

ORIGINAL ARTICLE

Body mass index, waist-to-hip ratio, waist circumference and waist-to-height ratio cannot predict male semen quality: a report of 1231 subfertile Chinese menJ.-C. Lu^{1,4}, J. Jing¹, J.-Y. Dai², A. Z. Zhao³, Q. Yao¹, K. Fan¹, G.-H. Wang¹, Y.-J. Liang¹, L. Chen¹, Y.-F. Ge¹ & B. Yao¹

1 Reproductive Medical Center, Nanjing Jinling Hospital, Nanjing University School of Medicine, Nanjing, China;

2 Key Laboratory of Animal Ecology and Conservation Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing, China;

3 Department of Gerontology, the First Affiliated Hospital, and the Center of Metabolic Disease Research, Nanjing Medical University, Nanjing, China;

4 Department of Laboratory Science, Nanjing Hospital, Jiangsu Corps, The Armed Police Force, PLA, Nanjing, China

Keywords

Obesity—reproductive hormone—semen parameter

CorrespondenceDr Bing Yao, The Reproductive Medical Center, Nanjing Jinling Hospital, Nanjing University School of Medicine, 305 Zhongshan East Road, Nanjing 210002, China.
Tel.: +86 25 8086 0174;

Fax: +86 25 8086 0174;

E-mail: 2424572228@qq.com

and

Dr Jin-Chun Lu, Department of Laboratory Science, Nanjing Hospital, Jiangsu Corps, The Armed Police Force, PLA, 256 He Yan Lu, Nanjing 210028, Jiangsu, China.
Tel.: +86 25 8561 8685;

Fax: +86 25 8555 2551;

E-mail: lujc@androl.cn

J.-C Lu, J. Jing and J.-Y Dai contributed equally to this work.

Accepted: September 23, 2014

doi: 10.1111/and.12376

Introduction

Overweight and obesity have become a major public health concern worldwide. The prevalence of overweight and obesity is increasing at an alarming rate. Increased body weight has been associated with a higher frequency of adverse health consequences including hypertension, cardiovascular disease, metabolic disorders, osteoarthritis, gallbladder stone disease, asthma as well as multiple malignancies (Chavarro *et al.*, 2010). Subfertility is also a severe health concern, which has affected at least 10% of population in developed countries (MacDonald *et al.*,

Summary

There were controversial results between obesity-associated markers and semen quality. In this study, we investigated the correlations between age, obesity-associated markers including body mass index (BMI), waist-to-hip ratio (WHR), waist-to-height ratio (WHtR) and waist circumference (WC), the combination of age and obesity-associated markers, semen parameters and serum reproductive hormone levels in 1231 subfertile men. The results showed that BMI, WC, WHR and WHtR were positively related to age, and there were also positive relations between BMI, WHR, WC and WHtR and between sperm concentration (SC), total sperm count (TSC), progressive motility (PR), sperm motility and per cent of normal sperm morphology (NSM). However, age, each of obesity-associated markers and the combination of obesity-associated markers and age were unrelated to any of semen parameters including total normal-progressively motile sperm count (TNPMS). Age, BMI, WHR, WC and WHtR were negatively related to serum testosterone and SHBG levels. However, only serum LH and FSH levels were negatively related to sperm concentration, NSM and sperm motility. In a conclusion, although age and obesity have significant impacts on reproductive hormones such as testosterone, SHBG and oestradiol, semen parameters related to FSH and LH could not be influenced, indicating that obesity-associated markers could not predict male semen quality.

2010). It was reported that obesity was associated with lower fertility (Cabler *et al.*, 2010). Excess body weight in women has been associated with an increased rate of polycystic ovary syndrome, menstrual cycle disturbances, infertility, miscarriage and multiple complications of pregnancy including gestational diabetes, pre-eclampsia, macrosomic foetus and Caesarean delivery. However, the reproductive consequences of excess body weight in men have been poorly understood. Most studies have been focused on the association between body mass index (BMI) and semen parameters; however, their results remained controversial (Macdonald *et al.*, 2013).

Moreover, the markers used to reflect obesity include not only BMI, but also waist circumference (WC), waist-to-hip ratio (WHR) and waist-to-height ratio (WHtR). All these markers have also been used in clinical and epidemiological studies (Shen *et al.*, 1990; Visscher *et al.*, 2001; Feldstein *et al.*, 2005; Nyamdorj *et al.*, 2008).

Several studies (Li *et al.*, 2009; Paasch *et al.*, 2010; Rybar *et al.*, 2011; Eskandar *et al.*, 2012) showed that semen quality was influenced by age, that is, sperm concentration and motility significantly decreased with increasing age. In this study, we observed the correlations of all of obesity-associated markers including BMI, WC, WHR and WHtR with each of semen parameters. We further analysed the impact of obesity-associated markers, in combination with age, on semen parameters. Semen volume, sperm concentration, total sperm count (TSC), sperm motility, progressive motility (PR) and per cent of normal sperm morphology just can reflect one aspect of semen quality. However, the determinative factor for male fertility is the spermatozoa with motility and normal morphology. Therefore, we introduced a new parameter – total normal-progressively motile sperm count (semen volume \times sperm concentration \times progressive motility \times per cent of normal sperm morphology, TNPMS), and further explored the correlations of each of obesity-associated markers and the combination of obesity-associated markers and age with TNPMS.

It was suggested that obesity leads to the change of semen quality by the most possible way to affect reproductive hormones (Chavarro *et al.*, 2010; MacDonald *et al.*, 2010; Tunc *et al.*, 2011; Macdonald *et al.*, 2013; Sermondade *et al.*, 2013). Therefore, we further analysed the relations of obesity-related markers with serum reproductive hormone levels and the relations of reproductive hormone levels with standard semen analysis parameters, thus accumulated the evidence for understanding the relationships of obesity-associated markers, reproductive hormones and semen quality.

Materials and methods

Study population

Subfertile men, aged from 18 to 55 years and whose partners had not conceived within 12 months after stopping use of contraception, were from the outpatient clinic at Nanjing Jingling Hospital between August 2012 and February 2014. All participants were asked to complete a questionnaire to provide information on occupation, medical and reproductive history and lifestyle factors including intake of alcohol and smoking history. Then, all participants underwent physical examination, and obesity-associated markers were measured, semen samples

were collected, and venous blood were drawn during 8:00 am and 10:00 am. We employed stringent exclusion criteria, excluding regular alcohol drinkers, heavy smokers and men with chronic diseases, urogenital infections, varicocele and other diseases which might lead to dyspermia. Thousand two hundred and thirty-one men were enrolled in this study. This study was approved by the Human Subject Committees of Nanjing Jinling Hospital, and informed consent was obtained from all participants.

Measurement of obesity-associated markers

Height and weight were measured with the participants standing without shoes and heavy outer garments. Waist circumference was measured at the level midway between the lower rib margin and the iliac crest with participants in standing position without heavy outer garments and with emptied pockets, breathing out gently. Hip circumference was recorded as the maximum circumference over the buttocks. BMI was calculated as weight divided by height squared (kg m^{-2}). Waist-to-hip ratio (WHR) was calculated as the ratio of waist circumference over the hip circumference. Waist-to-height ratio (WHtR) was calculated as the ratio of waist circumference over height.

Criteria for obesity

With regard to the current Chinese men criteria (Cai *et al.*, 2013), a BMI under 18.5 kg m^{-2} was considered underweight; BMI between 18.5 and 23.99 kg m^{-2} as normal weight; BMI between 24 and 27.99 kg m^{-2} as overweight; and BMI $\geq 28 \text{ kg m}^{-2}$ as obesity. Generalised obesity and abdominal obesity were defined using WHO Asia Pacific guidelines with WC cut-off as ≥ 90 cm (Jia *et al.*, 2003), WHR cut-off as ≥ 0.9 (Alberti & Zimmet, 1998), and WHtR cut-off as 0.5 (Raman *et al.*, 2010; Cai *et al.*, 2013).

Analysis of semen parameters

Semen specimens were collected after a period of 2–7 days of sexual abstinence and were allowed to liquefy for 30 min at 37°C . After liquefaction, semen volume was measured by weighing the sample, sperm concentration, total motility and progressive motility were analysed by computer-aided sperm analysis (CASA) system (CFT-9201; Jiangsu Rich Life Science Instrument Co., Ltd., Nanjing, China) (Lu *et al.*, 2014), and sperm morphology was evaluated using Diff-Quik staining. For each specimen, at least 200 spermatozoa were analysed in each replicate. If the difference between the two replicates was acceptable (within 95% confidence interval), report the average. If the difference was too high, take two new

aliquots from the semen sample and repeat the assessment (World Health Organization, 2010). Then, TNPMS was calculated. The criteria for oligozoospermia, asthenozoospermia and teratozoospermia were in accordance with the World Health Organization guidelines (World Health Organization, 2010).

Determination of serum reproductive hormones

A nonfasting blood sample was drawn the same day that the semen sample was produced. Blood was centrifuged and serum was stored at -80°C until analysis. Sera were then thawed and analysed for total testosterone (TT), luteinising hormone (LH), follicle-stimulating hormone (FSH), oestradiol (E2) and sex hormone-binding globulin (SHBG) levels. TT, LH, FSH, E2 and SHBG levels were determined by chemiluminescence assay using an automated Unicel Dxi 800 Access Immunoassay System (Beckman Coulter, Inc., USA). The assay sensitivities were 0.35 nmol l^{-1} for TT, 0.2 IU l^{-1} for FSH, 0.2 IU l^{-1} for FSH, 73 pmol l^{-1} for E2 and 0.33 nmol l^{-1} for SHBG. The intra-assay coefficients of variation (CV) for LH, FSH, TT, E2 and SHBG were all <5%, and the inter-assay CVs were all <8%. Then, the ratio of TT and E2 (TT/E2) was calculated.

Statistical analysis

All analyses were conducted using SPSS 11.0 software (SPSS Inc., Chicago, IL, USA). First, nonparametric tests (one-sample Kolmogorov–Smirnov test) were used to determine whether analysed parameters were normally distributed. If the parameter was consistent with normal distribution, correlations between and within obesity-associated markers, semen parameters and reproductive hormone levels were examined by Pearson test. If the parameter was consistent with non-normal distribution, correlations were examined by Spearman's rho test. The differences between categories were assessed by one-way ANOVA test or LSD *t*-test. The differences between two groups with different number of samples were analysed by independent-samples *t*-test. *P*-value < 0.05 was considered statistically significant.

Results

Correlations of obesity-associated markers and the combination of both obesity-associated markers and age with semen parameters

Of the 1231 subfertile men recruited into the study, 26 azoospermic men, 12 men with 100% of spermatia, 15 men with 100% of teratospermia and 46 men with incomplete data were excluded, and 1 132 men were

enrolled in study. Clinical and semen test results for 1132 subfertile men were summarised in Table 1. All these parameters were consistent with non-normal distribution, so correlations between these parameters were analysed by Spearman's rho test, and obtained results showed in Table 2.

We further observed the correlations between the combination of both obesity-associated markers and age and each of semen parameters, especially TNPMS. The results showed that there was no any correlation between the combination of each of obesity-associated markers and age and all semen parameters ($P > 0.05$).

Dichotomised analyses for obesity-associated markers, semen parameters and the combination of both obesity-associated markers and age

On the basis of BMI, men were grouped as underweight ($<18.5\text{ kg m}^{-2}$), normal ($18.5\text{--}23.99\text{ kg m}^{-2}$), overweight ($24\text{--}27.99\text{ kg m}^{-2}$) and obese ($\geq 28\text{ kg m}^{-2}$). The results showed that sperm concentration and TSC in underweight and obese men were lower than that in normal

Table 1 Clinical, semen and reproductive hormone test results for subfertile men

Variable	<i>n</i>	Mean (SD)	Range
Age (years)	1132	29.07 (4.83)	18–55
BMI (kg m^{-2})	1132	23.90 (3.01)	15.56–41.03
WHR	1132	0.85 (0.056)	0.70–1.13
WC (cm)	1132	83.33 (8.95)	59–131
WHtR	1132	0.48 (0.051)	0.34–0.74
BMI \times age	1132	697.31 (156.66)	363.32–1484.43
WHR \times age	1132	24.86 (4.93)	13.32–48.56
WC \times age	1132	24.34 (5.39)	12.60–50.50
WHtR \times age	1132	14.00 (3.12)	6.77–29.51
Sperm concentration (10^6 per ml)	1132	72.91 (60.39)	0.32–445.13
Total sperm count (10^6 per ejaculate)	1132	234.70 (201.53)	1.35–1899.30
Progressive motility (%)	1132	30.68 (12.39)	1.00–96.99
Sperm motility (%)	1132	45.50 (19.48)	1.43–97.59
Normal sperm morphology (%)	1132	5.42 (2.27)	0.41–17.84
TNPMS (10^6 per ejaculate)	1132	4.25 (4.90)	0.01–46.69
LH (IU l^{-1})	287	5.02 (3.05)	1.07–33.86
FSH (IU l^{-1})	287	5.02 (2.77)	1.46–25.06
TT (nmol l^{-1})	287	15.36 (7.34)	4.16–105.00
E2 (pmol l^{-1})	287	101.58 (53.18)	6.00–480.00
TT (pmol l^{-1})/E2 (pmol l^{-1})	287	229.65 (515.17)	49.17–7358.09
SHBG (nmol l^{-1})	287	31.05 (15.17)	6.73–137.10

TNPMS, total normal-progressively motile sperm count; SHBG, sex hormone-binding globulin.

Table 2 Nonparametric (Spearman) correlation coefficients for relationships between age, obesity-associated markers and semen parameters

Variable	Age	BMI	WHR	WC	WHtR	SC	TSC	PR	MOT	NSM
BMI	0.214 ^a									
WHR	0.314 ^a	0.596 ^a								
WC	0.289 ^a	0.796 ^a	0.823 ^a							
WHtR	0.322 ^a	0.788 ^a	0.853 ^a	0.955 ^a						
SC	-0.001	-0.002	-0.004	-0.008	0.000					
TSC	-0.012	-0.009	-0.016	-0.021	-0.020	0.901 ^a				
PR	-0.055	-0.042	0.016	0.049	0.041	-0.080 ^b	-0.078 ^b			
MOT	-0.050	0.052	0.027	0.060 ^b	0.055	0.208 ^a	0.179 ^a	0.906 ^a		
NSM	-0.044	-0.005	-0.010	-0.005	-0.003	0.415 ^a	0.406 ^a	0.076 ^b	0.234 ^a	
TNPMS	-0.036	0.014	0.002	0.007	0.008	0.773 ^a	0.841 ^a	0.323 ^a	0.546 ^a	0.644 ^a

SC, sperm concentration; TSC, total sperm count; PR, progressive motility; MOT, sperm motility; NSM, normal sperm morphology; TNPMS, total normal-progressively motile sperm count. ^a $P \leq 0.001$; ^b $P \leq 0.05$. BMI, WC, WHR and WHtR were positively related to age. There was a significantly positive relationship between BMI, WHR, WC and WHtR and between sperm concentration, total sperm count (TSC), sperm motility and normal sperm morphology, while a negative relationship between progressive motility (PR) and sperm concentration and TSC. There was also a suggestion of decreased sperm concentration, motility, PR, TSC, per cent of normal morphology and TNPMS with increasing age, but these results did not reach statistical significance. There was no any significant correlation between obesity-associated markers and semen parameters except a modest relationship between WC and sperm motility ($r = 0.060$, $P < 0.05$).

weight men but without significant difference and that there was no significant difference in sperm motility, normal sperm morphology and TNPMS between BMI categories. However, sperm progressive motility in underweight [(35.73 \pm 15.85)%] and obese [(32.84 \pm 12.68)%] men were significantly higher than that in normal weight [(29.82 \pm 11.70)%] men ($P < 0.05$).

Waist circumference was divided into two groups with the cut-off as ≥ 90 cm, and there was no significant differences for all semen parameters except sperm motility in obese men significantly higher than that in normal WC men [(48.19 \pm 20.18)% versus (44.69 \pm 19.20)%, $t = -2.562$, $P = 0.011$]. WHR and WHtR were also divided into two groups with the cut-offs as ≥ 0.9 and ≥ 0.5 , respectively, and there was no any statistical difference for all semen parameters between groups.

Based on the WHO5 standard (World Health Organization, 2010), we compared the differences of obesity-associated markers between oligospermia (sperm concentration $<15 \times 10^6$ per ml), asthenospermia (progressive motility $<32\%$) and teratospermia (per cent of normal sperm morphology $<4\%$) and their corresponding normal groups and found that there was no significant difference for all obesity-associated markers including BMI, WHR, WC and WHtR between groups.

Considering the combination effect of age and obesity-associated markers on semen quality, we further compared the differences of semen parameters dichotomised as the mean of the cross-product of obesity-associated markers and age. The results showed that there was no significant difference for each of semen parameters between groups.

Correlations of obesity-associated markers and the combination of both obesity-associated markers and age with serum reproductive hormone levels

We also determined serum reproductive hormone levels from 287 subfertile men, which were summarised in Table 1. The correlations between obesity-associated markers and serum reproductive hormone levels were showed in Table 3. The correlations between the combination of both obesity-associated markers and age and serum reproductive hormone levels were showed in Table 3.

Dichotomised analyses for obesity-associated markers

We also compared serum reproductive hormone levels based on the dichotomised analyses for BMI, WHR, WC and WHtR and found that overweight and obese men had significantly lower serum TT and SHBG levels than normal weight men (Table 4) and that obese men also had lower serum oestradiol level than normal weight men when dichotomised analyses for WC and WHtR.

Correlations between serum reproductive hormone levels and semen parameters

We analysed the correlations between semen parameters and serum reproductive hormone levels and found that serum LH and FSH levels were negatively related to sperm concentration, sperm motility, normal sperm morphology and TNPMS, but serum testosterone, oestradiol

Table 3 Correlation coefficients for relationships between age, obesity-associated markers and serum reproductive hormone levels

Variable	Age	BMI	WHR	WC	WHtR	LH	FSH	TT	E2	TT/E2	SHBG
BMI	0.283 ^a										
WHR	0.389 ^a	0.620 ^a									
WC	0.356 ^a	0.782 ^a	0.820 ^a								
WHtR	0.375 ^a	0.790 ^a	0.856 ^a	0.956 ^a							
LH	0.023	-0.038	-0.090	-0.070	-0.071						
FSH	0.012	-0.038	-0.014	-0.025	-0.026	0.329 ^a					
TT	-0.247 ^a	-0.368 ^a	-0.354 ^a	-0.411 ^a	-0.427 ^a	0.236 ^a	0.164 ^b				
E2	0.031	-0.046	-0.103	-0.076	-0.084	0.008	-0.120 ^b	0.233 ^a			
TT/E2	-0.159 ^b	-0.172 ^b	-0.111	-0.174 ^b	-0.171 ^b	0.155 ^b	0.202 ^a	0.347 ^a	-0.787 ^a		
SHBG	-0.058	-0.402 ^a	-0.315 ^a	-0.403 ^a	-0.418 ^a	0.163 ^b	0.124 ^b	0.555 ^a	0.069	0.270 ^a	
BMI × age						-0.019	-0.012	-0.390 ^a	-0.018	-0.198 ^a	-0.272 ^a
WHR × age						-0.002	-0.001	-0.335 ^a	-0.012	-0.175 ^b	-0.170 ^b
WC × age						-0.026	-0.007	-0.390 ^a	-0.021	-0.201 ^a	-0.260 ^a
WHtR × age						-0.019	-0.006	-0.394 ^a	-0.025	-0.197 ^a	-0.260 ^a

SHBG, sex hormone-binding globulin. ^a $P \leq 0.001$; ^b $P \leq 0.05$. Age was positively related to BMI, WHR, WC and WHtR. Age, BMI, WHR, WC and WHtR were inversely related to testosterone level and TT/E2. BMI, WHR, WC and WHtR were all negatively related to SHBG, but unrelated to LH, FSH and E2 levels. There was a positive relation between testosterone level and LH, FSH, E2 and SHBG levels and between SHBG level and LH and FSH levels, while a negative relation between FSH and E2 levels. The cross-product of all obesity-associated markers and age was significantly negatively related to serum testosterone and sex hormone-binding globulin levels, but not related to serum LH, FSH and oestradiol levels.

and SHBG levels unrelated to any of semen parameters (Table 5).

Discussion

Our data showed that BMI, WC, WHR and WHtR were positively related to age, indicating that adult men's obesity degree made more serious with increasing age. Likewise, age was slightly negatively related to all semen parameters, indicating that semen quality decreased with increasing age. However, the descending degree of semen quality in men of reproductive age (the age range of men in our study was 18–55 years old) was less serious than the incremental degree of obesity with increasing age. It was interesting that both the obesity-associated markers and the combination of obesity-associated markers and age were unrelated to semen parameters negatively but WC slightly positively related to sperm motility. Qin *et al.* (2007) found that compared with normal weight and overweight men, underweight men had lower semen quality, that is, decreased sperm concentration, total sperm count and per cent of normal sperm morphology (Jensen *et al.*, 2004) and thought that overweight might be a protective factor against lower sperm concentration and total sperm count (Qin *et al.*, 2007). Likewise, Chavarro *et al.* (2010) found that overweight men had slightly higher total progressively motile sperm count than normal weight men. Although Chavarro *et al.* (2010) thought that it might be an occasional result, we combined our results and other researchers' reports and deemed that in reproductive age, overweight men but not obesity might

be beneficial for male fertility. Dichotomised analyses for BMI suggested that progressive motility in underweight and obesity groups was significantly higher than that in normal weight group. Dichotomised analyses for WC showed no significant difference for all semen parameters except higher sperm motility in obese men than normal weight ($P = 0.011$). These results once again demonstrated that moderately overweight might be good for male fertility, and at least may improve sperm motility. Whether this phenomenon is associated with adequate energy metabolism of spermatozoa needs to be further investigated.

Our results showed that serum testosterone, SHBG and oestradiol levels and the ratio of testosterone and oestradiol (TT/E2) influenced by obesity were unrelated to semen parameters; however, serum FSH and LH levels that had no correlation with obesity-associated markers significantly affected semen parameters, which further explained the reason that obesity might affect serum reproductive hormone levels but did not change semen parameters. Similar results were reported (Aggerholm *et al.*, 2008; Chavarro *et al.*, 2010; MacDonald *et al.*, 2010; Anifandis *et al.*, 2013). The changes of serum reproductive hormone levels could not explain the correlations between obesity-associated markers and semen parameters. First, other factors except for endocrine hormones might influence semen quality (Qin *et al.*, 2007; Sermondade *et al.*, 2013). Second, spermatogenesis is driven mainly by the action of testosterone, in the form of free testosterone, and FSH (MacDonald *et al.*, 2010). Although testosterone decreased significantly in obese

Table 4 Comparisons of serum reproductive hormone levels based on the dichotomised analyses for BMI, WHR, WC and WHtR

Variable	<i>n</i>	LH (IU l ⁻¹)	FSH (IU l ⁻¹)	TT (nmol l ⁻¹)	E2 (pmol l ⁻¹)	TT/E2	SHBG (nmol l ⁻¹)
BMI (kg m ⁻²)							
<18.5	5	5.29 ± 1.07	4.97 ± 2.33	17.05 ± 1.67	97.60 ± 47.73	209.88 ± 95.83	42.13 ± 15.09
18.5–23.99	151	5.03 ± 2.90	5.15 ± 3.10	16.44 ± 5.66	105.01 ± 59.50	223.57 ± 365.77	35.20 ± 16.79
24–27.99	107	5.11 ± 3.53	4.97 ± 2.44	14.46 ± 9.54 ^a	98.69 ± 46.29	241.86 ± 706.88	26.59 ± 11.23 ^b
≥28	24	4.48 ± 1.67	4.47 ± 1.90	12.21 ± 4.30 ^a	93.67 ± 40.28	217.58 ± 349.34	22.55 ± 9.66 ^b
<i>F</i>		0.292	0.436	3.279	0.498	0.034	11.375
<i>P</i>		0.831	0.727	0.021	0.684	0.992	<0.001
WC (cm)							
<90	227	5.12 ± 3.28	5.09 ± 2.93	16.05 ± 7.91	106.29 ± 55.80	234.45 ± 566.80	32.79 ± 15.68
≥90	60	4.63 ± 1.93	4.75 ± 2.03	12.74 ± 3.54	83.75 ± 37.03	211.49 ± 235.54	24.48 ± 10.84
<i>t</i>		1.115	0.852	3.151	2.959	0.307	3.868
<i>P</i>		0.266	0.395	0.002	0.003	0.759	<0.001
WHR							
<0.9	231	4.97 ± 2.66	5.01 ± 2.75	15.45 ± 5.20	104.24 ± 55.27	210.67 ± 324.41	31.86 ± 15.49
≥0.9	56	5.19 ± 4.34	5.10 ± 2.88	15.00 ± 12.91	90.58 ± 42.14	307.93 ± 965.52	27.74 ± 13.36
<i>t</i>		-0.479	-0.218	0.409	1.731	-0.744	1.832
<i>P</i>		0.632	0.827	0.683	0.084	0.460	0.068
WHtR							
<0.5	193	5.05 ± 2.79	5.08 ± 2.93	16.64 ± 8.33	106.04 ± 57.84	250.83 ± 612.62	34.04 ± 15.99
≥0.5	94	4.95 ± 3.54	4.91 ± 2.42	12.73 ± 3.46	92.41 ± 40.79	186.16 ± 196.21	24.93 ± 11.09
<i>t</i>		0.263	0.465	5.594	2.048	0.998	5.613
<i>P</i>		0.793	0.643	<0.001	0.041	0.319	<0.001

SHBG, sex hormone-binding globulin. LSD *t*-test showed that overweight and obese men had lower serum testosterone and SHBG levels than underweight and normal weight men (^a*P* < 0.05; ^b*P* < 0.001).

Table 5 Correlation coefficients for relationships between semen parameters and serum reproductive hormone levels in 287 infertile men

Variable	Sperm concentration	Total sperm count	Progressive motility	Sperm motility	Normal sperm morphology	TNPMS
LH	-0.164 ^b	-0.112	-0.085	-0.148 ^b	-0.202 ^a	-0.190 ^a
FSH	-0.121 ^b	-0.063	-0.069	-0.110	-0.118 ^b	-0.102
TT	-0.034	0.007	-0.085	-0.086	-0.071	-0.040
E2	-0.050	-0.092	-0.076	-0.054	0.038	-0.107
TT/E2	0.022	0.094	0.012	-0.012	-0.085	0.072
SHBG	-0.089	-0.070	-0.019	0.047	-0.096	-0.080

SHBG, sex hormone-binding globulin; TNPMS, total normal-progressively motile sperm count. ^a*P* ≤ 0.001; ^b*P* ≤ 0.05.

men, free testosterone decreased slightly. Moreover, our data showed that obesity-associated markers were not related to FSH. Third, although serum reproductive hormone levels were influenced significantly by obesity, the local reproductive hormone levels in testis might remain stable. It is possible that the spermatogenesis is not completely controlled by hormonal regulation. Instead, it may be a biological process driven by a minimum endocrine and thus independent of hormone levels (MacDonald *et al.*, 2010). Finally, only in the men with extreme levels of obesity or morbid obesity may the changes of reproductive hormone levels negatively influence male reproductive potential (Chavarro *et al.*, 2010; Håkonsen *et al.*, 2011). However, it was reported that

surgery-induced massive weight loss in 20 morbidly obese men did not interfere with sperm quality (Reis *et al.*, 2012), while it improved the quality of sexual function, total testosterone and FSH. Therefore, it is not completely understood to what extent these hormonal changes affect a man's reproductive potential.

In summary, our results showed that age was the major factor leading to obesity and reduction in serum testosterone level and that obesity could lead to significant changes of serum testosterone, SHBG and oestradiol levels, especially the former two. However, obesity did not influence serum FSH and LH levels that significantly related to semen parameters, suggesting that obesity was unrelated to semen parameters. Our study was based on

prospective design, and the results were blindly evaluated. The analyses for all of semen parameters and serum reproductive hormones were performed with strict quality control. Moreover, all of obesity-associated markers used at present were compared with semen parameters and serum reproductive hormone levels, and a new marker, which could reflect whole semen quality, total normal-progressively motile sperm count, was adopted. Therefore, these efforts ensured the data obtained in this study complete and reliable. We deemed that obesity-associated markers including BMI, WC, WHR and WHtR could not predict male semen quality. However, because obesity was associated with erectile dysfunction and many other kinds of diseases, and increased general adult morbidity and mortality (MacDonald *et al.*, 2010), we advised that overweight and obese men should control their obesity-associated markers in normal range.

Acknowledgement

This study was supported by State Key Development Program for Basic Research of China (2013CB945200).

References

- Aggerholm AS, Thulstrup AM, Toft G, Ramlau-Hansen CH, Bonde JP (2008) Is overweight a risk factor for reduced semen quality and altered serum sex hormone profile? *Fertil Steril* 90:619–626.
- Alberti KG, Zimmet PZ (1998) Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med* 15:539–553.
- Anifandis G, Dafopoulos K, Messini CI, Polyzos N, Messini IE (2013) The BMI of men and not sperm parameters impact on embryo quality and the IVF outcome. *Andrology* 1:858–859.
- Cabler S, Agarwal A, Flint M, du Plessis SS (2010) Obesity: modern man's fertility nemesis. *Asian J Androl* 12:480–489.
- Cai L, Liu A, Zhang Y, Wang P (2013) Waist-to-height ratio and cardiovascular risk factors among Chinese adults in Beijing. *PLoS One* 8:e69298.
- Chavarro JE, Toth TL, Wright DL, Meeker JD, Hauser R (2010) Body mass index in relation to semen quality, sperm DNA integrity, and serum reproductive hormone levels among men attending an infertility clinic. *Fertil Steril* 93:2222–2231.
- Eskandar M, Al-Asmari M, Babu Chaduvula S, Al-Shahrani M, Al-Sunaidi M, Almushait M, Donia O, Al-Fifi S (2012) Impact of male obesity on semen quality and serum sex hormones. *Adv Urol* 2012:407601.
- Feldstein CA, Akopian M, Olivieri AO, Kramer AP, Nasi M, Garrido D (2005) A comparison of body mass index and waist-to-hip ratio as indicators of hypertension risk in an urban Argentine population: a hospital-based study. *Nutr Metab Cardiovasc Dis* 15:310–315.
- Häkonsen LB, Thulstrup AM, Aggerholm AS, Olsen J, Bonde JP, Andersen CY, Bungum M, Ernst EH, Hansen ML, Ernst EH, Ramlau-Hansen CH (2011) Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reprod Health* 8:24.
- Jensen TK, Andersson AM, Jørgensen N, Andersen AG, Carlsen E, Petersen JH, Skakkebaek NE (2004) Body mass index in relation to semen quality and reproductive hormones among 1,558 Danish men. *Fertil Steril* 82:863–870.
- Jia WP, Lu JX, Xiang KS, Bao YQ, Lu HJ, Chen L (2003) Prediction of abdominal visceral obesity from body mass index, waist circumference and waist-hip ratio in Chinese adults: receiver operating characteristic curves analysis. *Biomed Environ Sci* 16:206–211.
- Li Y, Lin H, Ma M, Li L, Cai M, Zhou N, Han X, Bao H, Huang L, Zhu C, Li C, Yang H, Rao Z, Xiang Y, Cui Z, Ao L, Zhou Z, Xiong H, Cao J (2009) Semen quality of 1346 healthy men, results from the Chongqing area of southwest China. *Hum Reprod* 24:459–469.
- Lu JC, Huang YF, Lü NQ (2014) Computer-aided sperm analysis: past, present and future. *Andrologia* 46:329–338.
- MacDonald AA, Herbison GP, Showell M, Farquhar CM (2010) The impact of body mass index on semen parameters and reproductive hormones in human males: a systematic review with meta-analysis. *Hum Reprod Update* 16:293–311.
- Macdonald AA, Stewart AW, Farquhar CM (2013) Body mass index in relation to semen quality and reproductive hormones in New Zealand men: a cross-sectional study in fertility clinics. *Hum Reprod* 28:3178–3187.
- Nyamdorj R, Qiao Q, Söderberg S, Pitkaniemi J, Zimmet P, Shaw J, Alberti G, Nan H, Uusitalo U, Pauvaday V, Chitson P, Tuomilehto J (2008) Comparison of body mass index with waist circumference, waist-to-hip ratio, and waist-to-stature ratio as a predictor of hypertension incidence in Mauritius. *J Hypertens* 26:866–870.
- Paasch U, Grunewald S, Kratzsch J, Glander HJ (2010) Obesity and age affect male fertility potential. *Fertil Steril* 94:2898–2901.
- Qin DD, Yuan W, Zhou WJ, Cui YQ, Wu JQ, Gao ES (2007) Do reproductive hormones explain the association between body mass index and semen quality? *Asian J Androl* 9:827–834.
- Raman R, Rani PK, Gnanamoorthy P, Sudhir RR, Kumaramanikavel G, Sharma T (2010) Association of obesity with diabetic retinopathy: Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study (SN-DREAMS Report no. 8). *Acta Diabetol* 47:209–215.
- Reis LO, Zani EL, Saad RD, Chaim EA, de Oliveira LC, Fregonesi A (2012) Bariatric surgery does not interfere with sperm quality—a preliminary long-term study. *Reprod Sci* 19:1057–1062.

- Rybar R, Kopecka V, Prinosilova P, Markova P, Rubes J (2011) Male obesity and age in relationship to semen parameters and sperm chromatin integrity. *Andrologia* 43:286–291.
- Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, Van Wely M, Cao J, Martini AC, Eskandar M, Chavarro JE, Koloszar S, Twig J, Ramlau-Hansen CH, Borges E Jr, Lotti F, Steegers-Theunissen RP, Zorn B, Polotsky AJ, La Vignera S, Eskenazi B, Tremellen K, Magnusdottir EV, Fejes I, Hercberg S, Lévy R, Czernichow S (2013) BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Hum Reprod Update* 19:221–231.
- Shen DC, Shieh SM, Fuh MT, Chen YD (1990) Comparison of the effects of differences in ratio of waist to hip girth and body mass index on carbohydrate metabolism in Chinese females. *Horm Metab Res* 22:533–536.
- Tunc O, Bakos HW, Tremellen K (2011) Impact of body mass index on seminal oxidative stress. *Andrologia* 43:121–128.
- Visscher TL, Seidell JC, Molarius A, van der Kuip D, Hofman A, Witteman JC (2001) A comparison of body mass index, waist-hip ratio and waist circumference as predictors of all-cause mortality among the elderly: the Rotterdam study. *Int J Obes Relat Metab Disord* 25:1730–1735.
- World Health Organization (2010) Laboratory Manual for the Examination and Processing of Human Semen, 5th edn. World Health Organization, Geneva, pp 10–56.