Evaluation of testicular hormones in obese adolescent males

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Abstract

Background: Previous studies have suggested an association between obesity and testicular function. Aim to Investigate the effect of obesity on testicular function by evaluating the reproductive hormones inhibin B and INSL3.

Subjects and Methods: This study was a case control study carried out in the "Obesity Clinic of the Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU)", Pediatric Hospital, Cairo University. It included sixty adolescent males complaining of obesity (BMI≥ 95 percentile) along with twenty normal subjects (BMI= 15-< 85 percentile) matched for age and sex as a control group. Their age was ranged from 12 to 18 years with Tanner stage from 2 to 4. Anthropometric assessment; weight and height; were recorded, and BMI was calculated. Laboratory investigations: inhibin B and INSL3 were measured.

Results: Obese boys had significant higher values in BMI and Insulin growth factor 3 (p= 0.001), and significant lower value of Inhibin B than the control normal group. BMI had significant positive correlations with INL3 (p= 0.000) and significant negative correlation with inhibin B (p= 0.004).

Conclusion: Obesity is associated with increased INL3 (marker of Leydig cell function) and decreased inhibin B (marker of testicular Sertoli cell). Moreover, inhibin B had significant negative correlation with INL3.

Key wards: inhibin B, INSL3, adolescent boys, obesity.

تقييم وظيفة الخصية فى الذكور المراهقين البدناء

الهدف: تقييم مستويات الإنهيبين (B) والأنسولين مثل عامل ٣ (INSL- 3) لدى الاولاد الذين يعانون من السمنة فى مرحلة البلوغ.

المنهجيه: هذة الدراسة هى دراسة حالات والمجموعه الضابطه فى عيادة النمو السكرى والغدد الصماء وحدة طب الأطفال مستشفى طب الأطفال جامعة القاهرة. وتشمل ستين ذكر من الذكور البدناء وعشرين اخرين اصحاء من نفس المرحلة العمرية. تم أخذ قياسات أنثروبومترية معينة للأولاد محل الدراسة مثل الطول، الوزن، كما تم حساب مؤشر كتلة الجسم. تم قياس تركيزات هرمون الإنهيبين (B) وهرمون الأنسولين مثل عامل ٣ (INSL- 3).

النتائج: أظهرت الدراسة أن الاولاد البدناء لديهن أعلى القيم فى مستوى هرمون الأنسولين مثل عامل ٣ (INSL-3) وأقل القيم فى مستوى هرمون الإنهيبين (B). ووجد إرتباطا معنويا إيجابيا بين مستوى هرمون الأنسولين مثل عامل ٣ وبين مؤشر كتلة الجسم بينما يوجد إرتباطا معنويا سلبيا بين مؤشر كتلة الجسم وبين مستوى هرمون الانهيبين (B) بالاضافه الى انه وجد إرتباطا معنويا سلبيا بين هرمون الانهيبين (BINSL-3).

الخلاصه: تؤثر السمنه على خلايا ليدج وخلايا سرتولى للذكور عن طريق زياده افراز هرمون الأنسولين مثل عامل (INSL-3) وتقليل افراز هرمون الإنهيبين (B).

الكلمات الافتتاحيه: الاولاد البالغين– السمنه– الانهيبين (B)– الانسولين مثل عامل ٣.

Introduction:

Obesity defined; by the World Health Organization (WHO); as abnormal or excessive fat accumulation that may impair health. WHO estimates that more than 1.5 billion adults over the age of twenty are overweight and that 1 in 10 adults in the world are obese. It has been suggested that this rising trend of excessive adipose tissue accumulation has not only been caused by an increase in high- sugar and cholesterol-saturated diets, but also by an increase in sedentary lifestyles (Khullar et.al., 2012). While obesity has been associated with a host of cardiovascular disease, the metabolic syndrome, and a wide variety of endocrine abnormalities, recent research has suggested a potential link between obesity and testicular affection (Pasquali, 2006; Kasturi et.al., 2008; Ferris and Crowther, 2011).

During the first month of life a phenomenon called "Minipuberty" takes place. It consists of an activation of the hypothalamic- pituitary-testicular axis and it is followed by a period of relative quiescence with low serum levels of gonadotropins and sexual steroids until puberty ensues (Rosita et.al., 2014).

In males, Leydig cells produce testosterone and insulin like factor 3 (INSL-3) under stimulation of LH (Ferlin et.al., 2006); both testosterone and INSL-3 cause testicular descent before birth. INSL-3 serum concentrations are strongly influenced by the degree of Leydig cells differentiation, in turn, influenced by LH levels (Sadeghian et.al., 2005; Bay et.al., 2006). Mutations of the INSL-3 receptor (Relaxin Family Peptide 2, RXFP2) gene are associated with osteoporosis even in presence of normal serum testosterone concentrations (Ferlin et.al., 2008). In addition, INSL-3 increases significantly in a dose dependent manner cAMP in human osteoblasts and stimulate the proliferation of these cells (Ferlin et.al., 2009). Sertoli cells produce inhibin B which first increases during the minipuberty in response to FSH and subsequently during puberty (Rosita et.al., 2014). In adult life, the inhibin B serum levels reflect spermatogenesis and exert a negative feedback on FSH secretion (Jorgensen et.al., 2010).

The worldwide pandemic of childhood obesity has renewed interest in the relationship between body composition in childhood and the timing and tempo of puberty. Limited evidence suggests that earlier puberty is associated with a tendency towards central fat deposition, in the other direction, rapid early weight gain is associated with advanced puberty in both sexes, and a clear association exists between increasing BMI and earlier pubertal development in girls. Evidence in boys is less clear, with the majority of studies showing obesity to be associated with earlier puberty and voice break, although a subgroup of boys with obesity exhibits late puberty (Wagner et.al., 2012).

Aim of the Study:

The present study aims to investigate the effect of obesity on testicular function by Evaluating the reproductive hormones inhibin B and insulin-like 3 (INSL3).

Subjects And Methods:

This study was a case control study carried out in the "Obesity Clinic of the Diabetes Endocrine and Metabolism Pediatric Unit (DEMPU)", Pediatric Hospital, Cairo University. It included sixty adolescent males complaining of obesity (BMI≥ 95 percentile) along with twenty normal subjects (BMI= 15≦ 85 percentile) matched for age and sex as a control group. Their age was ranged from 12 to 18 years with Tanner stage from 2 to 4. Obese males were having exogenous obesity. We excluded males with any active medical illness, chronic illness as chronic renal failure, celiac disease, … with history of gonadal dysfunction, under any regular medication and with obesity due to endocrinal or syndromatic cause.

Ethical approval from the "Ethical committee of the National Research Centre" was taken. Informed consent was obtained from the child and the parents after explanation of the aim of the study and its possible benefits for identifying the effect of obesity on health.

Methods:

For each boy participated in the study, detailed history, clinical examination, anthropometric and hormonal assessment were done.

1. History:
   a. Birth Weight.
   b. Onset and duration of obesity.
   c. History of early or delayed puberty in the mother or father or any of his siblings.
   d. Family history of any chronic disease as diabetes mellitus especially in the mother.
   e. History of Playing Sports.
   f. History for the risk factors, morbidities and co- morbidities of obesity.
   g. Symptoms suggestive of secondary diabetes mellitus like polyuria, polydipsia and loss of weight of the child.
   h. Symptoms suggestive of hypertenstion like headache, epistaxis.
2. Clinical Examination:
   a. Complete clinical examination to exclude any chronic disease.
   b. Measurement of blood pleasure.
   c. Pubertal stage assignment and testicular volume estimation were performed using a Prader orchidometer and measuring stretched penile lenght. The pubertal stage was determined according to Marshall and Tanner 1970.
3. Anthropometric Measurements: Specific anthropometric measurements (Weight, Height and BMI), were calculated using standardized equipments, and following the recommendations of the International Biological Program by (Weiner and Lourie, 1969).
4. Laboratory Investigations:
   a. Venous blood samples were obtained between 8:00 and 10:00 hours after 10 h of fasting. After clotting the blood samples were centrifuged and the sera were separated and kept at –80 °C for batch assessments.
   b. Serum Inhibin B and INSL3 were measured by electro-

chemiluminescence (ECLIA).

Results:

The study included 60 obese pubertal boys and 20 normal (control) pubertal boys their age ranged between 12 and 18 years.

Comparison between the 2 groups regarding BMI and laboratory findings was presented in table (1).

Obese boys had significant higher values in BMI and Insulin growth factor 3 (p= 0.001), and significant lower value of Inhibin B than the control normal group

Table (1) Comparison between obese and normal boys as regard BMI, Inhibin B and INLS3

Parameters	Groups	N	Mean ± S. D.	P Value
zBMI	Normal	20	20.99 ±0.46	0.001
	Cases	60	0.58 ± 1.17	
Inhibin B (ng/L)	Normal	20	83.28 ± 27.66	0.001
	Cases	60	62.90 ± 17.85	
Insulin growth factor- 3 (ug/L)	Normal	20	3.80 ±0.75	0.001
	Cases	60	6.39 ± 1.04	

Table (2) shows correlations between BMI, age, inhibin B and INL3.

BMI had significant positive correlations with INL3 (p= 0.000) and significant negative correlation with inhibin B (p= 0.004). Moreover, inhibin B had significant negative correlation with INL3 (P= 0.027). Age had insignificant correlations with either INL3 (p= 0.162) or inhibin B (p= 0.973).

Table (2) Correlation between BMI, INL3 and Inhibin B

	BMI Z		Inhibin B		INL3	
	R	P	R	P	R	P
Age (Years)	0.162	0.162	0.000	0.973	0.16	0.162
Inhibin B (ng/L)	- 0.33	0.004				
INL3 (ug/L)	0.63	0.000	-.2 5	0.027		

Discussion:

In the present study, serum INSL3 level significantly increased about 2 folds in obese boys than normal weight ones. BMI also had significant positive correlations with INL3 (p= 0.000) Previous studies stated that serum INSL3 concentrations in adolescent boys showed a significant rise during pubertal stages and positively correlated with testicular growth (Ferlin et.al., 2006; Feng et.al., 2009; Wikström et.al., 2006). Continuous increase in INSL3 levels during puberty is evidence that INSL3 may be used as a Leydig cell- specific marker for onset and succession of puberty in boys (Bay et.al., 2007). We believe that obesity lead to significant more increase in INSL3, the marker of leydig cell function, than among pubertal normal weight boys. So, INS3/ or leydig cells are affected by obesity in the pubertal period but there are no other literatures support our finding.

In contrary, in the present study, we found that obese boys had significant lower value of serum inhibin B (p= 0.001) than non- obese boys. BMI had significant negative correlation with inhibin B (p= 0.004). Moreover, inhibin B had significant negative correlation with INL3 (P= 0.027).

In agreement with current results, Radicioni et.al., (2005) reported that inhibin B levels increase regularly during puberty until they reach adult levels in late puberty. They believed that inhibin B is increased in association with the progressive increase in testis volume and spermatogenesis occurring in late puberty. Aggerholm et.al. (2008) found that serum inhibin B concentrations were (25%- 32%) lower in obese men in comparison with normal weight men. Winters et.al. (2006) demonstrated that inhibin B levels declined with increasing obesity in young adult men while it was normal in prepubertal boys (Fu et.al., 2006). In the present study, we believed that inhibin B, the marker of Sertoli cell function is sensitive and Sertoli cells are affected by obesity in the pubertal period.

Current study revealed that age had insignificant correlations with either INL3 (p= 0.162) or inhibin B (p= 0.973). This confirms that age had no effect on the relations between these hormones and obesity or between each other.

Conclusions:

This study demonstrated that obesity is associated with increase INL3 (marker of Leydig cell function) and decrease inhibin B (marker of testicular Sertoli cell). Moreover, inhibin B had significant negative correlation with INL3.

References:

1. Bay K, Matthiesson KL, McLachlan RI and Andersson AM. The effects of gonadotropin suppression and selective replacement on insulin- like factor 3 secretion in normal adult men. **J. Clin Endocrinol Metab**. 2006; 91:1108- 1111.
2. Bay K1, Virtanen HE, Hartung S, Ivell R, Main KM, Skakkebaek NE, Andersson AM; Nordic Cryptorchidism Study Group and Toppari J. Insulin- like factor 3 levels in cord blood and serum from children: effects of age, Postnatal hypothalamic- pituitary- gonadal axis activation, and cryptorchidism. **J. Clin Endocrinol Metab**. 2007 Oct; 92(10): 4020- 7.
3. Condorelli RA, Calogero AE, Vicari E, Mongioi' L, Favilla V, Morgia G, Cimino S, Russo G and La Vignera S. The gonadal function in obese adolescents. **J. Endocrinol Invest**. Published online 2014.
4. Fu JF, Dong GP, Liang L, Jiang YJ, Chen LQ and Dayan C. Early activation of the inhibin B/ FSH axis in obese Tanner Stage G1PH1 boys. **Clin Endocrinol** 2006; 65: 327- 32.
5. Feng S1, Ferlin A, Truong A, Bathgate R, Wade JD, Corbett S, Han S, Tannour- Louet M, Lamb DJ, Foresta C and Agoulnik AI. INSL3/ RXFP2 signaling in testicular descent. **Ann N.Y. Acad Sci.** 2009 Apr; 1160: 197- 204.
6. Ferris, W. F. and Crowther N. J. "Once fat was fat and that was that: our changing perspectives on adipose tissue", **Cardiovasc J Afr** 2011; 22(3): 147- 154.
7. Ferlin A, Garolla A, Rigon F, Caldogno LR, Lenzi A and Foresta C. Changes in seruminsulin like 3 during normal male puberty. **J. Clin Endocrinol Metab**. 2006; 91: 3426- 3431.
8. Ferlin A, Pepe A, Gianesello L, Garolla A, Feng S, Facciolli A, Morello R, Agoulnik AI and Foresta C. New roles for INSL3 in adults. **Ann N.Y. Acad** Sci. 2009; 1160:215- 218.

9. Jørgensen N, Liu F, Andersson AM, Vierula M, Irvine DS, Auger J, Brazil CK, Drobnis EZ, Jensen TK, Jouannet P, Overstreet JW, Redmon JB, Sparks A, Toppari J, Wang C, Skakkebæk NE and Swan SH. Serum inhibin- b in fertile men is strongly correlated with low but not high sperm counts: a coordinated study of 1.797 **European and US men. Fertil Steril**. 2010; 94:2128- 2134.
10. Khullar K, Agarwal A and Plessis SD: A Hormonal, Physical, and Proteomic View of Obesity- Induced Effects on Male Infertility and Possible Lifestyle Modifications. **Asian Pacific Journal of Reproduction** 2012; 160- 168.
11. Kasturi SS, Tannir J and Brannigan RE. The metabolic syndrome and male infertility. **J Androl**. 2008; 29(3): 251- 9.
12. Marshall WA and Tanner JM. Variations in the pattern of pubertal changes in boys. **Arch Dis Child**. 1970; 45:13- 23.
13. Pasquali, R. **"Obesity, fat distribution and infertility"** Maturitas 2006; 54(4): 363- 371.
14. Sadeghian H, Anand- Ivell R, Balvers M, Relan V and Ivell R. Constitutive regulation of the Insl3 gene in rat Leydig cells. **MolCell Endocrinol**. 2005; 241:10- 20.
15. Wikström AM, Bay K, Hero M, Andersson AM and Dunkel L. Serum insulin- like factor 3 levels during puberty in healthy boys and boys with klinefelter syndrome. **J Clin Endocrinol Metab** 2006; 91:4705- 8.
16. Weiner JS and Lourie JA. **Human Biology. A Guide to Field Methods**. Published for the International Biological Program.
17. Blackwell Scientific Publications. Oxford, Edinburgh. 1969. World Health Organization, 2002. Malnutrion. **Nutrition for Health and development**, pp: 11- 12.
18. Wagner IV, Sabin MA and Pfäffle RW, Hiemisch A, Sergeyev E, Körner A, Kiess W. Effects of obesity on human sexual development. **Nat Rev Endocrinol**.