVID-19 pandemic. Stress may act as a more powerful trigger on GnRH secreting neurons in girl with additional risk factors as a sedentary lifestyle already evident before the pandemic.

We observed that the overall use of electronic devices was greater in 2020 than in 2019 population. The total weekly time spent using electronic devices was increased due to their use for “e-learning” during the pandemic lockdown, with no concomitant decrease of daily screen time for leisure activities. Girls of the 2020 CPP group showed a more prolonged weekly use of electronic devices, already evident before the pandemic, compared to the rest of the 2020 population.

Different studies hypothesized that the progressive reduction in the age of pubertal onset and the increased incidence of precocious puberty over the last 20 years can be related to environmental factors including chemicals that mimic hormonal action. The so-called endocrine disruptors may have estrogen activity or may cause an increase of endogenous estrogen secretion (46-48). Flame retardants have been widely used in the last decades to reduce flammability of electronic devices. Their effect as endocrine disruptors interfering with pubertal development has been demonstrated in animal and human studies (49-51).

A different exposure to endocrine disruptors during the pandemic is unprovable. On the other hand, endocrine disruptors exert their action after having slowly accumulated in the human body due to prolonged environmental exposure. Therefore, they are unlikely related to the rapid increase in the incidence of precocious puberty during the pandemic.

In our study, the increase of new cases of precocious puberty did not involve boys. Male precocious puberty is overall less common and it is more often the result of predisposing genetic mutations or organic disorders of the hypothalamic-pituitary axis. On the other hand, the impact of environmental triggers on pubertal timing in males is still not fully understood. The clarification of
the reasons for this sexual dimorphism would require prolonged observation and a greater number of cases.

We are aware that the retrospective design of our study, together with the fact that living habits and physical activity were self-reported, represent clear limitations in the evaluations of the results. However, it was a multicenter study with a relatively large sample size and similar increases of precocious puberty were observed across the five Italian centers involved (located in different geographic regions).

To our knowledge, no previous studies have analyzed the impact of daily lifestyle changes on puberty. The sharp increase in cases of precocious puberty observed during the first COVID-19 pandemic wave suggests that rapid changes in physical activity, use of electronic devices and pandemic-related stress may trigger the GnRH pulsatile secretion leading to puberty onset.
Declarations of Interest

Conflicts of interest
The authors have no relevant financial or non-financial interests to disclose

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Data availability
The data that support the findings of this study are available on request from the corresponding author, CB.

Author contributions
All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by LC, CB, MV, DF, LP, CG, MS, DC, and NdI. The first draft of the manuscript was written by LC and CB, all authors commented on previous versions of the manuscript. MC, MM and SL reviewed the manuscript. All authors read and approved the final manuscript.
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Figure Legend

Figure 1. Flow chart summarizing the study design.

Figure 2. Upward mismatch between mean birth weight and BMI SDS in 2019 and 2020 subgroups (TT: transient thelarche, NPP: non-progressive precocious puberty, CPP: central precocious puberty, EP: early puberty. * p<0.05)

Figure 3. A: Overall weekly use of electronic devices in 2019 and 2020 populations. B: Pre-pandemic weekly use of electronic devices in 2020 subgroups (CPP: central precocious puberty; Other: transient thelarche, early puberty and non-progressive precocious puberty subgroups)

Figure 4. Overall weekly physical activity in 2019 and 2020 populations.

Figure 5. A: Pandemic weekly physical activity in 2020 subgroups. B: Pre-pandemic weekly physical activity in 2020 subgroups (CPP: central precocious puberty; Other: transient thelarche, early puberty and non-progressive precocious puberty subgroups)
Table 1: Anthropometric and laboratory parameters of 2019 and 2020 female populations.

| Subgroups                  | 2019 Population | 2020 Population |
|----------------------------|-----------------|-----------------|
|                            | TT  | NPP  | CPP  | EP  | TT  | NPP  | CPP  | EP  |
| Number (%)                 | 31  | 48   | 37   | 24  | 64  | 97   | 135  | 12  |
| Age at consultation (years)| 6.88±0.89       | 7.49±0.88°      | 7.58±0.94 | 8.91±0.45° | 7.03±0.94 | 7.19±0.85° | 7.39±0.84 | 8.64±0.44° |
| Age at the onset of pubertal signs (years) | 6.60±0.77 | 7.14±0.80 | 7.04±0.85 | 8.28±0.32 | 6.79±0.96 | 6.89±0.85 | 7.04±0.80 | 8.32±0.37 |
| Birth Weight SDS           | -0.05±1.12      | 0.05±1.09       | 0.02±0.90 | -0.09±0.82 | -0.18±1.02 | -0.03±0.95 | -0.16±1.02 | -0.12±0.87 |
| BMI SDS – Birth Weight SDS | 0.53±1.49       | 0.30±1.09       | 0.48±1.22 | 0.53±1.37 | 0.02±1.03 | 0.19±0.99 | 0.44±1.26 | 0.25±1.05 |
| Height SDS                 | 0.57±1.00       | 0.96±1.16°      | 1.08±1.14 | 0.92±0.89 | 0.43±1.06 | 0.54±1.13° | 0.82±1.01 | 0.59±1.16 |
| Weight SDS                 | 0.58±1.01       | 0.60±1.05       | 0.77±1.00 | 0.67±0.94 | 0.04±0.99 | 0.34±0.98 | 0.52±1.01 | 0.26±1.07 |
| BMI SDS                    | 0.47±1.02*      | 0.31±1.10       | 0.51±0.95 | 0.45±1.01 | -0.17±0.99* | 0.15±1.28 | 0.24±1.54 | 0.10±1.05 |
| Bone Age (years)           | 7.73±1.04       | 8.50±1.31       | 9.18±1.30 | 10.14±1.13 | 7.61±1.20 | 7.89±1.09 | 8.87±1.22 | 9.95±1.01 |
| Bone Age Advance (years)   | 0.75±0.85       | 1.02±0.89       | 1.65±0.94 | 1.20±1.01 | 0.63±1.05 | 0.75±0.81 | 1.45±0.94 | 1.37±0.87 |
| Basal FSH (IU/L)           | 2.12±0.91       | 1.98±1.14       | 3.44±1.83 | 4.66±1.81 | 2.22±1.18 | 2.28±1.89 | 3.95±2.12 | 3.76±1.98 |
| Basal LH (IU/L)            | 0.01±0.05       | 0.10±0.38       | 1.33±1.64 | 2.57±2.66 | 0.13±0.33 | 0.09±0.19 | 1.21±1.72 | 0.80±1.26 |
| FSH peak (IU/L)            | 12.31±4.26      | 10.97±4.73      | 13.16±4.74 | NA      | 12.32±4.81 | 11.96±4.68 | 13.39±6.08 | NA      |
| LH peak (IU/L)             | 3.92±2.76       | 2.80±1.10       | 16.09±14.67 | NA      | 5.07±4.14 | 3.63±2.13 | 17.15±14.34 | NA      |
| FSH/LH ratio               | 4.12±1.91       | 4.36±1.72       | 1.32±1.03 | NA      | 3.33±1.92 | 3.88±1.77 | 1.25±1.15 | NA      |
| 17beta Estradiol (pg/mL)   | 0.39±1.65       | 2.50±5.78       | 9.03±17.38 | 14.80±18.45 | 0.85±2.67 | 0.95±3.18 | 12.13±20.17 | 9.00±16.53 |
| 17OH Progesterone (ng/mL)  | 0.89±0.33       | 1.02±0.39       | 0.98±0.46 | 0.89±0.65 | 0.52±0.41 | 0.59±0.62 | 0.75±0.55 | 0.76±0.52 |
| Uterine longitudinal diameter (mm) | 31.10±6.63 | 32.44±5.48 | 42.89±9.63 | 46.56±15.85 | 33.28±7.36 | 33.37±5.26 | 39.48±8.14 | 39.27±7.91 |

Legend 1. Parameters are expressed as mean ± SD if not differently indicated. Independent Student’s t-test was used to compare continuous variables. Statistical differences between prevalences in 2019 and 2020 subgroups were assessed using the Chi-square test. TT: transient thelarche, NPP: non-progressive precocious puberty, CPP: central precocious puberty, EP: early puberty. *p<0.01 °p<0.05
Figure 1. Flow chart summarizing the study design.

170x132mm (72 x 72 DPI)
Upward mismatch between mean birth weight and BMI SDS in 2019 and 2020 subgroups

338x190mm (96 x 96 DPI)
A: Overall weekly use of electronic devices in 2019 and 2020 populations. B: Pre-pandemic weekly use of electronic devices in 2020 subgroups (CPP: central precocious puberty; Other: transient thelarche, early puberty and non-progressive precocious puberty subgroups)

174x52mm (300 x 300 DPI)
Overall weekly physical activity in 2019 and 2020 populations.

95x58mm (300 x 300 DPI)
A: Pandemic weekly physical activity in 2020 subgroups. B: Pre-pandemic weekly physical activity in 2020 subgroups (CPP: central precocious puberty; Other: transient thelarche, early puberty and non-progressive precocious puberty subgroups)