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Inhibin-B Levels in Healthy Young Adult Men and Prepubertal Boys: Is Obesity the Cause for the Contemporary Decline in Sperm Count Because of Fewer Sertoli Cells?

STEPHEN J. WINTERS,*† CHENXI WANG,* EIMAN ABDELRAHAMAN,* VENUS HADEED,† MARY ANN DYKY,†‡ AND ADAM BRUFSKY†

*From the *Department of Medicine, University of Louisville, Louisville, Kentucky; and the †Department of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania.*

ABSTRACT: Inhibin-B is a heterodimeric glycoprotein produced by Sertoli cells. Although inhibin-B levels are low when seminiferous tubules are damaged, studies in normal monkeys reveal that inhibin-B levels also correlate positively with Sertoli cell number. In this study, we measured inhibin-B levels in healthy young adult men aged 18–24 years and in prepubertal boys aged 5–9 years in relation to body mass index (BMI). Inhibin-B levels declined with increasing obesity in young adult men; values were 26% lower in men who were obese compared to normal-weight men. Sex hormone–binding globulin and total testosterone, but not free testosterone, were also lower with increasing BMI; serum follicle-stimulating hormone and luteinizing hormone levels were unaffected by obesity. In prepubertal boys, by contrast, inhibin-B was unaffected by obesity. We propose that reduced levels of inhibin-B indicate that obese men have fewer Sertoli cells than men of normal weight. Moreover, normal values in obese prepubertal boys suggest that the effect of obesity on inhibin-B is established during puberty. Finally, because each Sertoli cell is thought to support a finite number of germ cells, fewer Sertoli cells in obesity may predispose to a lower sperm count in adulthood. We speculate that the escalating prevalence of obesity and insulin resistance among adolescents might negatively influence male reproductive function for the next generation.

Key words: Male infertility, testis, puberty.

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Many studies have reported a decline in semen quality over the past 60 years (Sharpe, 1993; Jouannet et al, 2001). The explanation most offered for this trend has been the introduction into the environment of chemical disruptors with the properties of estrogens, androgens, or antiandrogens that might exert deleterious effects on pituitary testicular function (Akingbemi and Hardy, 2001). But whether the trace amounts of substances that migrate from commercial products into the environment truly influence male reproductive health remains unproven (Sharpe and Irvine, 2004).

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Correspondence to: Dr Stephen J Winters, Division of Endocrinology, Metabolism and Diabetes, University of Louisville, ACB-A3G11, 550 Jackson St, Louisville, KY, 40202 (e-mail: sjwint01@louisville.edu).

Present address: Genentech, Inc, So. San Francisco, CA.

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Sertoli cells provide developing germ cells with structural and hormonal support (Mruk and Cheng, 2004). Experiments in newborn rats in which Sertoli cell proliferation was suppressed by treatment with cytosine arabinoside produced adult rats with a parallel reduction in round spermatids, whereas the number of spermatids per Sertoli cell was unaffected (Orth et al, 1988). These observations and others have led to the idea that the number of germ cells in adulthood is related directly to the number of functional Sertoli cells (Sharpe et al, 2003).

In humans, only about 10% of the adult complement of 4 billion Sertoli cells is present at birth (Cortes et al, 1987). Controlled studies in the nonhuman primate revealed that the number of Sertoli cells increases between the neonatal and the juvenile period, with a further increase during puberty (Simorangkir et al, 2003). Both the neonatal and the pubertal increase in Sertoli cell number are paralleled by a rise in circulating levels of inhibin-B (Andersson et al, 1998; Winters and Plant, 1999), and in normal adult rhesus monkeys, inhibin-B is strongly positively correlated with the number of Sertoli cells (Ramaswamy et al, 1999).

Obesity is known to suppress adult Leydig cell function (Vermeulen, 1996). In this study, we measured serum inhibin-B levels in 2 populations of normal male

volunteers, prepubertal boys and young adult men, and have related inhibin-B to measures of overweight. From our findings, we propose that obesity suppresses Sertoli cell proliferation during puberty, and hypothesize that the rising prevalence of obesity has contributed to the contemporary decline in sperm production.

Materials and Methods

Study Subjects

Blood samples were analyzed from 2 cross-sectional studies that were designed originally to examine racial differences in circulating androgens and sex hormone–binding globulin (SHBG). One study enrolled 74 African American and Caucasian young adult men ages 18–24 years who were recruited by advertisement from the undergraduate and graduate students at the University of Pittsburgh (Winters et al, 2001). Subjects with any active medical illness, history of gonadal dysfunction, daily use of alcohol, or status as elite athletes were excluded. The second study, conducted in Louisville, Ky, enrolled 48 African American and Caucasian boys between the ages of 5 and 9 years at the time of a routine school physical examination (Abdelrahaman et al, 2005). No subject had a chronic illness or was taking any regular medication. A physical examination that included height, weight, blood pressure, waist and hip circumference, and Tanner staging was performed, and a blood sample was obtained between 0830 and 1030 hours. Informed consent was obtained according to protocols approved by the Institutional Review Boards of the University of Pittsburgh and the University of Louisville.

Outcome Measures

In each study, testis size was estimated by 1 examiner using an orchidometer. Inhibin-B levels were measured with an Inhibin-B ELISA kit from DSLabs (Webster, Tex). The minimal detectable dose was 15 pg/mL, and the within- and between assay coefficients of variation were less than 7.5% and 14.8%, respectively. Serum levels of testosterone, SHBG, estradiol, luteinizing hormone (LH), and follicle-stimulating hormone (FSH) were measured using established immunoassays described previously (Abdelrahaman et al, 2005). Free testosterone was calculated from the levels of total testosterone and SHBG (Sodergard et al, 1982). Body mass index (BMI) was calculated as the ratio of weight (kg) to the square of height (m), and was plotted onto the BMI growth curve for boys (www.cdc.gov/growthcharts) to determine the BMI percentile. Boys were categorized as overweight if BMI percentile was greater than or equal to 85, or obese if BMI percentile was greater than or equal to 95.

Statistical Analysis

Data are presented as mean ± SEM. Mean hormone concentrations in normal-weight (BMI < 25 kg/m^2), overweight (BMI 25–30 kg/m^2), and obese (BMI > 30 kg/m^2) men were compared by analysis of variance and Tukey's test. Scattergrams of inhibin-B were plotted by BMI, and subsequent Pearson correlation coefficients were computed to assess any linear relation between the variables. Inhibin-B was also regressed on BMI adjusting for age.

Results

Of the 72 young adult men studied, 29% were overweight and 11% were obese. The Table compares the hormone levels in these men stratified according to BMI. As in many previous studies (Glass et al, 1981), SHBG levels decreased with increasing BMI. Total testosterone levels were slightly lower in obese men, but the difference did not achieve statistical significance. Inhibin-B levels declined with increasing BMI, and the levels in obese men were 26% less ($P < .05$) than those of normal-weight men. Serum free testosterone, LH, FSH, and estradiol were unaffected by BMI.

The scattergram in Figure 1 shows that a high BMI was associated with a reduced level of inhibin-B ($r = -.226$), although low levels were also found among normal-weight men. A plot of inhibin-B levels versus waist circumference produced a similar result ($r = -.23$; not shown). For the group as a whole, the level of inhibin-B decreased 4.57 pg per unit of BMI increase (P

*Endocrine profiles in healthy young men in relation to body mass index**

	BMI, kg/m^2		
	<25 (n = 43)	25–30 (n = 21)	>30 (n = 8)
Testosterone (ng/dL)	423 ± 20	423 ± 66	353 ± 33
Free testosterone (pmol/L)	164 ± 11	195 ± 18	170 ± 31
SHBG (nmol/L)	88.6 ± 5.3	76.3 ± 8.0	63.2 ± 4.6†
Inhibin-B (pg/mL)	248 ± 83	231 ± 98	183 ± 46†
FSH (mIU/mL)	2.38 ± 0.15	2.54 ± 0.30	2.62 ± 0.25
LH (mIU/mL)	8.3 ± 0.44	7.31 ± 0.52	8.9 ± 0.98
Estradiol (pg/mL)	14.4 ± 1.4	15.8 ± 2.3	15.4 ± 2.6

* Data are mean ± SEM. BMI indicates body mass index; SHBG, sex hormone–binding globulin; FSH, follicle-stimulating hormone; and LH, luteinizing hormone.

† $P < .05$ vs normal-weight men, ANOVA and Fischer's test.

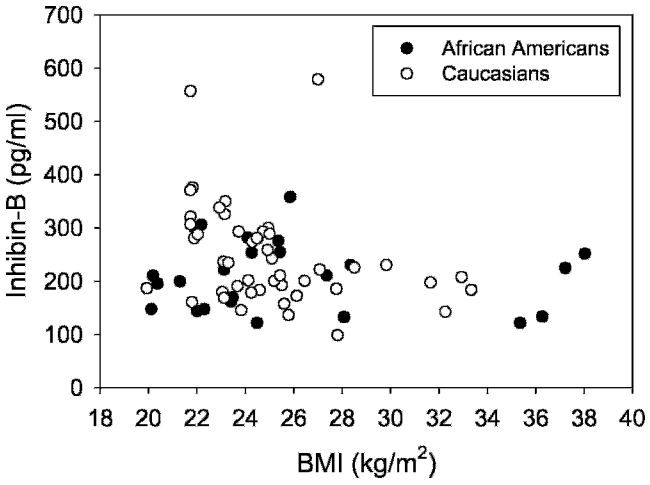

Figure 1. Relationship between body mass index and inhibin-B in 72 healthy young adult men.

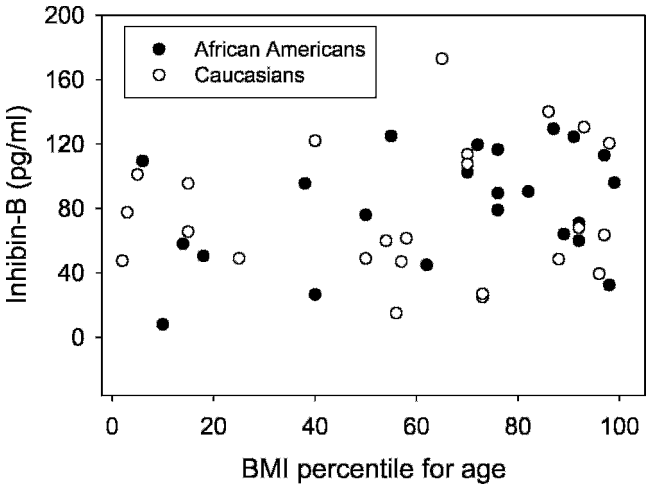

Figure 2. Relationship between BMI and inhibin-B in 48 healthy young boys aged 5–9 years. BMI was calculated as the ratio of weight (kg) to the square of height (m), and was plotted onto the BMI growth curve for boys (www.cdc.gov/growthcharts) to determine the BMI percentile for age.

= .075). In whites, the BMI effect was more pronounced (inhibin-B decreased 10.00 pg per unit of BMI increase, $P = .03$) whereas in blacks the effect was not significant ($P = .63$).

The 2000 Centers for Disease Control and Prevention BMI-for-Age Growth Chart was used to define boys as overweight or obese; according to this classification, 10 boys (21.7%) were overweight and 5 boys (10.9%) were obese. Inhibin-B levels in the 5 obese boys (74 ± 13 pg/mL) were comparable to the values in boys whose BMI was below the 95th percentile for age (79 ± 6 pg/mL). Figure 2 shows that inhibin-B levels in boys tend to rise ($r = .21$) with increasing BMI adjusted for age. There was no difference between African Americans and Caucasians. Furthermore, no relationship between inhibin-B and BMI ($r = .09$) or waist circumference ($r = -.09$) was found in boys (not shown).

Discussion

In order to study the impact of obesity on Sertoli cells in normal children and young adults, in whom testicular biopsy cannot be performed for research purposes, we measured the levels of inhibin-B in serum. The results reveal that inhibin-B levels are lower in obese young adult men than in normal-weight men, whereas inhibin-B is unrelated to BMI among prepubertal boys. The variable relationship observed between inhibin-B and BMI among African American and Caucasian men is intriguing, and could be a consequence of racial differences in adipose tissue distribution (Hill et al, 1999), but conclusions will require confirmation in a larger study population.

Inhibin-B levels vary fourfold among fertile men (Andersson et al, 2004), but the determinants of this between-subject variability have not been defined. There is no major moment-to-moment variation in circulating inhibin-B, although some men exhibit a diurnal variation that parallels that of testosterone (Carlsen et al, 1999). All blood samples in this study were drawn in the morning. Inhibin-B levels rise twofold to threefold during puberty to plateau by pubertal stage II–III (Andersson and Skakkebaek, 2001). Because inhibin-B levels are very low in men with congenital hypogonadotropic hypogonadism (Anawalt et al, 1996; Seminara et al, 1996), and increase as Sertoli cell number increases in juvenile monkeys administered gonadotropin-releasing hormone (GnRH), FSH, or LH (Marshall and Plant, 1996; Majumdar et al, 1997), the rise in inhibin-B during puberty is presumably attributable to Sertoli cell proliferation with increased GnRH-LH-FSH activation. From these findings, and the strong positive correlation between inhibin-B with the number of Sertoli cells (Ramaswamy et al, 1999), we propose that lower levels of inhibin-B among obese young adult men, together with unchanged values in boys, reflect suppressed Sertoli cell proliferation during puberty.

Although inhibin-B levels were reduced in obese men, FSH levels were normal. Inhibin functions to down-regulate the level of expression of the FSH-β gene (Attardi et al, 1989). Accordingly, serum FSH levels tend to be elevated when the testes are damaged and circulating inhibin-B is reduced (Jensen et al, 1997). The finding of normal FSH levels in obese men in this study could reflect a limitation in study design, because LH and FSH are released into the circulation in pulses, and the single blood samples obtained may have provided insufficiently accurate information to identify small

between-group differences. On the other hand, FSH levels are reduced in morbidly obese men (Strain et al, 2003), and the mechanisms responsible for that difference may have prevented a rise in FSH. Gonadotropin insufficiency is unlikely to have caused the low levels of inhibin-B, however, because LH and free testosterone as well as FSH were normal. Furthermore, inhibin-B levels in normal men declined only slightly during long-term gonadotropin suppression by testosterone (Matthiesson et al, 2003) or testosterone together with medroxyprogesterone acetate (McLachlan et al, 2002).

Low testosterone is an established consequence of obesity in adult men (Vermeulen, 1996), and is partly explained by a low level of SHBG (Glass et al, 1981). Free testosterone declines with extreme obesity, although the mechanisms for this decline are not well understood. SHBG and total testosterone were lower in the moderately obese subjects (BMI 31–38 kg/m^2) in this study, whereas free testosterone was normal. Mean LH levels (Strain et al, 2003) and LH pulse amplitude tend to decline in extremely obese adult men (BMI $>$ 40 kg/m^2), whereas LH pulse frequency was reported to be normal (Giagulli et al, 1994). Decreased LH pulse amplitude could reflect less GnRH secreted per burst or reduced pituitary responsiveness to GnRH; however, reports of a normal response to administered GnRH (Glass et al, 1977) favor the former mechanism. Like free testosterone, LH levels were normal in the moderately obese subjects in our study.

Little information is so far available concerning the reproductive consequences of obesity in men. In a study from Argentina, 40% of men attending an infertility clinic were overweight (Oliva et al, 2001). In a recent study of military recruits from Denmark, men with BMI exceeding 25 kg/m^2 had a 23.9% lower total sperm count than men with a BMI of 20–25. Semen volume and the percentage of motile sperm were, on the other hand, unrelated to BMI. Serum FSH and inhibin-B levels, as well as SHBG and total testosterone, also decreased with increasing BMI in that study (Jensen et al, 2004). In a preliminary study, Pauli et al (2004) reported an inverse correlation between BMI and both FSH and inhibin-B, but no relation of BMI to the parameters of the semen analysis. In an earlier study of semen samples from 16 men who were 52%–332% above ideal body weight, the authors concluded that spermatogenesis in obese men was not different from that of historical controls (Strain et al, 1982). Globerman et al (2005) recently reported low levels of inhibin-B in some morbidly obese men that failed to rise after gastroplasty, although weight loss was associated with a rise in testosterone. Their findings support the notion that inhibin-B levels are a surrogate marker for Sertoli cell number.

The prevalence of obesity among US adolescents has increased from 5% to 15.5% between the 1960s and 1999–2001 (Ogden et al, 2002). If our interpretation of the data is correct, the negative impact of obesity on Sertoli cell proliferation during puberty might substantially compromise male reproductive function for the next generation.

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References

Abdelrahaman E, Raghavan S, Baker L, Weinrich M, Winters SJ. Racial difference in circulating sex hormone-binding globulin levels in prepubertal boys. *Metabolism*. 2005;54:91–96.

Akingbemi BT, Hardy MP. Oestrogenic and antiandrogenic chemicals in the environment: effects on male reproductive health. *Ann Med*. 2001;33:391–403.

Anawalt BD, Bebb RA, Matsumoto AM, Groome NP, Illingworth PJ, McNeilly AS, Bremner WJ. Serum inhibin B levels reflect Sertoli cell function in normal men and men with testicular dysfunction. *J Clin Endocrinol Metab*. 1996;81:3341–3345.

Andersson AM, Muller J, Skakkebaek NE. Different roles of prepubertal and postpubertal germ cells and Sertoli cells in the regulation of serum inhibin B levels. *J Clin Endocrinol Metab*. 1998;83:4451–4458.

Andersson AM, Petersen JH, Jorgensen N, Jensen TK, Skakkebaek NE. Serum inhibin B and follicle-stimulating hormone levels as tools in the evaluation of infertile men: significance of adequate reference values from proven fertile men. *J Clin Endocrinol Metab*. 2004;89:2873–2879.

Andersson AM, Skakkebaek NE. Serum inhibin B levels during male childhood and puberty. *Mol Cell Endocrinol*. 2001;180:103–107.

Attardi B, Keeping HS, Winters SJ, Kotsuji F, Maurer RA, Troen P. Rapid and profound suppression of messenger ribonucleic acid encoding follicle-stimulating hormone beta by inhibin from primate Sertoli cells. *Mol Endocrinol*. 1989;3:280–287.

Carlsen E, Olsson C, Petersen JH, Andersson AM, Skakkebaek NE. Diurnal rhythm in serum levels of inhibin B in normal men: relation to testicular steroids and gonadotropins. *J Clin Endocrinol Metab*. 1999;84:1664–1669.

Cortes D, Muller J, Skakkebaek NE. Proliferation of Sertoli cells during development of the human testis assessed by stereological methods. *Int J Androl*. 1987;10:589–596.

Giagulli VA, Kaufman JM, Vermeulen A. Pathogenesis of the decreased androgen levels in obese men. *J Clin Endocrinol Metab*. 1994;79:997–1000.

Glass AR, Burman KD, Dahms WT, Boehm TM. Endocrine function in human obesity. *Metabolism*. 1981;30:89–104.

Glass AR, Swerdloff RS, Bray GA, Dahms WT, Atkinson RL. Low serum testosterone and sex-hormone-binding-globulin in massively obese men. *J Clin Endocrinol Metab*. 1977;45:1211–1219.

Globerman H, Shen-Orr Z, Karnieli E, Aloni Y, Charuzi I. Inhibin B in men with severe obesity and after weight reduction following gastroplasty. *Endocr Res*. 2005;31:17–26.

Hill JO, Sidney S, Lewis CE, Tolan K, Scherzinger AL, Stamm ER. Racial differences in amounts of visceral adipose tissue in young adults: the CARDIA (Coronary Artery Risk Development in Young Adults) study. *Am J Clin Nutr*. 1999;69:381–387.

Jensen TK, Andersson AM, Hjollund NH, Scheike T, Kolstad H, Giwercman A, Henriksen TB, Ernst E, Bonde JP, Olsen J, McNeilly A, Groome NP, Skakkebaek NE. Inhibin B as a serum marker of spermatogenesis: correlation to differences in sperm concentration and follicle-stimulating hormone levels. A study of 349 Danish men. *J Clin Endocrinol Metab*. 1997;82:4059–4063.

Jensen TK, Andersson AM, Jorgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE. Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril*. 2004;82:863–870.

Jouannet P, Wang C, Eustache F, Kold-Jensen T, Auger J. Semen quality and male reproductive health: the controversy about human sperm concentration decline. *Apmis*. 2001;109:333–344.

Majumdar SS, Winters SJ, Plant TM. A study of the relative roles of follicle-stimulating hormone and luteinizing hormone in the regulation of testicular inhibin secretion in the rhesus monkey (Macaca mulatta). *Endocrinology*. 1997;138:1363–1373.

Marshall GR, Plant TM. Puberty occurring either spontaneously or induced precociously in rhesus monkey (Macaca mulatta) is associated with a marked proliferation of Sertoli cells. *Biol Reprod*. 1996;54:1192–1199.

Matthiesson KL, Robertson DM, Burger HG, McLachlan RI. Response of serum inhibin B and pro-alphaC levels to gonadotrophic stimulation in normal men before and after steroidal contraceptive treatment. *Hum Reprod*. 2003;18:734–743.

McLachlan RI, O'Donnell L, Stanton PG, Balourdos G, Frydenberg M, de Kretser DM, Robertson DM. Effects of testosterone plus medroxyprogesterone acetate on semen quality, reproductive hormones, and germ cell populations in normal young men. *J Clin Endocrinol Metab*. 2002;87:546–556.

Mruk DD, Cheng CY. Sertoli-Sertoli and Sertoli-germ cell interactions and their significance in germ cell movement in the seminiferous epithelium during spermatogenesis. *Endocr Rev*. 2004;25:747–806.

Ogden CL, Flegal KM, Carroll MD, Johnson CL. Prevalence and trends in overweight among US children and adolescents, 1999–2000. *JAMA*. 2002;288:1728–1732.

Oliva A, Spira A, Multigner L. Contribution of environmental factors to the risk of male infertility. *Hum Reprod*. 2001;16:1768–1776.

Orth JM, Gunsalus GL, Lamperti AA. Evidence from Sertoli cell-depleted rats indicates that spermatid number in adults depends on numbers of Sertoli cells produced during perinatal development. *Endocrinology*. 1988;122:787–794.

Pauli EM, Legro RS, Dodson WC, Kunselman AR, Lee PA. Diminished paternity and gonadal function with increasing obesity in normal males. In: *86th Annual Meeting of the Endocrine Society*. New Orleans, Louisiana, 2004:P3–P508.

Ramaswamy S, Marshall GR, McNeilly AS, Plant TM. Evidence that in a physiological setting Sertoli cell number is the major determinant of circulating concentrations of inhibin B in the adult male rhesus monkey (*Macaca mulatta*.). *J Androl*. 1999;20:430–434.

Seminara SB, Boepple PA, Nachtigall LB, Pralong FP, Khoury RH, Sluss PM, Lecain AE, Crowley WF Jr. Inhibin B in males with gonadotropin-releasing hormone (GnRH) deficiency: changes in serum concentration after shortterm physiologic GnRH replacement—a clinical research center study. *J Clin Endocrinol Metab*. 1996;81:3692–3696.

Sharpe RM. Declining sperm counts in men—is there an endocrine cause? *J Endocrinol*. 1993;136:357–360.

Sharpe RM, Irvine DS. How strong is the evidence of a link between environmental chemicals and adverse effects on human reproductive health? *BMJ*. 2004;328:447–451.

Sharpe RM, McKinnell C, Kivlin C, Fisher JS. Proliferation and functional maturation of Sertoli cells, and their relevance to disorders of testis function in adulthood. *Reproduction*. 2003;125:769–784.

Simorangkir DR, Marshall GR, Plant TM. Sertoli cell proliferation during prepubertal development in the rhesus monkey (*Macaca mulatta*) is maximal during infancy when gonadotropin secretion is robust. *J Clin Endocrinol Metab*. 2003;88:4984–4989.

Sodergard R, Backstrom T, Shanbhag V, Carstensen H. Calculation of free and bound fractions of testosterone and estradiol-17 beta to human plasma proteins at body temperature. *J Steroid Biochem*. 1982;16:801–810.

Strain GW, Zumoff B, Kream J, Strain JJ, Deucher R, Rosenfeld RS, Levin J, Fukushima DK. Mild hypogonadotropic hypogonadism in obese men. *Metabolism*. 1982;31:871–875.

Strain GW, Zumoff B, Miller LK, Rosner W. Sex difference in the effect of obesity on 24-hour mean serum gonadotropin levels. *Horm Metab Res*. 2003;35:362–366.

Vermeulen A. Decreased androgen levels and obesity in men. *Ann Med*. 1996;28:13–15.

Winters SJ, Brufsky A, Weissfeld J, Trump DL, Dyky MA, Hadeed V. Testosterone, sex hormone-binding globulin, and body composition in young adult African American and Caucasian men. *Metabolism*. 2001;50:1242–1247.

Winters SJ, Plant TM. Partial characterization of circulating inhibin-B and pro-alpha C during development in the male rhesus monkey. *Endocrinology*. 1999;140:5497–5504.