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Original Research

Testicular volume in 268 children and adolescents followed-up for childhood obesity—a retrospective cross-sectional study

R. Cannarella,[1,*] M. Caruso,[2] R.A. Condorelli,[1] T.A. Timpanaro,[2] M.A. Caruso,[1] S. La Vignera,[1] and A.E. Calogero[1]

[1]Department of Clinical and Experimental Medicine, University of Catania, Catania 95123, Italy
[2]Pediatric Endocrinology, Department of Clinical and Experimental Medicine, University of Catania, Catania 95123, Italy

***Corresponding author:** Department of Clinical and Experimental Medicine, University of Catania, via S. Sofia 78, Catania 95123, Italy.
Email: rossella.cannarella@phd.unict.it

Abstract

Context: Prevalence of obesity in childhood has increased over the past few decades. The impact of obesity and of obesity-related metabolic disorders on testicular growth is unknown.

Objective: To evaluate the impact of obesity, hyperinsulinemia, and insulin resistance on testicular volume (TV) in pre-pubertal (<9 years), peri-pubertal (9-14 years), and post-pubertal (14-16 years) periods.

Methods: We collected data on TV, age, standard deviation score (SDS) of the body mass index (BMI), insulin, and fasting glycemia in 268 children and adolescents followed-up for weight control.

Results: Peri-pubertal boys with normal weight had a significantly higher TV compared to those with overweight or obesity. No difference was found in the other age ranges when data were grouped according to BMI. Pre- and post-pubertal children/adolescents with normal insulin levels had significantly higher TV compared to those with hyperinsulinemia. Peri-pubertal boys with hyperinsulinemia had significantly higher TV compared to those with normal insulin levels. Post-pubertal adolescents with insulin resistance had lower TV and peri-pubertal boys had higher TV compared to those without insulin resistance. No difference was found in pre-puberty.

Conclusions: Closer control of the body weight and the associated metabolic alterations in childhood and adolescence may maintain testicular function later in life.

Keywords: obesity, insulin, puberty, testicular volume, childhood obesity

Significance

Although the prevalence of childhood obesity is increasing worldwide, the impact of obesity and associated metabolic disorders on testicular growth is unknown. We report here data on testicular volume (TV) in 268 children and adolescents, 206 of whom were overweight or obese and 62 normal-weight controls. In this study, we found that being overweight or obese was associated with a lower peri-pubertal TV. In addition, obesity-related comorbidities, such as hyperinsulinemia and insulin resistance, have been found to influence TV in pre- and post-puberty. Therefore, more careful control of body weight in childhood could represent a prevention strategy for maintaining testicular function later in life.

Introduction

Couple infertility represents an important public problem that weighs on both the psychological health and the economic and social aspects of couples of childbearing age. The global patterns of infertility analysis, shown in the World Health Organization (WHO) report conducted by examining 277 health surveys, revealed that 48 million couples were affected by infertility in 2010.[1] Although the male infertility factor is often overlooked,[2] it is implicated as an etiological factor in about half of all infertility cases.[3,4] Based on a WHO multicenter study published in the 1980s, 20% of infertile couples are solely due to the male, 38% to the female, 27% to both partners, and 15% are unexplained.[5] American epidemiological data indicate a prevalence of isolated male factor of 17.1% and total male infertility of 34.6%[6] although the true extent of male infertility is probably underestimated due to the lack of evaluation of the male partner.[2]

Despite a comprehensive diagnostic workup, the diagnosis remains elusive in a quarter of infertile patients who are, therefore, identified as idiopathic.[7–13] According to a monocentric German study that evaluated the etiology of infertility in over 20 000 male patients who were referred to a fertility center, no diagnosis could be made in about 70% of them.[14] Worryingly, a similar percentage was reported in a Dutch single-center retrospective study on 1737 patients with reduced sperm

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counts.[15] Therefore, a relevant percentage of infertile patients appears to have an unexplained etiology of infertility. Meta-regression data also indicate a trend toward decreasing sperm concentration and total sperm count over the past 40 years.[16] Specifically, by combining evidence from 185 studies including 42 935 men who provided semen samples from 1973 to 2011, the authors showed an overall decrease of 52.4% and 59.3% in sperm concentration and total sperm count, respectively, in this time interval.[16] Again, the causes of this decline have not been clearly understood and this requires urgent and careful research.

Italian surveys have shown the presence of testicular hypotrophy (<12 mL) in 14%-23% of young men aged 18-19 whose future fertility is, therefore, at risk.[17,18] This indicates the susceptibility of the testicular function to undergo damage starting from the first years of life.

Undeniably, various environmental conditions (eg, exposure to endocrine-disrupting chemicals [EDCs] and heavy metals) and personal habits (eg, sedentary lifestyle and eating disorders) have changed dramatically over the past few decades. According to the WHO, the prevalence of childhood obesity has increased worldwide from 32 to 42 million[19] in parallel with the decline in sperm count. Predictive modeling also indicates that 60% of children will be obese by the age of 35.[20] However, it is currently unknown whether childhood obesity can affect the testicular function and increase the risk of future infertility.

The testis was classically considered quiescent in childhood. However, recent studies have shown the presence of testicular metabolic activity in the early stages of life.[21–25] The prepubertal testis is made up primarily of Sertoli cells, which are immature, actively proliferating, and release anti-Müllerian hormone (AMH). When puberty begins, they switch from an immature to a mature state, losing their proliferative capacity and acquiring the ability to release mitogens that support the differentiation of germ cells. Since each Sertoli cell can support the differentiation of a finite number of germ cells, their reduced proliferation during childhood resulting in a reduced number of Sertoli cells at puberty could potentially cause irreversible (and defined as unexplained) oligozoospermia in adulthood.[25] Sertoli cells are also sensitive to exogenous stimuli, such as EDCs[26,27] or heavy metals.[28,29] They also express the insulin receptor, and insulin can affect pre-pubertal Sertoli cell proliferation and their hormonal release.[30,31]

On this basis, this study aimed to evaluate whether overweight, obesity, and their related comorbidities, such as hyperinsulinemia, insulin resistance, and type 2 diabetes mellitus (T2DM), can have an impact on testicular volume (TV) in prepuberty (<9 years), peri-puberty (9-14 years), and post-puberty (14-16 years).

Methods

Study protocol

This is a retrospective, cross-sectional study conducted on children and adolescents aged 2-18 years, who were referred to the Unit of Pediatric Endocrinology, University of Catania, for the control of body weight. Data on TV, Tanner stage, age, body mass index (BMI) standard deviation score (SDS), insulin, homeostatic model assessment (HOMA) index, fasting glycemia, blood glycemia 2 h after oral glucose tolerance test (OGTT), and glycated hemoglobin (HbA1c) of children and adolescents were collected retrospectively.

The primary outcome was TV, which was analyzed in age-matched subgroups based on (1) SDS BMI and presence of (2) hyperinsulinemia, (3) insulin resistance, and (4) T2DM. In addition, data on the Tanner stage were collected to assess the percentage of children/adolescents in each Tanner stage in conditions i-iv.

In detail, normal weight was considered for SDS BMI values between −1 and 1, overweight for values between 1 and 2, obesity between 2 and 3, and severe obesity ≥3.[32,33] Hyperinsulinemia was diagnosed for insulin serum levels ≥20 μIU/μL, which is the cutoff value of the central hospital laboratory where all measurements were made. The HOMA index was calculated using the formula: [glycemia (mg/dL) × insulin (μIU/mL)]/405. Insulin resistance was diagnosed for HOMA index values ≥2.5[34] in pre- and post-pubertal periods and for values >3.2 in the pubertal period.[35] Pre-diabetes was diagnosed with fasting glycemia between 100 and 125 mg/dL, or HbA1c between 6% and 6.4%, or 2-h post-OGTT glycemia between 145 and 199 mg/dL. Type 2 diabetes mellitus was diagnosed when fasting glycemia was ≥26 mg/dL, HbA1c ≥6.5%, or 2-h post-OGTT glycemia ≥200 mg/dL.[36] Finally, TV was measured using a Prader orchidometer, and measurements were made by the same two pediatric endocrinologist experts (M.C. and T.A.T.). They also evaluated the Tanner stages of genital and pubic hair.

Patient selection

The inclusion criterion was the presence of information on TV, in a cohort of children and adolescents who were referred to the Pediatric Endocrinology Unit for the control of body weight. A cohort of healthy normal-weight children/adolescents in which TV data were available served as control. The following exclusion criteria were used: genetic abnormalities, possible drug intake, radiotherapy, chemotherapy, head or testicular trauma, systemic diseases (eg, kidney and/or liver diseases), endocrine disorders such as hypogonadism, hyperprolactinemia, Cushing syndrome, acromegaly, hypopituitarism, hypostaturalism, underweight (SDS BMI ≤−1), and female sex. Finally, patients with puberty disorders were excluded. These included patients with precocious puberty (onset of puberty before age 9) or with delayed puberty (onset of puberty after age 14).[37,38]

Hormonal measurements

All patients underwent a 1-day hospitalization for the collection of study parameters. In particular, the blood tests were performed after 12 h of fasting and the samples were analyzed at the central laboratory of the University-Teaching Hospital Policlinico, University of Catania (Catania, Italy). The hormonal evaluation was performed by electrochemiluminescence (Hitachi–Roche equipment, Cobas 6000, Roche Diagnostics, Indianapolis, IN, USA). Reference values were as follows: fasting glycemia 55-99 mg/dL, fasting insulin 1.5-20 μIU/μL, and HbA1c <6.0%.

Statistical analysis

The patients were classified into three groups based on their age: <9 years (a group made of children), 9-14 years (a group made of children and adolescents), and 14-16 years (a group made of adolescents).[37,38] Results are reported as mean ± SD throughout the study. For each group, differences in TV and

Tanner stage were analyzed based on the SDS BMI (between −1 and 1, 1 and 2, 2 and 3, and ≥3), the HOMA index (insulin resistance was considered absent for values <2.5 during pre- and post-pubertal periods, while it was considered present for values ≤3.2 in the peri-pubertal period), the level of insulin (<20 μIU/μL or ≥20 μIU/μL), and the presence of T2DM. The normality of the variables was evaluated with the Shapiro–Wilk test. Testicular volume values were log-transformed before intergroup differences were evaluated using the two-way analysis of variance (ANOVA). Differences in the prevalence of each Tanner stage in the various groups were assessed using the χ^2 test. Finally, a stepwise multiple regression analysis was performed to assess the correlation between TV and SDS BMI, glucose and insulin levels, HOMA index, HbA1c, and age. The regression analysis was performed for the overall sample of each age group and each Tanner stage. A *P*-value ≤.05 was accepted as statistically significant. Statistical analysis was performed using MedCalc Software Ltd. (Version 19.6—64 bit). The sample size was estimated at 198 children and adolescents, considering a mean TV of 6.38 mL and a SD of 8.09 referring to a cohort of healthy boys whose TV was measured using the Prader orchidometer,[39] a type I error (alpha) of 0.05, a type II error (beta, 1-power) of 0.20, and a null hypothesis value of 8 mL.

Ethical approval

This study was conducted at the Division of Endocrinology, Metabolic Diseases and Nutrition and Pediatric Endocrinology of the University-Teaching Hospital Policlinico "G. Rodolico," University of Catania (Catania, Italy). The protocol was approved by the internal Institutional Review Board. Informed consent was obtained from parents, tutors, or any legal representatives after a full explanation of the purpose and nature of all procedures used. Children and adolescents older than 8 years old gave their assent. The study has been conducted according to the principles expressed in the Declaration of Helsinki.

Results

Descriptive analysis

The initial cohort for this study consisted of 290 children and adolescents. Of these, 22 were excluded because they did not meet the inclusion criteria. In the final analysis, 268 children and adolescents were included (age range: 1.6-17.6 years). The clinical characteristics and hormone values of the enrolled cohort by age group and Tanner stage are reported in Tables 1 and 2, respectively. In detail, 62 participants were normal weight, 54 overweight, 79 obese, and 73 severely obese. The Tanner stage was available for 242 participants; 126 were in Tanner stage 1, 49 in Tanner stage 2, 30 in Tanner stage 3, 23 in Tanner stage 4, and 14 in Tanner stage 5. Regarding hyperinsulinemia and insulin resistance, 115 children/adolescents had insulin levels <20 μIU/mL, 45 had insulin levels ≥20 μIU/mL, 78 had no insulin resistance, and 80 had insulin resistance. Finally, 139 had no T2DM, 22 had pre-diabetes, and only 3 had T2DM.

Testicular volume analysis based on age group

Testicular volume in the subanalysis based on SDS BMI or the presence of hyperinsulinemia, insulin resistance, or T2DM are shown in Table S1.

The TV in the group of children younger than 9 years did not differ significantly in normal-weight, overweight, obese, and severely obese participants. Interestingly, when the 9-14 age group was considered, the normal-weight participants showed significantly higher TV than those who were overweight, obese, or severely obese. In contrast, TV did not differ significantly in adolescents (group of 14-16 years) (Figure 1A).

When the data were analyzed based on hyperinsulinemia presence or absence, we found significantly lower TV in the hyperinsulinemic subgroup compared to that with normal insulin levels in children <9 years. In contrast, TV resulted higher in hyperinsulinemic children/adolescents compared to those with normal insulin in the 9-14 age group. Finally, TV was significantly lower in the hyperinsulinemic subgroup than in the normal-insulinemic one of the 14-16 age group (Figure 1B).

Insulin resistance did not have a significant impact on TV in the subgroup of children <9 years. However, at the age range 9-14, patients with insulin resistance had significantly higher TV compared with those with no insulin resistance. Furthermore, when the 14-16-year age group was considered, TV was significantly lower in adolescents with insulin resistance compared to those without (Figure 1C).

Finally, the analysis of TV based on the presence of T2DM did not show any significant difference (Figure 1D).

Analysis of the Tanner stages

We then analyzed the percentages of participants at Tanner stages 1-5 according to the SDS BMI or the presence of hyperinsulinemia, insulin resistance, or T2DM. Since all the children aged <9 years were staged as Tanner 1, the analysis was restricted to the participants ≥9 years.

The percentage of participants in Tanner stage 1 was significantly lower in the normal-weight subgroup (17.7%) compared to overweight (48.5%), obese (41.7%), or severely obese (36.6%) subgroups ($P = .05$). The percentage of participants in Tanner stage 2 was similar among the subgroups (normal weight: 23.5%, overweight: 33.3%, obese: 25.0%, and severely obese: 26.8%). In contrast, the percentage of boys on Tanner stage 3 was significantly higher in normal-weight adolescents (32.4%) than in overweight (6.1%), obese (16.7%), or severely obese (12.20%) ones ($P = .02$). The percentage of participants in Tanner stage 4 did not differ significantly among normal-weight (20.6%), overweight (6.1%), obese (12.5%), and severely obese (12.2%) adolescents. Finally, Tanner stage 5 showed a similar frequency in the four subgroups (normal weight: 5.9%, overweight: 6.1%, obese: 4.2%, and severely obese: 12.2%) (Figure 2A).

The frequency of each Tanner stage was similar in the subgroups with normal or high insulin serum levels (Figure 2B).

The analysis evaluating the frequency of each Tanner stage in patients with or without insulin resistance showed an upward trend to a lower frequency of Tanner stage 1 in the insulin-resistant subgroup (46.2% vs 29.4%, $P = .08$). No difference was found in the frequency of Tanner 2, 3, 4, and 5 in the other three subgroups (Figure 2C).

Finally, the percentage of patients on each Tanner stage based on the presence of T2DM included only non-diabetic patients and those with pre-diabetes, since only three participants had T2DM. Accordingly, the analysis did not reveal any difference in the frequency of each Tanner stage between the two subgroups (Figure 2D).

Table 1. Clinical and hormonal characteristics of the enrolled cohort based on age.

	<9 years	9-14 years	14-16 years	16-19 years
Whole cohort				
n	72	162	30	4
Age (years)	6.49 ± 1.78	11.52 ± 1.38	14.61 ± 0.68	16.43 ± 0.77
BMI (*z* score)	1.99 ± 1.85	1.91 ± 1.41	2.08 ± 1.20	2.58 ± 1.66
HOMA index	3.90 ± 4.07	3.74 ± 3.81	3.79 ± 3.04	2.85 ± 0.39
Fasting glycemia (mg/dL)	83.30 ± 9.73	83.96 ± 8.66	83.96 ± 8.66	90.0 ± 1.41
Fasting insulin (μIU/mL)	18.11 ± 17.04	24.35 ± 71.02	17.79 ± 13.54	12.8 ± 1.56
HbA1c (%)	5.45 ± 0.51	5.49 ± 0.40	5.51 ± 0.27	5.20 ± 0.85
WC (*z* score)	0.64 ± 0.10	0.63 ± 0.12	0.62 ± 0.07	0.63 ± 0.03
Normal weight				
n	23	32	6	1
Age (years)	6.29 ± 1.87	11.74 ± 1.49	14.53 ± 0.37	16.12
BMI (*z* score)	−0.15 ± 0.60	0.02 ± 0.70	0.25 ± 0.57	0.190
HOMA index	2.07[a]	4.62[a]	1.89 ± 0.88	—
Fasting glycemia (mg/dL)	70.0[a]	85.5 ± 0.71	84.67 ± 4.73	—
Fasting insulin (μIU/mL)	12.0[a]	22.0[a]	9.17 ± 4.68	—
HbA1c (%)	—	4.5[a]	—	—
WC (*z* score)	—	—	—	—
Overweight				
n	12	34	8	0
Age (years)	7.53 ± 0.93	11.47 ± 1.39	14.41 ± 0.83	—
BMI (*z* score)	1.52 ± 0.42	1.50 ± 0.56	1.67 ± 0.25	—
HOMA index	2.96 ± 2.89	4.20 ± 7.13	3.23 ± 1.71	—
Fasting glycemia (mg/dL)	91.0 ± 1.41	87.71 ± 16.80	86.0 ± 10.50	—
Fasting insulin (μIU/mL)	13.05 ± 12.66	16.69 ± 18.08	15.07 ± 7.21	—
HbA1c (%)	6.0 ± 1.27	5.58 ± 0.54	5.38 ± 0.33	—
WC (*z* score)	0.59 ± 0.04	0.56 ± 0.07	0.57 ± 0.03	—
Obesity				
n	19	52	8	0
Age (years)	6.42 ± 2.07	11.38 ± 1.44	14.53 ± 0.83	—
BMI (*z* score)	2.53 ± 0.25	2.45 ± 0.29	2.51 ± 0.29	—
HOMA index	4.09 ± 5.22	3.28 ± 2.20	4.77 ± 4.14	—
Fasting glycemia (mg/dL)	81.60 ± 9.75	81.61 ± 8.97	85.75 ± 0.47	—
Fasting insulin (μIU/mL)	18.80 ± 20.79	33.19 ± 13.40	22.36 ± 19.10	—
HbA1c (%)	5.35 ± 0.47	5.50 ± 0.28	5.54 ± 0.10	—
WC (z score)	0.65 ± 0.15	0.65 ± 0.15	0.61 ± 0.03	—
Severe obesity				
n	18	44	8	3
Age (years)	6.64 ± 1.56	11.55 ± 1.31	14.91 ± 0.70	16.01 ± 0.01
BMI (*z* score)	4.44 ± 0.76	3.42 ± 0.28	3.46 ± 0.29	3.70 ± 0.02
HOMA index	3.96 ± 2.76	4.16 ± 2.85	4.32 ± 3.30	2.85 ± 0.08
Fasting glycemia (mg/dL)	84.87 ± 9.72	86.53 ± 8.00	79.33 ± 7.12	90.0 ± 1.41
Fasting insulin (μIU/mL)	18.60 ± 13.83	19.40 ± 12.93	19.72 ± 13.19	12.80 ± 1.56
HbA1c (%)	5.52 ± 0.40	5.48 ± 0.42	5.58 ± 0.43	5.20 ± 0.85
WC (*z* score)	0.64 ± 0.06	0.65 ± 0.11	0.675 ± 0.08	0.64 ± 0.03

Data are presented as mean ± SD. Normal weight: −1≤ SDS BMI <1; overweight: 1≤ SDS BMI <2; obesity: 2≤ SDS BMI <3; and severe obesity: SDS BMI ≥3.
Abbreviations: BMI, body mass index; HOMA, Homeostatic Model Assessment; WC, waist circumference.
[a]Information available only in one patient.

Testicular volume analysis based on the Tanner stages

Testicular volume values were then analyzed for each Tanner stage based on the SDS BMI or the presence of hyperinsulinemia, insulin resistance, or T2DM (Table S2).

When the data were analyzed according to the SDS BMI, we found no significant difference in TV in the normal-weight subgroup compared to overweight, obese, or severely obese children/adolescents for any of the Tanner stages (Figure 3A). Regarding the effects of hyperinsulinemia on TV, the analysis showed the presence of significantly lower TV in children/adolescents with normal insulin levels than in those with hyperinsulinemia in Tanner stage 2. No significant differences were found in the other Tanner stages (Figure 3B). In contrast, insulin resistance affected TV in both Tanner stages 2 and 4. Indeed, TV values were significantly lower in children/adolescents without insulin resistance than in those with insulin resistance in both Tanner stages 2 and 4 (Figure 3C). Finally, the results of the TV analysis based on the presence of T2DM showed no significant differences in any of the Tanner stages (Figure 3D).

Correlation analysis

Testicular volume of the overall cohort was included as an independent variable in a stepwise regression analysis, using age, Tanner stage, SDS BMI, glycemia, serum insulin levels, HOMA index, and HbA1c as independent variables. As expected, TV correlated positively with the Tanner stage (Table S3), while other variables were excluded from the

Table 2. Clinical and hormonal characteristics of the enrolled cohort, based on the Tanner stages.

	Tanner 1	Tanner 2	Tanner 3	Tanner 4	Tanner 5
Whole cohort					
n	126	49	30	23	14
Age (years)	8.48 ± 2.56	11.92 ± 1.75	12.34 ± 1.57	13.61 ± 1.10	14.27 ± 1.47
BMI (*z* score)	2.03 ± 1.56	1.90 ± 1.46	1.38 ± 1.47	1.59 ± 1.67	2.29 ± 1.24
HOMA index	4.04 ± 5.02	3.85 ± 2.42	4.25 ± 2.38	4.16 ± 3.71	2.95 ± 3.20
Fasting glycemia (mg/dL)	84.71 ± 12.58	85.09 ± 8.89	84.87 ± 8.44	84.77 ± 7.35	79.0 ± 6.95
Fasting insulin (μIU/mL)	17.89 ± 16.80	18.27 ± 10.99	20.07 ± 10.27	19.68 ± 17.38	16.37 ± 12.88
HbA1c (%)	5.51 ± 0.46	5.47 ± 0.45	5.49 ± 0.18	5.53 ± 0.34	5.38 ± 0.32
WC (z score)	0.62 ± 0.76	0.63 ± 0.08	0.62 ± 0.05	0.63 ± 0.61	0.61 ± 0.07
Normal weight					
n	26	9	13	8	3
Age (years)	4.25 ± 2.46	12.07 ± 1.61	12.22 ± 1.53	13.36 ± 1.58	14.97 ± 1.19
BMI (*z* score)	−0.24 ± 0.67	0.13 ± 0.74	0.09 ± 0.62	−0.21 ± 0.63	0.62 ± 0.37
HOMA index	2.07[a]	2.88[a]	—	1.20[a]	1.58[a]
Fasting glycemia (mg/dL)	70[a]	81[a]	86[a]	90[a]	83[a]
Fasting insulin (μIU/mL)	12.0[a]	14.40[a]	—	5.40[a]	7.70[a]
HbA1c (%)	—	—	—	—	—
WC (*z* score)	—	—	—	—	—
Overweight					
n	30	13	2	2	3
Age (years)	9.57 ± 1.94	11.66 ± 2.23	12.01 ± 0.42	14.60 ± 0.85	13.50 ± 2.27
BMI (*z* score)	1.43 ± 0.59	1.67 ± 0.41	1.43 ± 0.22	1.59 ± 0.36	1.61 ± 0.27
HOMA index	4.89 ± 8.55	3.25 ± 0.98	—	1.81 ± 1.52	2.52[a]
Fasting glycemia (mg/dL)	88.53 ± 19.07	89.29 ± 10.19	—	79.50 ± 13.44	81.0[a]
Fasting insulin (μIU/mL)	18.56 ± 22.97	14.74 ± 3.79	—	8.65 ± 6.29	12.6[a]
HbA1c (%)	5.77 ± 0.65	5.30 ± 0.47	—	5.0[a]	5.30[a]
WC (*z* score)	0.57 ± 0.06	0.59 ± 0.05	—	—	0.52[a]
Obesity					
n	37	14	9	7	3
Age (years)	8.80 ± 2.55	11.87 ± 1.87	12.64 ± 2.01	13.80 ± 0.72	14.65 ± 1.91
BMI (*z* score)	2.46 ± 0.29	2.47 ± 0.27	2.42 ± 0.33	2.70 ± 0.29	2.57 ± 0.16
HOMA index	3.83 ± 4.09	4.13 ± 3.62	3.40 ± 1.74	3.99 ± 2.46	1.30 ± 1.83
Fasting glycemia (mg/dL)	82.59 ± 11.0	80.31 ± 7.26	84.0 ± 19.46	85.83 ± 6.08	74.0[a]
Fasting insulin (μIU/mL)	17.60 ± 16.29	20.35 ± 16.50	15.97 ± 7.25	18.87 ± 12.0	14.2[a]
HbA1c (%)	5.41 ± 0.40	5.53 ± 0.24	5.47 ± 0.18	5.62 ± 0.24	5.60[a]
WC (*z* score)	0.61 ± 0.09	0.64 ± 0.07	0.61 ± 0.03	0.60 ± 0.02	0.60 ± 0.02
Severe obesity					
n	33	13	6	6	5
Age (years)	8.87 ± 2.49	12.22 ± 1.62	11.72 ± 1.23	13.29 ± 0.82	14.56 ± 1.34
BMI (*z* score)	3.90 ± 0.74	3.49 ± 0.35	3.36 ± 0.25	3.34 ± 0.15	3.46 ± 0.23
HOMA index	3.96 ± 2.96	3.98 ± 1.30	5.81 ± 2.30	6.35 ± 5.59	4.66 ± 4.56
Fasting glycemia (mg/dL)	85.67 ± 8.64	88.17v8.10	86.80 ± 9.09	84.5 ± 8.02	78.75 ± 9.03
Fasting insulin (μIU/mL)	18.62 ± 13.85	18.40 ± 5.69	27.42 ± 11.15	29.98 ± 25.03	20.03 ± 16.81
HbA1c (%)	5.52 ± 0.40	5.54 ± 0.57	5.50 ± 0.28	5.55 ± 0.42	5.33 ± 0.42
WC (m)	0.64 ± 0.06	0.65 ± 0.11	0.66 ± 0.03	0.67 ± 0.07	0.64 ± 0.07

Data are presented as mean ± SD. Normal weight: −1≤ SDS BMI <1; overweight: 1≤ SDS BMI <2; obesity: 2≤ SDS BMI <3; severe obesity: SDS BMI ≥3.
Abbreviations: BMI, body mass index; HOMA, Homeostatic Model Assessment; WC, waist circumference.
[a]Information available only in one patient.

model. When the analysis was repeated for each age group, we found a positive correlation between age in children <9 years and serum insulin levels, and the Tanner stages for the 9-14- and 14-16-year age groups. The analysis could not be performed for the age group 16-19 years due to the insufficient number of observations. Analysis was also performed for each Tanner stage. A positive correlation with age was found for Tanner stage 1, while, for Tanner stage 2, we found a positive correlation with the HOMA index. It was not possible to run the analysis for Tanner's stages 3 and 5 due to a lack of data. Finally, none of the independent variables correlated with TV for Tanner stage 4 (Table S3).

Discussion

Childhood and adolescence represent an important time window for testicular development. Therefore, these phases should be considered a critical moment for the prevention of andrological diseases that may arise later in life. Current literature indicates that male children and adolescents have great exposure to high-risk substances and unhealthy behaviors. These include EDCs, cigarette smoking, alcohol, drugs, unprotected sexual intercourse, and a sedentary lifestyle. The latter is responsible for the higher prevalence of obesity, insulin resistance, and T2DM.[40] It is noteworthy that testicular hypotrophy (<12 mL) is observed in 14%-23% of male youngsters aged 18-19 years[17,18] and up to 9.6% of its variance can be explained by exposure to health risk behaviors that, in turn, can predict testicular hypotrophy.[18]

Overweight and obesity in childhood and adolescence appear to have significantly increased worldwide, with a prevalence of 23.8% in developed countries and 12.9% in developing countries in 2013.[41] Imperial College of London and WHO reported a 10-fold increase in the number of

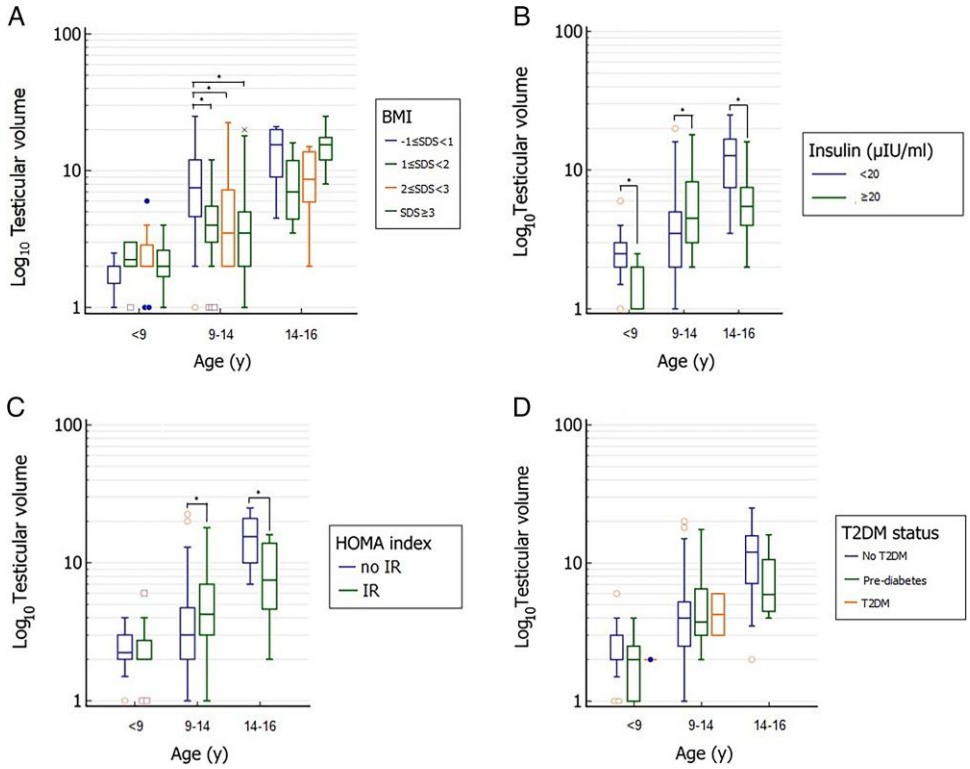

Figure 1. Testicular volume in each age range, according to the standard deviation score (SDS) body mass index (BMI) and the presence of hyperinsulinemia, insulin resistance, and type 2 diabetes mellitus (T2DM). (A) shows the analysis of logarithm (Log)-transformed testicular volume (TV) based on SDS BMI. Significant differences of Log TV were found in the 9-14-year age group, where normal-weight participants (−1≤ SDS <1) showed significantly higher values compared to those overweight (1≤ SDS <2), obese (2≤ SDS <3), or severely obese (SDS ≥3). No difference was found in the other age groups. (B) shows the analysis of Log TV based on the presence of hyperinsulinemia (insulin ≥20 µIU/mL) or normal insulin serum levels (<20 µIU/mL). Log TV was significantly higher in the participants with normal insulin levels than those with hyperinsulinemia in <9-year age group and in that of 14-16 years. In the group 9-14 years, the Log TV was significantly lower in participants with normal insulin levels compared to those with hyperinsulinemia. (C) shows the analysis of Log TV based on the presence of insulin resistance (IR) (homeostatic model assessment [HOMA] index ≥2.5 for age <9 years and 14-16 years, >3.2 for age 9-14 years) or its absence (HOMA index <2.5 for age <9 years and 14-16 years, ≤3.2 for age 9-14 years). Differences in Log TV were found in the 14-16 age group whose participants without insulin resistance had significantly higher Log TV compared to those with insulin resistance. In the 14-16 age group, a significantly lower Log TV was found in participants without insulin resistance. No difference was found in the <9 age group. (D) shows the Log TV based on the absence of T2DM, the presence of pre-diabetes, or the presence of T2DM. No difference in Log TV was found in the subgroups analyzed.

children and adolescents with obesity between the ages of 5 and 19.[42] Therefore, it is crucial to question the impact of obesity and related metabolic diseases on TV in childhood and adolescents. Analyzing the TV of 264 children and adolescents followed for weight control, we found that 9-14-year-old boys with normal weight had significantly higher TVs than those who were overweight or obese. Furthermore, children/adolescents with normal insulin levels in both pre- and post-pubertal phases had significantly higher TVs than those with hyperinsulinemia. In contrast, peripubertal boys with hyperinsulinemia had significantly higher TV compared to those with normal insulin levels. In post-puberty, adolescents with insulin resistance had lower TV than those without insulin resistance, while no differences were found in the other age ranges. Therefore, children/adolescents with metabolic disorders, characterized by overweight/obesity, hyperinsulinemia, and insulin resistance, appear to have lower TV than normal-weight peers. When the data were analyzed by Tanner stages, the difference did not reach the statistical significance although the CI of the TVs was higher in the normal-weight subgroup than in the overweight, obese, or severely obese for Tanner stages 2-5. These results could be due to the sample size, which, with this analysis, was significantly reduced for each group. On the other hand, boys with normal insulin levels at Tanner stage 2 had significantly lower TV than those with hyperinsulinemia. Similarly, TVs were significantly lower in patients without insulin resistance compared to those with insulin resistance at Tanner stage 2. However, at Tanner stage 5, patients without insulin resistance showed a significantly higher TV than those with insulin resistance.

A limited number of studies have so far focused on the effect of overweight and obesity on testicular function (Table 3). Particularly, a longitudinal study carried out on boys born in 1989-1991 followed up to the age of 17 and 20 years found that non-alcoholic fatty liver disease (NAFLD), a trait of metabolic syndrome, was associated with a 50% reduction in sperm count. Furthermore, the presence of insulin resistance was associated with a 20% reduction in TVs at 20 compared to 17 years.[43] However, this study did not provide data on patients younger than 17 years. In contrast, a recent study on 351 overweight/obese boys of 5-19 years has evaluated inhibin B and testosterone serum levels with those of healthy normal-weight controls of similar Tanner stage. The authors documented a reduction in inhibin B levels starting from the age of 12 and testosterone levels starting from the age of 14 in overweight and obese boys compared to controls.[44] Similarly, another study on 121 obese and 38 normal-weight

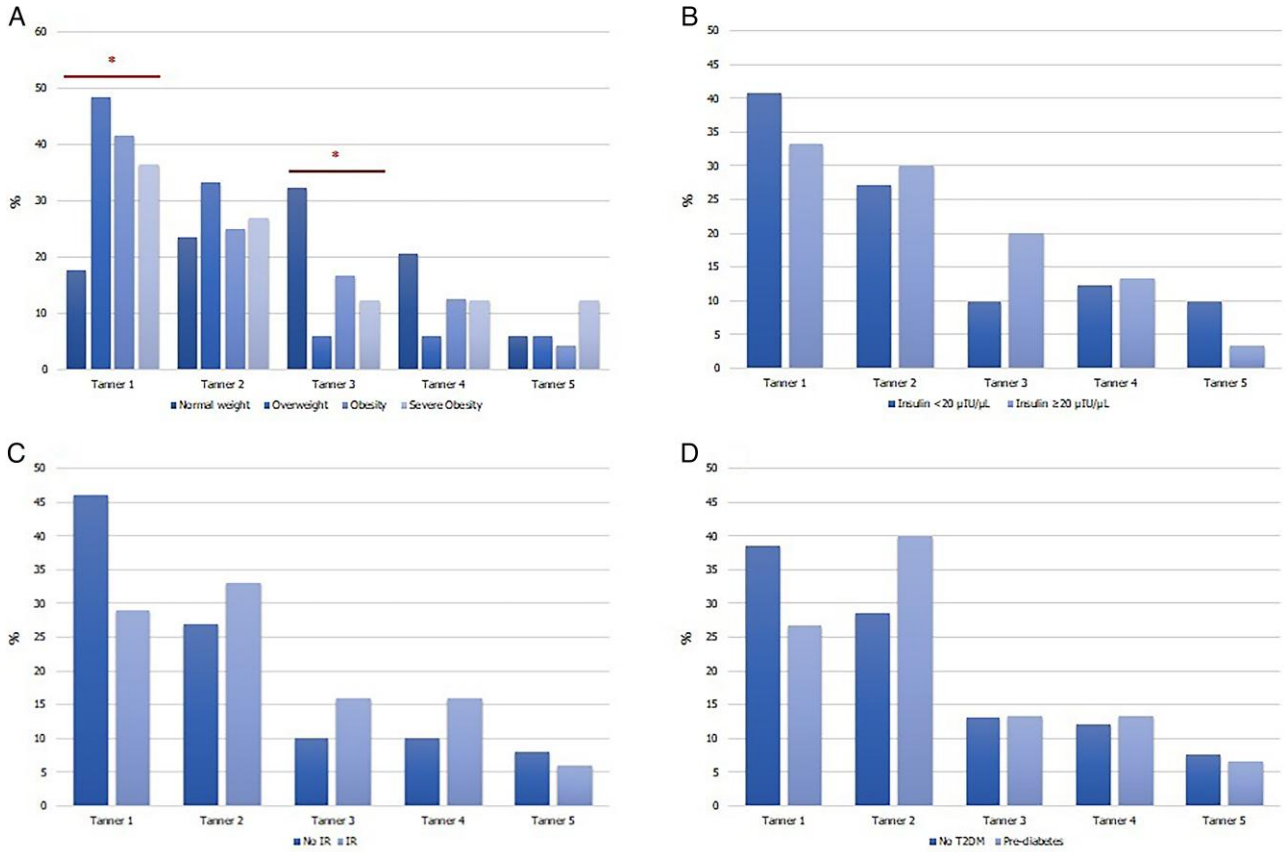

Figure 2. Percentage of participants on Tanner stages 1-5 based on standard deviation score (SDS) body mass index (BMI) and the presence of hyperinsulinemia, insulin resistance, or type 2 diabetes mellitus (T2DM) in <9-year children. (A) shows the percentage of participants in each Tanner stage based on SDS BMI. The percentage of normal-weight (−1≤ SDS <1) participants on Tanner stage 1 was significantly lower in the normal-weight group than in overweight (1≤ SDS <2), obese (2≤ SDS <3), and severely obese (SDS ≥3) groups. The frequency of Tanner stage 3 was significantly higher in normal weight than in the other groups. No difference was found for the other Tanner stages. (B) shows the percentage of participants in each Tanner stage based on the presence of hyperinsulinemia (insulin ≥20 µIU/mL) or normal insulin serum levels (<20 µIU/mL). The frequency of each Tanner stage was similar between the two subgroups. (C) shows the percentage of participants in each Tanner stage based on the presence of insulin resistance (IR) (homeostatic model assessment [HOMA] index ≥2.5 for age <9 years and 14-16 years, >3.2 for age 9-14 years) or its absence (HOMA index <2.5 for age <9 years and 14-16 years, ≤3.2 for age 9-14 years). Compared to the insulin-resistant group, a trend toward a higher percentage of patients on Tanner stage 1 was found in the HOMA index <2.5 group. A significantly higher frequency of Tanner stage 2 was found in the group with insulin resistance than in that without. No difference in the frequency of the other Tanner stages was found between the two groups. (D) shows the percentage of participants in each Tanner stage based on the absence of T2DM or the presence of pre-diabetes. No difference in the percentage of each Tanner stage was found.

adolescents reported a significant decrease in AMH, inhibin B, and total testosterone serum levels in obese boys vs controls.[45] Decreased levels of inhibin B and testosterone were also reported in NAFLD–obese vs non-NAFLD–obese and control adolescents, though TVs did not show significant differences in obese and non-obese boys.[46] These findings indicate a negative influence of higher body weight on both Sertoli and Leydig cell function in adolescence. Unfortunately, data on TV were not reported in these studies.[44,45]

Another study, conducted on 80 male boys (20 with obesity and 20 with normal weight in Tanner stage 2 and 20 with obesity and 20 with normal weight in Tanner stage 4), did not find significant differences in TVs between obese and controls in both Tanner stages (Tanner 2: group 1 [obese] 5.3 ± 1.3 mL, group 2 [non-obese] 5.5 ± 2.0 mL; Tanner 4: group 1 [obese] 17.0 ± 3.0 mL, group 2 [non-obese] 17.1 ± 4.5 mL). However, the same authors reported significantly lower insulin-like peptide 3 (INSL3) levels in the obese group. The levels of this hormone negatively correlated with serum leptin, thus supporting the deleterious influence of obesity on Leydig cell function.[47] The apparent discrepancy in the results of this study could be reasonably explained considering the low sample size of the groups compared.

Regarding the age of puberty onset, the percentage of Tanner stage 1 children we enrolled in the present study was significantly lower in the normal-weight subgroup than in the overweight, obese, or severely obese subgroups. In contrast, the prevalence of adolescents in pubertal Tanner stage 3 was significantly higher in the normal-weight subgroup than in those who were overweight, obese, or severely obese. These data suggest that obesity delays the onset of puberty. However, the evidence on this point is still unclear as some studies support a delaying effect of obesity on the timing of puberty onset,[49,50] while others suggest an anticipatory effect[50] and others found no effect of obesity on the age of puberty onset.[48] Speculatively, the diverging results may be due to the differences between the populations studied and/or to the different definitions of obesity used in the various studies (Table 3).

Interestingly, regardless of BMI, we found a significant difference in TV across all subgroups based on the presence or absence of hyperinsulinemia. Indeed, hyperinsulinemia is associated with higher TVs in pre-pubertal (<9 years) and post-

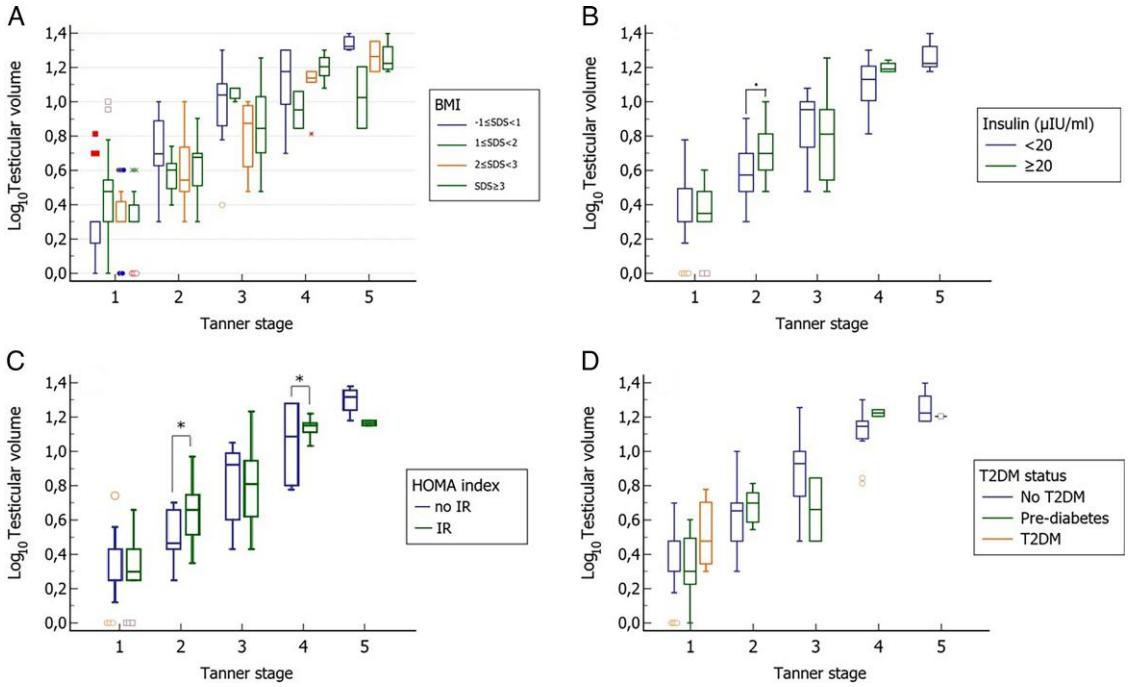

Figure 3. Testicular volume of the participants at Tanner stages 1-5 based on standard deviation score (SDS) body mass index (BMI) and the presence of hyperinsulinemia, insulin resistance, or type 2 diabetes mellitus (T2DM). (A) shows the difference of logarithm (Log)-transformed testicular volume (TV) based on SDSBMI. Although the Log TV of normal-weight (−1≤ SDS <1) participants appear higher than that of overweight (1≤ SDS <2), obese (2≤ SDS <3), or severely obese (SDS ≥3) participants in Tanner stages 2-5, the statistical significance was not reached. (B) shows the analysis of the Log TV based on the presence of hyperinsulinemia (insulin ≥20 µIU/mL) or normal insulin serum levels (<20 µIU/mL). In the participants at Tanner stage 2, Log TV was significantly lower in the participants with normal insulin levels than in those with hyperinsulinemia. (C) shows the analysis of the Log TV based on the presence of insulin resistance (IR) (homeostatic model assessment [HOMA] index ≥2.5 for age <9 years and 14-16 years, >3.2 for age 9-14 years) or its absence (HOMA index <2.5 for age <9 years and 14-16 years, ≤3.2 for age 9-14 years). Differences in the values of Log TV occurred in the participants at Tanner stage 2 where the patients without insulin resistance had significantly lower Log TV compared to those with insulin resistance and in participants at Tanner stage 5 where the patients without insulin resistance had a significantly higher Log TV compared to those with insulin resistance. No differences were found in the other Tanner stages. (D) shows the analysis of the Log TV based on the absence of T2DM and the presence of pre-diabetes or T2DM. No difference was found in any of the subgroups analyzed.

pubertal (14-16 years) boys, but not in peri-pubertal boys (9-14 years), where it is associated with elevated TVs compared to matched controls. Interestingly, insulin did not affect the Tanner stage, as the prevalence of boys in Tanner stages 1-5 did not differ significantly between children/adolescents with hyperinsulinemia and those with normal insulin serum levels. To the best of our knowledge, this is the first study to provide such evidence, as no studies have evaluated whether insulin levels may affect TV in childhood and adolescence. This finding is important, as we have reported that insulin can impair the function of porcine pre-pubertal Sertoli cells in terms of AMH and inhibin B release and cell proliferation.[31] These results obtained in the experimental animal suggest an influence of hyperinsulinemia on the function of Sertoli cells in obese children. The family of insulin-like growth factors (IGF) (to whom insulin belongs) is able to influence testicular development and function,[30] as well as the follicle-stimulating hormone (FSH) signaling pathways.[51] Indeed, the signaling pathways of FSH and those of the family of tyrosine kinase receptors to which the receptor for insulin and that for IGF1 belong are linked.[52] In this regard, evidence in humans suggests that the treatment of insulin resistance improves response to exogenous FSH administration in infertile male patients.[53] Therefore, insulin resistance can adversely affect the testicular responsiveness to FSH, even in peri-puberty.

In this study, we chose an insulin value above 20 µIU/µL to identify children/adolescents with hyperinsulinemia. To the best of our knowledge, there is no clear agreement on what the cutoff for insulin serum should be during childhood and puberty, especially considering the physiological rise in insulin during the pubertal period. Ballerini et al.[34] have suggested a fasting blood insulin level of 10 µIU/mL in pre-pubertal children and of 17 and 13 µIU/mL in pubertal girls and boys, respectively, as cutoff values in healthy children. Peplies et al.[54] reported a median value of 43.0 pmol/L (6.192 µIU/mL) in normal-weight boys aged 10.5-11 years. However, the 99th percentile was 123.97 pmol/L in the same age range, corresponding to 17.9 µIU/mL, suggesting a broad distribution of values in this population. Similarly, Moran et al.[55] reported a mean of 84 pmol/L (12.1 µIU/mL) in Tanner 1-5 boys of normal weight. However, also in this study, the SD indicated a broad distribution of values, especially as the Tanner stage increased. With these premises, since there is no solid agreement in the literature and considered the wide distribution of the values in normal-weight children and pubertal boys, we have chosen the value of 20 µIU/µL to identify the truly hyperinsulinemic subjects. In fact, no study has reported mean or median insulin levels equal to 20 µIU/µL in healthy and normal-weight children/adolescents. Choosing this cutoff prevented us from overestimating the results. Interestingly, even after choosing this value, we found a clear relationship between high serum insulin levels and TVs.

Among the reasons for taking the results of this study with caution is the retrospective design, which does not allow for

Table 3. Review of the literature.

First author	Year	Population	Ethnicity	Criteria used to define obesity	Measure outcomes	Method used to measure TV	Results[a]
Hart[42]	2019	648 late adolescent boys	NR (patients come from the Raine study)	WHO criteria for adults[b]	TV, semen parameters, testosterone, LH, FSH, inhibin B	Ultrasound	High metabolic risk at 20 years: 14.7 (12.3-16.9, 9.0-23.8); low risk at 20 years: 15.2 (13.0-17.4, 7.6-28.4)[c] High metabolic risk at 17 years: 15.6 (13.3-17.5, 10.1-23.2); low risk at 17 years: 14.7 (12.6-17.1, 8.0-28.4)[c] HOMA >4 at 20 years: 12.8 (11.1-14.7, 10.0-16.9); HOMA ≤4 at 20 years: 15.2 (13.0-17.4, 7.6-28.4)[c]*
Rerat[43]	2022	351 obese and overweight (5-19 years) vs 652 controls	NR	Overweight: SDS BMI >+ 1 and < +2 Obesity: SDS BMI > +2 (WHO 2006a, b)	Testosterone, inhibin B	—	—
Buyukinan[44]	2018	121 obese vs 38 healthy lean adolescents	NR (patients were enrolled in the Konya Research Hospital)	BMI of ≥95th percentile (reference curves for Turkish children and adolescents were used)	Testosterone, inhibin B, AMH	—	—
Kurku[45]	2019	119 obese and 78 non-obese adolescent	Not specified	Obesity was defined as BMI ≥95th percentile according to the reference curves for Turkish adolescents	TV, testosterone, LH, FSH, inhibin B, AMH	Prader's orchidometer	NAFLD obese boys: 11.3 ± 7.5 mL Non-NAFLD obese boys: 11.1 ± 6.2 mL Control boys: 11.2 ± 6.3 mL
Taneli[46]	2010	80 adolescents		Non-obesity: BMI of <85th percentile Obesity: BMI of ≥95th percentile	TV, total and free testosterone, LH, FSH INSL3, estradiol, SHBG, leptin	Prader's orchidometer	Tanner 2, group 1 (obese): 5.3 ± 1.3 mL, group 2 (non-obese): 5.5 ± 2.0 mL Tanner 4 group 1: 17.0 ± 3.0 mL, group 2: 17.1 ± 4.5 mL
Lee[47]	2016	3872 boys	White, African–American, Hispanic	Normal weight: BMI 5th-84th percentile; overweight: BMI ≥85th and <95th percentile; obese: BMI ≥95th percentile	Age at TV ≥3 mL or ≥4 mL	Prader's orchidometer	The author found evidence for earlier puberty for overweight compared with normal-weight boys for white boys but not for African–American or Hispanic boys
Tomova[48]	2015	4030 boys (7-19 years)	Caucasian	Normal weight: BMI 5th-84th percentile; overweight: BMI ≥85th and <94.99th percentile; obese: BMI ≥95th percentile	Age at TV of 3 mL,12 mL, Tanner stage 2	Prader's orchidometer	Earlier maturing boys were heavier than their peers
He[49]	2017	782 boys (6-17 years)	East Asian	Overweight: BMI ≥85th and <95th percentile; obese: BMI ≥95th percentile	Timing of puberty in normal-weight, overweight, and obese groups	Prader's orchidometer	Body density, percentage of body fat, fat mass, and fat-free mass of boys did not significantly influence the timing of puberty. Delayed puberty was negatively correlated with obesity
Busch[50]	2020	218 obese vs 660 healthy boys	Not specified	Obesity: SDS BMI >+2	Timing of testicular volume ≥4 mL, genital stage ≥2, and pubarche	Prader's orchidometer	Testicular volume ≥4 mL occurred significantly earlier in obese boys compared to controls. No difference in the timing of genital stage ≥2 and pubarche was observed

Abbreviations: AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; INSL3, insulin-like peptide 3; NAFLD, non-alcoholic fatty liver disease; NR, not reported; TV, testicular volume; SHBG, sex hormone-binding globulin.
[a]Only the results related to differences in TV and timing of puberty are shown.
[b]https://www.who.int/westernpacific/health-topics/obesity#:~:text=In%20adults%2C%20a%20body%20mass,the%20WHO%20Growth%20Reference%20median.
[c]Data are expressed as median (interquartile range, range).
*$P < .05$.

any causal relationship. In particular, the cross-sectional design does not allow us to exclude that the results during the various pubertal periods are due to a temporal difference in pubertal maturation. However, as mentioned before, obesity appears to affect the timing of puberty onset, but the evidence on this is still contradictory. Indeed, some authors reported a delaying effect,[49,50] others an anticipatory effect,[48] and some no effect.[56] Other limitations of the study are the small number of post-pubescent boys (14-16 years), as well as that of the overall cohort, and the absence of data on serum gonadotropin, testosterone, AMH, and inhibin B levels. Furthermore, as reported in "Methods," TV was measured using the Prader orchidometer. However, the gold standard for measuring TV is the ultrasound scan. In fact, the orchidometer also measures the epididymis as well as the scrotal skin and, consequently, overestimates TV, especially when the testis is small and the epididymis is relatively larger than the total TV.[38] On the other hand, the same two expert pediatric endocrinologists made the orchidometer measurements, and this helped in minimizing the error. Further prospective studies in obese children and adolescents are needed to confirm our findings. In addition, it will be important to extend the follow-up to adulthood to acquire data of semen analysis in men who had an increased body weight and its related comorbidities during childhood and/or adolescence.

Conclusion

Children and adolescents with overweight/obesity, hyperinsulinemia, and insulin resistance have lower TVs than their age-matched controls. Since a lower TV is predictive of worse sperm production, these results help to understand the reason for the high prevalence of testicular hypotrophy in young men. We speculate that more careful control of body weight in this time window could represent a prevention strategy to pursue the maintenance of testicular function later in life. If further studies will confirm our findings, there will be room for primary prevention of male infertility in pediatric clinics (pediatric andrology). Indeed, an accurate assessment of TV at each visit and the construction of TV growth curves would help identify early deviations in TV growth in children and adolescents.

Acknowledgments

R.C. conceived the study, collected and analyzed the data, and wrote the manuscript; M.C. and T.A.T. visited the patients and collected the data; M.A.C. collected the data; R.A.C. and S.L.V. supervised the study; A.E.C. conceived the study, wrote the manuscript, and managed the project.

Supplementary material

Supplementary material is available at *European Journal of Endocrinology* online.

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Conflicts of interest: None declared.

Data availability

The data sets analyzed during the current study are not publicly available since they are part of a larger ongoing project, but are available from the corresponding author on reasonable request.

Patients' consent

Informed consent was obtained from parents, tutors, or any legal representatives after a full explanation of the purpose and nature of all procedures used. Children and adolescents older than 8 years old gave their assent. The study has been conducted according to the principles expressed in the Declaration of Helsinki.

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