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Features of the metabolic syndrome in late adolescence are associated with impaired testicular function at 20 years of age.

Short title Metabolic syndrome and testicular function

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Abstract

Study question: Are early signs of metabolic disorder in late adolescence associated with features of impaired testicular function many years before the majority are seeking parenthood?

Summary answer: Adolescents with features of metabolic disorder, as manifest by ultrasound evidence of non-alcoholic fatty liver (NAFL) at 17 years or systemic markers of inflammation, and/or insulin resistance measured by homeostasis model of insulin resistance (HOMA-IR) at 20 years of age, have reduced testicular volume, total sperm output, serum testosterone (T) and inhibin B (inhB) concentrations and a higher serum follicle stimulating hormone concentration (FSH), at 20 years of age, in comparison to their peers without metabolic disorder.

What is known already: Controversial evidence suggests a recent decline in sperm production potentially linked to environmental influences, but its cause remains unclear. The concomitant increases in obesity and diabetes suggests that lifestyle factors may contribute to this decline in testicular function. Although obesity has been associated with adverse testicular function in some studies, it remains unclear whether poor testicular function reflects, or causes, poor metabolic health. If metabolic disorder were present in adolescence, prior to the onset of obesity, this may suggest that metabolic disorder may lead to impaired testicular function

Study design, size, duration: The Western Australian Pregnancy Cohort (Raine) Study is a longitudinal study of children born in 1989-1991 who have undergone detailed physical assessments since birth (1454 male infants born). The purpose of this current sub-study was to perform a testicular assessment at 20 years of age (913 were contactable).

At 17 years of age 490 underwent a hepatic ultrasound examination, serum cytokine assessment (n=520) and a metabolic assessment (n=544). A further metabolic assessment was performed at 20 years (n=608). Testicular assessment was performed on 20 year participants who consented to inclusion; 609 had reproductive hormones measured, 404 underwent a testicular ultrasound and 365 produced a semen sample.

Participants/materials, setting, methods: Testicular volume was estimated by ultrasonography, and semen analysis performed by WHO methods. Serum was analysed to determine concentrations of

luteinising hormone (LH), FSH, inhB by immunoassays and T by liquid chromatography-mass spectrometry (LC-MS).

At 17 years of age a liver ultrasound examination was performed to determine the presence of NAFL, and serum analysed for the following cytokines; interleukin-18 (IL18), soluble tumour necrosis factor receptor 1 & 2 (sTNFR1, sTNFR2) concentrations.

At 17 and 20 years of age fasting blood samples were analysed for serum liver enzymes, insulin, glucose, triglycerides (TG), total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, high sensitivity (hs) CRP, and uric acid. HOMA was calculated and insulin resistance (IR) was defined by a HOMA >4, anthropometric data was collected and dual energy X-ray absorptiometry (DEXA) measurement was performed for lean and total fat mass. As at this young age the prevalence of metabolic syndrome was expected to be low, a two-step cluster analysis was used using waist circumference, TGs, insulin, and systolic blood pressure to derive a distinct high-risk group with features consistent with the metabolic syndrome.

Main results and the role of chance: Men who at age 17 years were at elevated cardiometabolic risk had lower concentrations of T (medians: 4.0ng/ml *vs* 4.9ng/ml) and inhB (medians: 193.2pg/ml *vs* 221.9pg/ml) ($p<0.001$ for both) compared to those within the low risk metabolic cluster. Furthermore, men with ultrasound evidence of NAFLD detected at 17 years (n=45, 9.8%) had reduced total sperm output (medians: 68.0 million *vs* 126.00 million, p=0.044), T (4.0ng/ml *vs* 4.7ng/ml, p=0.005) and inhB (209.1pg/ml *vs* 218.4pg/ml, p=0.032) concentrations at 20 years compared to men without NAFLD..

Men with higher concentrations of sTNFR1, at 17 years of age, had a lower sperm output and seminal volume, and serum concentrations of inhB, with an increase in LH and FSH at 20 years of age (all $p<0.05$ after adjustment for age, body mass index [BMI], abstinence and a history of cryptorchidism and varicocele, cigarette smoking, alcohol and drug use). Similarly, serum T was lower in men with a higher fasting serum insulin, hsCRP, HOMA and total fat mass, and higher in men with higher fasting HDL, iron at 20 years of age (all $p<0.05$). Multivariable regression analysis, adjusting for age and BMI at 20 years, cryptorchidism and presence of a varicocele examined the associations between NAFLD (at 17 years), and HOMA-IR >4 and metabolic cluster (at 20 years) with reproductive hormone concentrations at age 20 years, demonstrated that men in the high-risk metabolic cluster at 20 years

had a lower serum T and inhB (both p=0.012), and HOMA-IR >4 was associated with a lower serum T (p=0.002), .

Limitations, reasons for caution: This study is limited by the sample size and multiple comparisons, and causality cannot be proven from an observational study. Due to a three year interval between some metabolic assessments and assessment of testicular function, we cannot exclude an introduction of a bias into the study, as some of the participants and their testicular function will not have been fully mature at the 17 year assessment.

Wider implications of the findings: Irrespective of a proven causation, our study findings are important in that a significant minority of the men, prior to seeking parenthood, presented co-existent features of metabolic disorder and signs of testicular impairment. Of particular note is that the presence of NAFLD at 17 years of age, although only present in a minority of men, was associated with an almost 50% reduction in sperm output at 20 years of age, and that the presence of IR at 20 years was associated with a 20% reduction in testicular volume.

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Key words: Raine, metabolic, testicular function, semen, reproductive hormones, testicular volume.

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Introduction

There is an on-going debate in reproductive medicine as to whether there has been a general decline in sperm production in recent times, potentially linked to environmental influences (1-3). The parallel increase in the rates of lifestyle related disorders, such as obesity and diabetes (4), raises the possibility that lifestyle factors may contribute to any potential decline in sperm production. In populations of men seeking fertility treatment, obesity has been associated with adverse testicular function; such as reduced testicular volume, seminal volume, sperm output, sperm motility, serum testosterone concentration and sperm DNA damage (5-9), although this has been challenged (10).

The metabolic syndrome is a cluster of adverse cardiovascular features including central obesity, atherogenic dyslipidemia, insulin resistance, a prothrombotic state, elevated blood pressure and increased circulating proinflammatory markers. Some evidence exists for an association between the metabolic syndrome and impaired testicular function in sub-fertile men (9, 11). However, causality is unclear whether these disorders have a common origin in early life (12), or whether impaired testicular function may induce or result from the metabolic disorder.

With the increase in the prevalence of features of the metabolic syndrome in adolescent populations (13), many will have ultrasound evidence of a fatty liver (14). Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disorder, affecting almost 1 in 5 adolescents (15), and is a recognized antecedent of progressive liver disease and cardiometabolic disorder (16). Hyperinsulinemia, or the presence of a fatty liver (17), is associated with a reduction in hepatic synthesis of sex hormone binding globulin (SHBG), increasing the metabolic clearance of testosterone.

The metabolic syndrome is associated with a low grade inflammatory state, with increased C-reactive protein (CRP) and production of inflammatory cytokines, such as, Interleukin- 6 (IL-6), tumour necrosis factor -α (TNF-α), and their receptors 1 and 2 (TNFR1 and TNFR2), and the production of oxygen free radicals, all of which may impair sperm and testicular function (18, 19). We therefore proposed that impaired testicular function may reflect or cause poor metabolic health.

Our study was driven by the question; whether or not in a young adult population, representative of the Western Australian population (20), early signs of metabolic disorder are associated with a profile of impaired testicular function, many years before the majority of men seek paternity. Hence our aim was to relate antecedent and concurrent markers of adverse cardiometabolic health, in adolescence and early adulthood, to markers of testicular function within men at 20 years of age from the Western Australian Pregnancy Cohort (Raine) Study.

Materials and Methods

The Raine study

The Raine Study (www.rainestudy.org.au) was designed to measure the relationships between early life events and subsequent health and behaviour. The study recruited 2900 women around 18 weeks of gestation in 1989-91 (20, 21). 2868 children (including 1454 boys) born to 2804 mothers were retained to form the Raine Study cohort, and were studied every 2-3 years into early adulthood, including detailed cardiometabolic assessment at 17 and 20 years of age**,** and 423 men underwent testicular assessment by ultrasound and / or semen examinations (20, 22). Ethical approval was obtained from the University of Western Australia Human Research Ethics Committee, and all participants provided informed written consent for all aspects of the study.

Testicular function assessment

Clinical and testicular function assessment at 20 years of age

All male cohort members were invited to attend follow-up, which involved questionnaires, collection of anthropometric data (n=687), and collection of blood for analysis of serum testosterone, luteinizing hormone (LH), FSH and inhB concentrations (n=609). A testicular ultrasound examination was performed (n=404), and a semen sample (n=365) analysed at Fertility Specialists of Western Australia, as previously reported (22). Semen samples were analysed as per WHO semen manual guidelines (23) including sperm concentrations (million per ml), total sperm output (million per ejaculate), motility (%A grade + %B grade) and morphology. The sperm chromatin structural assay (SCSA) was performed as described (24) with slight modifications. The DNA fragmentation index represents the percentage of sperm within the sample with fragmented or damaged DNA. Serum inhB concentration was measured by Gen II ELISA (Beckman Coulter Inc. Brea, CA); LH and FSH were

measured by ELISA (IBL International, Hamburg, Germany), and testosterone was measured by liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described (25) (for further details refer to supplementary methods [sM]). Testicular ultrasonography was performed as described (22), and the volume of each testis calculated (26). Varicocele was defined as present when the maximal venous diameter was over 3mm, and increased with the Valsalva manoeuver (27).

Metabolic assessments

1. Metabolic assessment at 17 years of age

a. Hepatic ultrasound

The methods of hepatic ultrasound examinations conducted among 587 cohort members at age 17 years for diagnosing NAFL have been reported previously, and the data was used in this study (sM)] (14).

b. Cytokine assessment

The serum from 520 cohort members was stored at -80 C and was analysed for the following cytokines; interleukin-18 (IL18) by ELISA, soluble tumor necrosis factor receptor 1 & 2 (sTNFR1, sTNFR2). Plasma IL-18 was quantitated with a commercially available ELISA method. Plasma sTNFR1 and sTNFR2 were quantified using cytometric Bead Array. Individual cytokine concentrations were determined using FCAP Array software (BD Biosciences) (sM).

c. Cardiometabolic assessment

Data from previous publication (13) was extracted for the fasting blood samples from 549 cohort members which were analysed at the PathWest Laboratory at Royal Perth Hospital for serum liver enzymes, insulin, glucose, triglycerides (TG), total cholesterol, HDL and LDL cholesterol, hsCRP, and uric acid, as previously described (13, 29), excluding serum hsCRP concentrations >10mg/l (13, 29). Glucose, insulin, total cholesterol and triglycerides were measured by automated analysers (sM). HOMA was calculated by fasting insulin (microunits per milliliter) × fasting glucose (millimoles per liter)]/22.5, and insulin resistance (IR) was defined by a HOMA >4 (30). Resting blood pressure (BP) readings were taken (sM). The cardiometabolic data was used to derive a ‘high risk metabolic cluster’ phenotyped previously in this cohort (13), and described below.

2. Cardiometabolic assessment at 20 years of age

Fasting blood samples from 620 cohort members at 20 years of age were analysed according to the same protocol for the 17 year cardiometabolic assessment. To assess body fat distribution DEXA measurement was performed (31, 32).

Statistical Considerations

Derivation of metabolic cluster at 20 years of age

The two most frequently used definitions of the metabolic syndrome in adulthood are the National Cholesterol Education Program expert panel on detection, evaluation, and treatment of high blood cholesterol in adults (NCEP ATP-III) (33), and the International Diabetes Federation definition (IDF) (34), which differ significantly on the components of their definition (see details in sM). Hence, as there is no universally accepted definition, and it was expected that at this young age the prevalence of metabolic syndrome would be low, an alternative approach, a two-step cluster analysis was used (13, 35, 36). This is an effective tool used to define groups accounting for variables for where there is strong evidence of clustering. Within a single cluster, the subjects are relatively homogeneous, sharing similar traits and being dissimilar to subjects in other clusters. The technique uses a scalable cluster analysis algorithm (37), designed specifically to handle large data sets and has been used previously within this cohort (13, 35, 36). It preselects subjects into sub-clusters before further grouping into the desired number of clusters with use of log-likelihood distance. The cluster groups were formed with use of; waist circumference, TGs, insulin, and systolic BP measured at 20 years of age to derive distinct high-risk group with features consistent with the metabolic syndrome. This approach was used previously to identify those Raine study participants within a high risk metabolic cluster at 17 years of age (13).

Data analysis

Continuous data were summarized using medians and inter-quartile ranges (IQR), reported as Q1–Q3, when following a non-Gaussian distribution. Categorical data were summarized using frequency distributions. Multivariable linear regression analysis was used to examine associations between metabolic parameters and reproductive outcomes or hormone concentrations. Covariate adjustments

included abstinence, history of cryptorchidism, varicoceles and BMI. Regression results were summarized using standardised coefficients (β) and their 95% confidence intervals (CI). Effects of the metabolic parameters on outcomes were presented without (β_1) and with (β_2) adjustment for BMI. Supplementary analyses adjusting for waist circumference instead of BMI were performed with analogous results (data not shown). Reproductive outcomes had a non-Gaussian distribution, and were transformed to normality either via logarithmic or power transformations determined using the Box-Cox analysis.

Differences in reproductive parameters and hormone concentrations across low and high risk metabolic clusters, HOMA-IR, NAFLD, insulin and hsCRP were investigated univariately using Mann-Whitney test for two groups. When appropriate univariable analyses were supplemented with multivariable analyses to control for confounders age and BMI at 20 years, cryptorchidism and presence of a varicocele. All hypothesis tests were two-sided and *p*-values of <0.05 were considered statistically significant. No adjustments for multiple hypothesis testing were made in this exploratory study (38, 39). SPSS (version 22.0, IBM SPSS) statistical software was used for data analysis.

Results

Demographics

Of 913 male cohort members who were contactable, 365 (40%) provided a semen sample and represented 48% of the men who attended any of the assessments at 20 years of age and 404 underwent a testicular ultrasound and 609 had serum available for reproductive hormone assessment (Table 1). Most (587) had undergone a liver ultrasound at 17 years of age and/or a fasting metabolic assessment (544), and up to 608 had undergone some aspect of metabolic assessment at age 20 years of age (Table 2). Participants who took part in the testicular assessment (semen sample and/or testicular ultrasound) were similar clinically to those that declined participation (Table 2). There was no difference between the participants and the non-participants with respect to markers of socio-economic status (data not shown sM). The prevalence of metabolic syndrome among males within the participants was 4.1% by the NCEP-ATPIII definition (40) and 5.4% using the IDF definition (34).

Associations between markers of metabolic disorder and testicular parameters

Metabolic indices at 17 years.

Multivariable linear regression analysis adjusting for current age, abstinence, a history of cryptorchidism, presence of a varicocele, and BMI revealed the association of semen parameters with markers of systemic inflammation at 17 years of age: total sperm output was reduced in men at 20 years of age who had a higher serum IL18 (p=0.025), or sTNFR1 (p=0.036) and their sperm concentration was negatively associated with their serum IL18 concentration (p=0.020) measured at 17 years (Table 3A). In addition higher sTNFR1 was negatively associated with inhibin B (p=0.011), and positively associated with serum LH and FSH (p=0.015, and p=0.001 respectively) three years later (Table 4). When adjustment was performed for waist circumference instead of BMI the results were analogous (data not shown). We have previously shown that alcohol use, cigarette smoking and recreational drug use in this cohort had no influence on markers of testicular function (22), and that testicular volume was positively associated with height, and total soft and lean body mass(12)

Associations between metabolic cluster analysis at 17 years and subsequent testicular function at 20 years of age

At 17 years of age 70 of 439 participants (15.9%) who would subsequently undergo the male reproductive assessment were clustered within the high metabolic risk group.

In an unadjusted analysis of the reproductive hormones of men within the high risk metabolic cluster at 17 years of age, had median T and inhB concentrations significantly lower ($p<0.001$ for both), in comparison to the men within the low risk metabolic cluster (Table 5).

Metabolic indices at 20 years.

After adjustment for age, abstinence, a history of cryptorchidism, varicocele and BMI; diastolic blood pressure and serum insulin at 20 years of age were negatively associated with testicular volume (p=0.028 and p=0.004 respectively), although diastolic blood pressure was positively associated with total sperm output (p=0.020) and seminal volume (p=0.014). ALT and GGT were positively associated with sperm morphology (p=0.008 and p=0.028 respectively) (Table 3A).

Associations between markers of metabolic disorder at 20 years of age and sex hormones

Multivariable regression analyses with adjustment for history of cryptorchidism and presence of a varicocele shown that serum total testosterone (TT) was reduced in men with a higher fasting serum; triglycerides, insulin, hsCRP, ferritin, ALT, HOMA score, and DEXA indices of fat when measured concurrently at 20 years of age (Table 4) (all $p<0.05$). Serum TT was positively associated with serum HDL cholesterol, iron and transferrin saturation (all $p<0.05$). After simultaneous adjustment for BMI (and waist circumference-data not shown) positive associations with serum HDL, iron, transferrin saturation and lean mass remained and a negative association with hsCRP and serum insulin remained (all $p<0.05$).

Associations between metabolic cluster analysis at 20 years of age and testicular function

The number of men in the high risk metabolic cluster at 20 years of age, those insulin resistant as measured by the HOMA score and with NAFL, varied within the analyses due to the varying number of participants who took part in the various sub-studies (Table 1).

In an unadjusted analysis of the men within the high risk metabolic cluster at 20 years of age, their median T and inhB concentrations were lower than men within the low risk metabolic cluster (Table 5, supplementary figures [sF] 1a and 1b).

Associations between HOMA as a proxy for IR at 20 years of age and testicular function

IR (as defined by a HOMA>4) was present in 24 out of 616 men (3.9%) at 20 years of age. In an unadjusted analysis, in comparison to those participants who were not IR, their median testicular volume was smaller, and median T and inhB concentrations were lower, and their median serum FSH concentration was higher (Table 6, sF2a-d). Furthermore the 51 men, out of 609 (8.4%), who had a fasting serum insulin greater than 10 μU/ml (91st centile), at 20 years of age had lower median serum T and inhB concentrations, and their FSH concentration was greater (supplementary table [sT]1, sF3a-c).

Associations between presence of NAFLD at 17 years of age and subsequent testicular function

Ultrasound evidence of NAFLD was present in 44 men, out of 458 (9.6%) who subsequently underwent some assessment of testicular function. Compared to those participants without NAFLD, there were reductions in their; median total sperm output, serum T and inhB concentrations (sT2 and sF4a-c).

Associations between serum hsCRP either; above or below, the 75% percentile at 20 years of age and testicular function

Men whose serum hsCRP was greater than the 75% centile (1.62mg/l) at 20 years of age (after exclusion of concentrations >10mg/l), in comparison to those below, had a reduction in their median seminal volume, serum T, LH and FSH concentrations (sT3, sF5a-d).

Multivariable analysis

Multivariable analysis demonstrated that being in the high risk metabolic cluster at 20 years of age was associated with a lower serum testosterone and inhB, and HOMA-IR >4 was associated with a lower serum testosterone concentration at 20 years (sT4).

Discussion

The findings of this observational study of adult men at 20 years of age, demonstrated that despite the majority of men being of normal weight, a small minority already displayed features of metabolic disturbance which are associated with adverse cardiovascular outcomes at a much older age. Men with features of the metabolic syndrome, or who were IR at 20 years of age, or had ultrasound evidence of NAFLD at 17 years of age, displayed a picture consistent with a degree of primary hypogonadism as they had reductions in testicular volume, sperm output, and serum testosterone and inhB, with a reciprocal increase in serum FSH at 20 years of age. All of these variables are well established as adverse markers of reproductive potential (2, 22). Furthermore in considering potential mechanisms for the observed finding it is possible there are contrasting influences of metabolic disorder as; higher concentrations of the inflammatory markers sTNFR1 (and IL18 to a lesser degree), when measured at 17 years were associated with subsequent reductions in sperm output, seminal volume, sperm concentration, inhB, with reciprocal rises in LH and FSH, at 20 years of age, suggesting a direct gonadotoxic effect of adolescent inflammation on subsequent testicular function,

and in contrast higher concentrations of hsCRP at 20 years of age had a potential central negative influence on serum FSH secretion (and LH to a lesser degree), inducing a central hypogonadal state with reductions in serum testosterone and seminal volume, however without a concomitant reduction inhB and testicular volume, these could be chance associations.

One can speculate that if their cardiometabolic picture deteriorates over time, their testicular function might further deteriorate, leading to an adverse effect on their reproductive potential. Conversely if their metabolic picture improves, this may have a positive impact on their reproductive potential, although. it is interesting to note that already at 20 years of age, irrespective of BMI, the markers of cardiometabolic disorder; a higher fasting serum insulin, triglycerides, hsCRP, HOMA score and DEXA fat mass were negatively associated with the testicular hormones; serum testosterone and inhB, concentrations, while a higher total lean mass, serum HDL cholesterol and iron stores were positively associated with these hormones. These findings offer a potential link between metabolic and reproductive health, in that these adverse metabolic features recorded at 17 and 20 years of age may predispose a man to later impaired testicular function, irrespective of adiposity. Although the direction of causality will require further investigation, as it is established that a low circulating testosterone is associated with cardiometabolic disorder (41-43). However, data from one prospective study suggests that a low serum testosterone may be a risk marker for the development of cardiometabolic disorder, rather than a causative risk factor (44). Due to a three year interval between some metabolic assessments and assessment of testicular function, we cannot exclude an introduction of a bias into the study, as some of the participants and their testicular function will not have been fully mature at the 17 year assessment, and it is known that pubertal maturation can have a moderating impact on obesity associated inflammation (45). Irrespective of a proven causation, our study findings are important in that a significant minority of the men, prior to seeking parenthood, presented with some features of metabolic disorder and signs of testicular impairment.

These study findings warrant further study in other cohorts. Of particular note is that the presence of NAFLD at aged 17 years of age, although only present in a minority of men, was associated with an almost 50% reduction in sperm output at 20 years of age, and that the presence of IR at 20 years was

associated with; a 20% reduction in testicular volume, a 30% reduction in serum testosterone, and a 20% reduction in serum inhB concentrations.

Conclusion

This study has demonstrated an association of adverse cardiometabolic features with impaired testicular function at 20 years of age. Furthermore, it is notable that, despite the majority of the young men having apparently normal metabolic function, a significant minority were already showing some features of the metabolic syndrome.

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409 **Author contributions (supplementary data on line)**

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533

Supplementary Methods on line

Hepatic ultrasound examination

Ultrasonographers experienced in hepatic ultrasound performed the ultrasound examinations, as described (14), according to the protocol described by Hamaguchi (28), which provides 92% sensitivity and 100% specificity for the histological diagnosis of fatty liver (14). Boys with sonographic fatty liver and a self-reported weekly alcohol intake of less than 140g over the previous 12 months were classified as having NAFLD. Testing for hepatitis B or C virus infections was not performed due to low notification rates for these infections in local teenagers during the study period (14).

Sex hormone measurement; Serum inhibin B concentrations were measured in duplicate by Inhibin B Gen II ELISA from Beckman Coulter Inc. (Brea, CA), which had a limit of detection of 2.6 pg/ml. Luteinising hormone (LH), follicular stimulating hormone (FSH) levels were determined in duplicate using ELISA kits from IBL International, Hamburg, Germany. The limit of detection of the LH assay was 0.4 IU/L (calibrated against WHO IRP 80/552), while for FSH assay it was 0.2 IU/L (calibrated against NIBSC 92/510). The intra-assay precision (CV) of the ELISAs ranged from 8-11% based on the mean values for low and high value quality control samples from n=16-17 assays.

Measurement of serum inflammatory markers;

Cytokine assessment

The serum from 520 cohort members was stored at -80 C and was analysed for the following cytokines; interleukin-18 (IL18) by ELISA (Medical Biological Laboratories, Nagoya, Japan), soluble tumor necrosis factor receptor 1 & 2 (sTNFR1, sTNFR2). Plasma IL-18 was quantitated with a commercially available ELISA method (Medical Biological Laboratories, Nagoya, Japan). Plasma sTNFR1 and sTNFR2 were quantified using cytometric Bead Array (CBA) Flex sets (BD PharMingen, San Diego, CA) on the BD FACSArrayTM bioanalyser (BD Biosciences, San Jose, California, USA). Procedures followed the manufacturer's recommendations. Individual cytokine concentrations were determined using FCAP Array software (BD Biosciences). The IL-18: Intra- and inter-assay coefficients of variation (CV) were 5.6% and 7.6%, respectively, with a sensitivity: 12.5 pg/Ml.

c. Cardiometabolic assessment

Fasting blood samples from 454 cohort members were analysed at the PathWest Laboratory at Royal Perth Hospital for serum liver enzymes, insulin, glucose, triglycerides (TG), total cholesterol, high density lipoprotein (HDL) and low density lipoprotein (LDL) cholesterol, high sensitivity (hs) CRP, and uric acid, as previously described (14, 31), excluding serum hsCRP concentrations >10mg/l (14, 31). Glucose was measured by an automated Technicon Axon analyser (Bayer Diagnostics, Sydney, Australia) using a hexokinase method. Insulin was measured by automated radioimmunoassay (Tosoh, Tokyo, Japan). Total cholesterol and triglycerides were determined enzymatically on the Cobas MIRA analyzer (Roche Diagnostics) with reagents from Trace Scientific (Melbourne, Australia) (supplementary methods).

The intra-assay CV was 0.87% for total cholesterol, 1.92% for triglycerides, 1.04% for glucose, 1.78%. The intra- and inter-assay CV for insulin was 2.5%, sensitivity: 0.9 ng/Ml. For leptin the intra and inter-assay CV were 3.3% and 5.4%, respectively, with a sensitivity of 7.8 pg/mL.

Resting blood pressure (BP) readings were taken using an oscillometric sphygmomanometer (DINAMAP vital signs monitor 8100, DINAMAP XL vital signs monitor, or DINAMAP ProCare 100), Soma Technology Bloomfiled, Connecticut) with the subject seated. The monitor was set to automatically record readings every 2 minutes and the mean of the second and third readings was calculated.

Sociodemographic assessment

Family income when children were aged 14 years was obtained by questionnaire from the primary caregiver, and the highest maternal education was obtained by questionnaire when children were aged 8 years (20).

The commonest definitions of metabolic syndrome

The NCEP ATP III guidelines state that the metabolic syndrome may be diagnosed when a person has three or more of five components: central obesity (waist circumference), an elevated TG level, a reduced HDL-cholesterol level, an elevated BP or an elevated fasting glucose concentration (35). The

IDF definition requires a person to have central obesity plus any two of four additional factors: a raised TG level: a reduced HDL-cholesterol: a raised BP or a raised fasting plasma glucose (known type 2 diabetes) (36).

Supplementary tables on-line

Supplementary Table 1. Comparison of testicular volume, semen parameters and serum hormones by serum insulin concentration above or below 10μu/ml, all assessments made at 20 years of age. Data are represented as Median (IQR, R).

Variables of interest	N_{High}	**Insulin ≥ 10** μu/ml	N_{Normal}	**Insulin < 10** μu/ml	**p-value**
Testicular volume (ml)	28	14.8 (12.5-16.6, 10.0-23.8)	345	15.1 (12.7-17.3, 7.6-28.4)	0.646
Semen parameters					
Volume (ml)	22	2.9 (1.6-4.1, 0.8-7.2)	317	2.8 (2.0-3.7, 0.1-11.0)	0.799
Total sperm output (M)	22	134.4 (44.3-239.0, 0-436)	317	110.5 (51.6-206.8, 0.0-927.5)	0.578
Sperm concentration (M/ml)	22	45.5 (19.1-84.5, 0-160)	317	45.0 (22.5-70.5, 0-220)	0.795
SCSA (%)	21	2.9 (1.6-5.0, 1.1-10.8)	311	3.1 (1.8-5.2, 0.2-30.0)	0.658
Morphology (*N*, %)	21	6.0 (3.5-9.0, 2-17)	307	5 (3-7, 0-18)	0.159
Motility (a + b, %)	21	54.0 (44.5-69.0, 19-79)	314	58 (43-67, 1-88)	0.907
Serum hormones					
Testosterone (ng/mL)	51	3.6 (2.9-4.8, 1.1-7.1)	556	4.7 (3.7-5.9, 1.3-10.3)	**<0.001**
LH (IU/l)	51	9.9 (7.5-12.9, 5.6-18.8)	557	10.5 (8.3-13.1, 2.3-28.4)	0.287
FSH (IU/l)	51	5.2 (3.3-7.3, 1.1-25.8)	557	4.2 (2.9-6.0, 0.6-39.5)	**0.018**
InhB (pg/ml)	51	174.0 (136.7-222.3, 28.9-389.3)	558	218.6 (176.8-268.9, 4.5-543.9)	**<0.001**

Supplementary Table 2. Comparison of testicular volume, semen parameters and serum testicular hormones and gonadotrophins, assessed at 20 years of age, by ultrasound evidence of NAFLD, at 17 years of age. Data is represented as Median (IQR, R).

Outcome	N_{NAFLD}	NAFLD	N_{Normal}	No NAFLD	p-value
Testicular volume (ml)	28	15.0 (12.6-17.2, 10.5-23.2)	270	14.8 (12.6-17.3, 7.6-28.4)	0.821
Sperm parameters					
Semen volume (ml)	25	2.5 (1.6-3.3, 0.7-6.2)	255	2.8 (1.9-3.8, 0.3-11.0)	0.161
Total sperm output (M)	25	68.0 (23.2-170.9, 0.0-551.8)	255	126.0 (55.8-213.0, 0.0-927.5)	**0.044**
Sperm concentration (M/ml)	25	26 (15-69, 0-113)	255	47 (25-71, 0-220)	0.060
SCSA (%)	24	4.0 (1.8-5.9, 0.7-13.6)	249	3.0 (1.9-5.4, 0.2-30.0)	0.428
Sperm morphology (*N*, %)	24	5 (3-7, 1-17)	246	5 (3-7, 0-15)	0.979
Sperm motility (a + b, %)	24	56.5 (370-67.8, 6-82)	252	58 (43-67, 1-88)	0.591
Serum hormone concentrations					
Testosterone (ng/ml)	44	4.0 (3.2-5.0, 1.1-7.2)	391	4.7 (3.6-5.9, 1.3-9.9)	**0.005**
LH (IU/l)	44	8.8 (7.3-12.8, 5.4-18.9)	393	10.7 (8.6-13.4, 2.3-28.4)	0.054
FSH (IU/l)	44	3.6 (2.7-5.6, 1.2-14.3)	393	4.3 (3.1-6.3, 0.6-39.5)	0.428
InhB (pg/ml)	44	209.1 (145.85-253.2, 48.7-389.3)	393	218.4 (179.7-270.5, 4.5-543.9)	**0.032**

Supplementary Table 3. Comparison of testicular volume, semen parameters and serum hormones by serum hsCRP concentration by 75% percentile, all assessments made at 20 years of age. Data are represented as Median (IQR, R). Individuals with hsCRP>10 were excluded.

Variables of interest	N_{High}	**hsCRP ≥ 1.615 mg/l**	N_{Normal}	**hsCRP < 1.615 mg/l**	**p-value**
Testicular volume (ml)	60	15.4 (12.2-17.1, 8.9-23.5)	299	14.9 (12.9-17.2, 7.6-28.4)	0.955
Semen parameters					
Volume (ml)	53	2.5 (1.6-3.1, 0.7-6.8)	275	3.0 (2.0-3.8, 0.1-11.0)	**0.043**
Total sperm output (M)	53	84.8 (49.1-166.9, 0.0-639.2)	275	118.4 (50.4-214.6, 0.0-927.5)	0.127
Sperm concentration (M/ml)	53	35 (23-63, 0-180)	275	47.0 (21.5-74.0, 0-220)	0.209
SCSA (%)	51	2.9 (1.7-5.1, 0.7-10.8)	271	3.1 (1.9-5.4, 0.2-30.0)	0.606
Morphology (*N*, %)	51	5 (4-8, 2-13)	267	5 (3-7, 0-18)	0.239
Motility (a + b, %)	52	52.5 (40.3-65.8, 10.0-86.0)	273	58 (43-67, 1-88)	0.276
Serum hormones					
Testosterone (ng/ml)	126	4.0 (3.2-5.2, 1.1-8.1)	456	4.8 (3.8-6.0, 1.7-9.9)	**<0.001**
LH (IU/l)	126	9.5 (7.7-12.3, 4.3-19.5)	458	10.7 (8.6-13.2, 2.3-28.4)	**0.008**
FSH (IU/l)	126	3.9 (2.7-5.2, 0.6-14.3)	458	4.4 (3.0-6.4, 0.6-39.5)	**0.024**
InhB (pg/ml)	126	216.3 (156.0-260.2, 48.7-419.1)	458	216.2 (174.2-269.7, 4.5-543.9)	0.201

Supplementary Table 4 Multivariate regression analysis to derive which metabolic risk factor individually remains significant, after adjustments for age and BMI at 20 years, cryptorchidism, and presence of a varicocele, on the reproductive hormones measured at 20 years of age. Three separate models, with either; HOMA at 20 years, NAFLD or metabolic cluster at 20 years were examined for each outcome. Model summaries include univariable and multivariable (adjusted) regression coefficients with their corresponding 95% confidence intervals (95% CI) and p-values; together with R^2 obtained in univariable models and an increase in R^2 (denoted R^2 change) in adjusted models are shown. P-value for R^2 change is reported to indicate whether the simultaneous adjustment for other characteristics improves prediction of outcomes. All measurements other than assessment for presence of NAFL (assessed at 17 years of age), were taken at 20 years of age.

Outcome (Total n=260)	**Univariable**			**Multivariable**			
	β (95% CI)	***p*-value**	**R^2**	**Adjusted β (95% CI)**	***p*-value**	**R^2 change**	***p*-value for**
Testosterone							
HOMA-IR	-0.54 (-0.85, -0.24)	**<0.001**	0.036	-0.48 (-0.78,-0.18)	**0.002**	0.063	**<0.001**
NAFLD	-0.24 (-0.48, 0.009)	**0.004**	0.014	-0.12 (-0.37, 0.14)	0.364	0.050	**0.010**
Metabolic cluster	-0.43 (-0.62, -0.25)	**<0.001**	0.060	-0.28 (-0.50, -0.06)	**0.012**	0.026	0.056
FSH							
HOMA-IR	0.01 (-0.001, 0.034)	0.061	0.004	0.013 (-0.007, 0.034)	0.202	0.012	0.375
NAFLD	0.002 (-0.01, 0.01)	0.667	0.000	0.02 (-0.02, 0.01)	0.842	0.013	0.487
Metabolic cluster	0.003 (-0.01, 0.01)	0.547	0.002	0.01 (-0.01, 0.02)	0.546	0.015	0.275
Inhibin B							
HOMA-IR	-10.5 (-22.9, -6.15)	0.097	0.008	-8.7 (-20.8, 3.44)	0.160	0.074	**<0.001**
NAFLD	-3.0 (-12.7, 6.7)	0.545	0.001	1.1 (-8.9, 11.1)	0.829	0.075	**0.001**
Metabolic cluster	-11.3 (-22.0, -2.5)	**<0.001**	0.042	-11.3 (-22.0, -2.5)	**0.012**	0.050	**0.001**

NAFL = Non-alcoholic fatty liver diagnosed on ultrasound

HOMA = homeostasis model assessment insulin

Table 1. Flow of study participants. Total number of participants with measurements available are shown as (n=maximum number of participants) and the maximal number of participants for each outcome out of testicular volume, semen sample and blood sample assessment according to measurements taken during the various follow-ups listed. The presence of non-alcoholic fatty liver (NAFL) was derived from a previous study Ayonirynde et al (14) and the data identifying individuals within or without the high cardiometabolic risk cluster, derived using cluster analysis in a previous study Huang et al (13)

	N
Pregnant women enrolled in the Raine study	2900
Live births	2868
Male infants	1454
Female infants	1414

Male participants who had at least one of testicular ultrasound, semen or blood samples (n=648)		Testicular volume assessment performed (n=404)	Semen sample provided (n=365)	Serum available for reproductive hormones (n=609)
Participants who underwent 16/17 ± 20/21 follow-up	Total participants underwent assessment (n)	Testicular volume measurements available (n)	Semen sample parameters available (n)	Serum sample available for gonadotrophins and testosterone (n)
16/17 year follow up				
Serum cytokines assessment	(n=520)	319	290	478
Liver ultrasound for NAFL presence(14)	(n=587)	298	280	437
Serum available for full metabolic assessment (13)	(n=544)	289	264	439
20/21 year follow up				
Contactable	(n=913)			
Participated	(n=705)			
Anthropometric examination	(n=687)			
Blood pressure measured	(n=693)	391	360	603
Serum available for biochemistry	(n=620)	374	340	609
Serum available for full metabolic assessment	(n=608)	367	337	599
Serum for HOMA calculation	(n=618)	373	339	609
DEXA scan performed	(n=634)	362	333	557

NAFL = Non-alcoholic fatty liver diagnosed on ultrasound

HOMA = homeostasis model assessment insulin (fasting insulin [μu/ml] × fasting glucose [mM]/22.5).

DEXA = Dual energy X-ray absorptiometry

Table 2. Participant characteristics at 20 years of age – comparison between those who participated in at least one aspect of the testicular assessment and those who did not. Unless otherwise specified, data were collected at 20 years of age.

	Male participants n=648		**Male non-participants n=57**		***p*-value**
	N	**Median (IQR, R) or N (%)**	**N**	**Median (IQR, R) or N (%)**	
Age at 17 year follow-up	487	17.0 (16.9-17.1, 16.3-18.0)	33	17.0 (16.9-17.1, 16.7-17.3)	0.598
Age at 20 year follow-up	648	19.9 (19.7-20.3, 19.3-22.1)	57	19.9 (19.6-20.5, 19.4-21.7)	0.786
Anthropometric					
Height (cm)	632	180 (170-180, 162-199)	55	180 (180-190, 156-198)	0.550
Weight (kg)	632	75.9 (68.3-86.2, 52.2-137.5)	55	75.8 (69.0-86.1, 50.2-176.5)	0.884
BMI (kg/m^2)	632	23.6 (21.4-26.3, 16.7-48.9)	55	23.9 (21.5-25.5, 18.0-42.9)	0.887
under 25		405 (64.1%)		39 (70.9%)	0.543
25 - 30		155 (24.5%)		10 (18.2%)	
30 plus		72 (11.4%)		6 (10.9%)	
Waist circumference (cm)	632	80.5 (75.1-87.5, 43.8-145.5)	55	80.8 (74.3-88.3, 63.5-131.5)	0.995
Adiposity (DEXA)					
Total fat mass (g)	586	14935 (10519-2431, 3413-105957)	48	15349 (10604-20633, 6583-50244)	0.843
Total lean mass (g)	586	56702 (52052-61561, 33747-83318)	48	57479 (51526-63403, 38679-89622)	0.790
Soft tissue percentage[a]	586	21 (16-28, 6-63)	48	20 (16-28, 10-46)	0.771
Total fat percentage[b]	586	20 (15-27, 5-61)	48	19 (15-27, 10-45)	0.740
Biochemistry					
Fasting glucose (mmol/l)	616	5.0 (4.8-5.3, 3.1-8.2)	2	-	
Triglycerides (mmol/l)	616	1.0 (0.7-1.3, 0.3-17.8)	2	-	
HDL cholesterol (mmol/l)	616	1.2 (1.0-1.4, 0.6-2.6)	2	-	
LDL cholesterol (mmol/l)	616	2.4 (1.9-2.8, 0.2-5.3)	2	-	
Iron (umol/L)	617	16.1 (12.8-20.5, 3.0-40.7)	2	-	
Transferrin (umol/l)	617	31.6 (29.0-34.0, 21.2-46.5)	2	-	
Transferrin saturation (%)	617	26.2 (20.7-32.8, 4.7-84.1)	2	-	
Ferritin (ug/l)	392	87.8 (61.9-127.2, 6.3-326.9)	2	-	
Insulin (µu/ml)	616	2.0 (2.0-4.7, 2.0-64.3)	2	-	
High sensitivity CRP (mg/l)[c]	591	0.6 (0.3-1.4, 0.1-9.8)	2	-	
ALT (u/l)	616	30 (22-42, 10-372)	2	-	
GGT (u/l)	616	17 (14-23, 7-83)	2	-	
AST (u/l)	616	25 (22-31, 11-199)	2	-	
Adiponectin (mg/l)	616	7.6 (5.1-10.3, 0.6-34.6)	2	-	
Leptin (µg/l)	616	3.3 (1.7-7.0, 0-162.1)	2	-	
HOMA	616	0.5 (0.4-1.1, 0.3-16.3)	2	-	
HOMA>4[c]	616	24 (3.9%)	2	0	
Metabolic clusters					
High risk at 20yrs	606	76 (12.5%)	2	0	
High risk at 17 yrs	439	70 (15.9%)	15	2 (13.3%)	
Blood pressure					
Systolic (mm/Hg)	636	122 (114-132, 90-160)	57	123 (112-131, 91-152)	0.792
Diastolic (mm/Hg)	636	65 (59-71, 46-96)	57	64 (60-69, 47-90)	0.609
Serum reproductive hormones					
Testosterone (ng/mL)	607	4.6 (3.6-5.8, 1.1-10.3)			
LH (iu/l)	608	10.5 (8.3-13.0, 2.3-28.4)			
FSH (iu/l)	608	4.3 (3.0-6.2, 0.6-39.5)			
InhB (pg/ml)	609	216.4 (170.4-266.4, 4.5-543.9)			
Cytokines(at 17 yrs)					
IL18 (pg/ml)	496	288.4 (231.2-373.9, 0-3122)	23	263.6 (236.4-363.1, 153-1109)	0.802
sTNFR1 (pg/ml)	497	364.4 (286.1-462.8, 11-3549)	23	362.2 (293.7-420.4, 189-668)	0.874
sTNFR2 (pg/ml)	497	3180.4 (2636.3-3930.8, 24-9150)	23	3222.3 (2588.0-4057.2, 1930-5737)	0.853
Hepatic ultrasound (at 17 yrs)					
NAFLD	459	45 (9.8%)	31	4 (12.9%)	0.757
Tobacco and alcohol use					
Smoking^	494	78 (15.8%)	38	34 (15.8%)	1.000
Alcohol consumption^					
Nil	492	85 (17.3%)	37	6 (16.2%)	0.923
Moderate		249 (50.6%)		11 (29.7%)	
Binge		158 (32.1%)		58 (28.2%)	

[a] Total soft tissue fat percentage = fat mass x 100 / (fat mass + lean mass), [b] Total fat percentage = fat mass x 100 / (fat mass + lean mass + bone mineral content), [c] hsCRP>10 has been excluded. ^Smoking has 154 missing in the participants and 19 in the non-participants group. Alcohol consumption has 156 missing in the participants and 20 missing in the non-participants group.

Replaced with Table 3 and Table 3A

Replaced with new Table 4

Table 3A. Associations between reproductive and metabolic parameters at 20/21 years of age summarised using standardized beta coefficients and their 95% confidence intervals (CI). All analyses were adjusted for age at 20 years of age, history of cryptorchidism and varicocele (β_1), coefficients also adjusted for BMI at 20 years of age are shown as (β_2Semen parameters were also adjusted for abstinence period. Unless otherwise specified, data were collected at 20 years of age.

	Testis volume		Semen volume		Sperm output		Semen concentration	
	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)
Biochemistry								
Glucose	-0.043 (-0.143, 0.057)	-0.057 (-0.157, 0.044)	0.066 (-0.034, 0.166)	0.078 (-0.023, 0.179)	0.071 (-0.026, 0.168)	0.085 (-0.013, 0.182)	0.057 (-0.045, 0.159)	0.065 (-0.038, 0.168)
Triglycerides	-0.047 (-0.147, 0.053)	-0.070 (-0.173, 0.032)	-0.044 (-0.144, 0.057)	-0.029 (-0.132, 0.074)	-0.044 (-0.141, 0.053)	-0.027 (-0.127, 0.004)	-0.012 (-0.114, 0.090)	-0.003 (-0.108, 0.102)
HDL cholesterol	-0.027 (-0.126, 0.073)	-0.002 (-0.106, 0.103)	0.030 (-0.070, 0.130)	0.010 (-0.095, 0.115)	0.027 (-0.070, 0.124)	0.003 (-0.098, 0.105)	-0.010 (-0.092, 0.111)	-0.003 (-0.110, 0.103)
LDL cholesterol	0.028 (-0.073, 0.128)	0.009 (-0.094, 0.112)	-0.023 (-0.123, 0.078)	-0.007 (-0.111, 0.096)	-0.001 (-0.099, 0.096)	0.018 (-0.082, 0.118)	0.015 (-0.087, 0.118)	0.026 (-0.079, 0.131)
Iron	-0.019 (-0.119, 0.081)	-0.017 (-0.117, 0.083)	0.092 (-0.008, 0.192)	0.090 (-0.010, 0.191)	-0.012 (-0.010, 0.086)	-0.014 (-0.111, 0.084)	-0.059 (-0.161, 0.043)	-0.060 (-0.162, 0.042)
Transferrin	0.081 (-0.019, 0.180)	0.072 (-0.028, 0.172)	0.020 (-0.080, 0.120)	0.029 (-0.072, 0.129)	-0.067 (-0.167, 0.033)	-0.057 (-0.153, 0.040)	-0.075 (-0.177, 0.026)	-0.071 (-0.174, 0.031)
Transferrin saturation %	-0.046 (-0.147, 0.054)	-0.041 (-0.141, 0.059)	0.076 (-0.025, 0.177)	0.072 (-0.029, 0.173)	-0.001 (-0.098, 0.097)	-0.006 (-0.103, 0.092)	-0.039 (-0.141, 0.064)	-0.042 (-0.145, 0.061)
Ferritin	-0.040 (-0.141, 0.060)	-0.057 (-0.159, 0.045)	-0.049 (-0.150, 0.053)	-0.037 (-0.140, 0.066)	-0.060 (-0.158, 0.037)	-0.047 (-0.147, 0.052)	-0.031 (-0.133, 0.072)	-0.024 (-0.128, 0.081)
Insulin	**-0.116 (-0.215, -0.016)**	**-0.153 (-0.256, -0.049)**	-0.036 (-0.137, 0.065)	-0.017 (-0.122, 0.088)	-0.013 (-0.111, 0.084)	0.011 (-0.091, 0.113)	0.001 (-0.101, 0.104)	0.015 (-0.092, 0.122)
hsCRP†	-0.003 (-0.105, 0.099)	-0.029 (-0.135, 0.077)	**-0.113 (-0.216, -0.010)**	-0.101 (-0.208, 0.007)	-0.065 (-0.164, 0.035)	-0.046 (-0.149, 0.057)	-0.046 (-0.150, 0.058)	-0.037 (-0.146, 0.071)
ALT	-0.034 (-0.135, 0.066)	-0.061 (-0.165, 0.043)	**-0.106 (-0.207, -0.006)**	-0.093 (-0.202, 0.016)	-0.008 (-0.105, 0.090)	0.015 (-0.086, 0.117)	0.024 (-0.078, 0.127)	0.039 (-0.068, 0.145)
AST	-0.010 (-0.112, 0.092)	-0.041 (-0.149, 0.066)	-0.031 (-0.133, 0.072)	0.009 (-0.117, 0.098)	-0.045 (-0.143, 0.054)	-0.021 (-0.125, 0.082)	-0.031 (-0.135, 0.073)	-0.019 (-0.128, 0.090)
GGT	0.003 (-0.097, 0.103)	-0.003 (-0.103, 0.097)	-0.021 (-0.121, 0.080)	-0.015 (-0.116, 0.085)	-0.017 (-0.115, 0.080)	-0.011 (-0.108, 0.086)	-0.018 (-0.119, 0.084)	-0.014 (-0.117, 0.088)
Blood pressure								
Systolic	0.005 (-0.097, 0.103)	-0.028 (-0.132, 0.076)	0.094 (-0.003, 0.191)	**0.135 (0.032, 0.238)**	0.006 (-0.089, 0.100)	0.038 (-0.062, 0.139)	-0.039 (-0.138, 0.060)	-0.028 (-0.134, 0.078)
Diastolic	**-0.109 (-0.207, -0.011)**	**-0.124 (-0.223, -0.025)**	**0.112 (0.014, 0.209)**	**0.124 (0.026, 0.223)**	**0.100 (0.005, 0.194)**	**0.114 (0.018, 0.209)**	0.049 (-0.050, 0.149)	0.057 (-0.044, 0.157)
Cytokines at 16/17 yrs								
IL18	0.022 (-0.088, 0.132)	0.026 (-0.084, 0.135)	0.003 (-0.107, 0.112)	-0.001 (-0.110, 0.109)	**-0.116 (-0.222, -0.011)**	**-0.120 (-0.225, -0.015)**	**-0.129 (-0.239, -0.019)**	**-0.131 (-0.242, -0.011)**
sTNFR1	-0.089 (-0.197, 0.020)	-0.094 (-0.203, 0.014)	**-0.123 (-0.232, -0.015)**	**-0.118 (-0.228, -0.009)**	**-0.119 (-0.224, -0.013)**	**-0.113 (-0.219, -0.007)**	-0.045 (-0.157, 0.066)	-0.043 (-0.155, 0.068)
sTNFR2	-0.009 (-0.118, 0.101)	-0.013 (-0.122, 0.096)	-0.084 (-0.193, 0.026)	-0.081 (-0.190, 0.029)	-0.079 (-0.185, 0.027)	-0.076 (-0.181, 0.030)	-0.065 (-0.177, 0.048)	-0.063 (-0.176, 0.049)
DEXA								
Total fat %	-0.018 (-0.122, 0.086)	-0.129 (-0.266, 0.007)	-0.086 (-0.189, 0.018)	-0.068 (-0.205, 0.070)	-0.096 (-0.199, 0.007)	-0.073 (-0.206, 0.059)	-0.045 (-0.150, 0.060)	-0.029 (-0.169, 0.111)
Soft tissue fat %	-0.035 (-0.139, 0.069)	**-0.137 (-0.266, -0.008)**	-0.084 (-0.187, 0.020)	-0.064 (-0.201, 0.073)	-0.095 (-0.195, 0.005)	-0.072 (-0.205, 0.060)	-0.046 (-0.151, 0.060)	-0.030 (-0.170, 0.109)
Total fat mass	0.046 (-0.058, 0.149)	-0.041 (-0.196, 0.115)	-0.071 (-0.174, 0.032)	-0.040 (-0.196, 0.116)	**-0.102 (-0.202, -0.003)**	-0.094 (-0.245, 0.057)	-0.065 (-0.170, 0.039)	-0.076 (-0.234, 0.083)
Total lean mass	**0.299 (0.202, 0.397)**	**0.366 (0.249, 0.433) ***	0.096 (-0.005, 0.198)	**0.196 (0.076, 0.317)**	0.004 (-0.094, 0.103)	0.071 (-0.047, 0.189)	-0.055 (-0.159, 0.048)	-0.046 (-0.170, 0.079)
Metabolic syndrome								
HOMA	**-0.121 (-0.220, -0.021)**	**-0.157 (-0.260, -0.054)**	-0.032 (-0.133, 0.069)	-0.013 (-0.118, 0.092)	-0.004 (-0.101, 0.094)	0.021 (-0.081, 0.122)	-0.011 (-0.091, 0.114)	0.026 (-0.081, 0.132)

† hsCRP>10 are excluded (n=10); **effects significant at 0.05 level are shown in bold. *p<0.001**

Table 3B. (Table 3 continued) Associations between semen parameters and metabolic parameters at 20/21 years of age summarised using standardized beta coefficients and their 95% confidence intervals (CI). All analyses were adjusted for age at 20 years of age, history of cryptorchidism and varicocele (β_1), coefficients also adjusted for BMI at 20 years of age are shown as (β_2Semen parameters were also adjusted for abstinence period. Unless otherwise specified, data were collected at 20 years of age.

	SCSA		Morphology		Motility	
	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)
Biochemistry						
Glucose	0.011 (-0.094, 0.116)	0.006 (-0.100, 0.112)	0.042 (-0.064, 0.149)	0.038 (-0.070, 0.146)	0.080 (-0.023, 0.184)	0.081 (-0.024, 0.185)
Triglycerides	-0.065 (-0.170, 0.040)	-0.078 (-0.186, 0.030)	-0.018 (-0.124, 0.089)	-0.027 (-0.137, 0.083)	0.051 (-0.053, 0.154)	0.052 (-0.055, 0.159)
HDL cholesterol	-0.081 (-0.185, 0.024)	-0.077 (-0.186, 0.033)	0.031 (-0.076, 0.138)	0.045 (-0.067, 0.157)	-0.046 (-0.150, 0.057)	-0.048 (-0.157, 0.060)
LDL cholesterol	0.010 (-0.096, 0.115)	0.002 (-0.107, 0.110)	-0.039 (-0.147, 0.068)	-0.049 (-0.159, 0.061)	**0.117 (0.014, 0.221)**	**0.122 (0.016, 0.228)**
Iron	-0.011 (-0.117, 0.095)	-0.010 (-0.116, 0.095)	-0.054 (-0.162, 0.053)	-0.054 (-0.161, 0.054)	0.004 (-0.101, 0.108)	0.004 (-0.101, 0.108)
Transferrin	-0.026 (-0.130, 0.079)	-0.030 (-0.136, 0.075)	-0.011 (-0.117, 0.096)	-0.015 (-0.122, 0.093)	-0.055 (-0.159, 0.048)	-0.057(-0.161, 0.047)
Transferrin saturation %	-0.012 (-0.094, 0.118)	0.014 (-0.092, 0.121)	-0.055 (-0.162, 0.052)	-0.053 (-0.161, 0.055)	0.020 (-0.084, 0.125)	0.021 (-0.084, 0.126)
Ferritin	0.047 (-0.059, 0.153)	0.041 (-0.066, 0.149)	-0.065 (-0.173, 0.042)	-0.073 (-0.183, 0.036)	0.065 (-0.040, 0.169)	0.065 (-0.041, 0.172)
Insulin	-0.006 (-0.112, 0.100)	-0.018 (-0.129, 0.092)	0.048 (-0.059, 0.156)	0.042 (-0.070, 0.155)	0.037 (-0.067, 0.141)	0.038 (-0.071, 0.147)
hsCRP†	-0.027 (-0.134, 0.081)	-0.040 (-0.152, 0.072)	0.053 (-0.056, 0.162)	0.048 (-0.066, 0.161)	-0.008 (-0.115, 0.098)	-0.011 (-0.122, 0.100)
ALT	0.006 (-0.100, 0.111)	0.005 (-0.115, 0.105)	**0.149 (0.043, 0.255)**	**0.151 (0.040, 0.262)**	0.012 (-0.093, 0.116)	0.010 (-0.098, 0.119)
AST	-0.030 (-0.137, 0.077)	-0.045 (-0.158, 0.067)	0.069 (-0.043, 0.181)	0.067 (-0.051, 0.185)	-0.010 (-0.116, 0.096)	-0.014 (-0.125, 0.098)
GGT	-0.044 (-0.149, 0.061)	-0.047 (-0.152, 0.058)	**0.122 (0.016, 0.228)**	**0.120 (0.013, 0.226)**	0.024 (-0.079, 0.128)	0.024 (-0.080, 0.128)
Blood pressure						
Systolic	-0.001 (-0.103, 0.101)	-0.016 (-0.125, 0.093)	-0.002 (-0.106, 0.102)	-0.015 (-0.126, 0.096)	0.024 (-0.077, 0.124)	0.024 (-0.084, 0.132)
Diastolic	-0.007 (-0.110, 0.096)	-0.012 (-0.117, 0.092)	-0.049 (-0.154, 0.056)	-0.055 (-0.166, 0.057)	0.059 (-0.043, 0.160)	0.059 (-0.044, 0.161)
Cytokines at 16/17 yrs						
IL18	0.017 (-0.098, 0.132)	0.019 (-0.097, 0.134)	-0.009 (-0.126, 0.108)	-0.007 (-0.124, 0.110)	0.0003 (-0.113, 0.114)	0.001 (-0.113, 0.115)
sTNFR1	-0.030 (-0.145, 0.084)	-0.033 (-0.148, 0.082)	-0.034 (-0.150, 0.083)	-0.036 (-0.153, 0.081)	-0.093 (-0.206, 0.019)	-0.094 (-0.207, 0.019
sTNFR2	0.071 (-0.044, 0.186)	0.070 (-0.046, 0.185)	0.009 (-0.108, 0.127)	0.008 (-0.110, 0.125)	-0.050 (-0.163, 0.064)	-0.050 (-0.164, 0.064
DEXA						
Total fat %	-0.012 (-0.121, 0.097)	-0.064 (-0.209, 0.080)	0.062 (-0.048, 0.172)	0.070 (-0.077, 0.216)	0.052 (-0.056, 0.159)	0.082 (-0.060, 0.225)
Soft tissue fat %	-0.010 (-0.119, 0.099)	-0.061 (-0.205, 0.083)	0.061 (-0.050, 0.171)	0.067 (-0.079, 0.213)	0.051 (-0.057, 0.158)	0.080 (-0.062, 0.222)
Total fat mass	0.007 (-0.102, 0.115)	-0.049 (-0.214, 0.115)	0.045 (-0.065, 0.155)	0.046 (-0.121, 0.213)	0.038 (-0.069, 0.145)	0.074 (-0.088, 0.237)
Total lean mass	0.043 (-0.064, 0.150)	0.033 (-0.096, 0.161)	-0.019 (-0.127, 0.090)	-0.054 (-0.184, 0.077)	-0.038 (-0.144, 0.067)	-0.061 (-0.188, 0.066)
Metabolic syndrome						
HOMA	-0.002 (-0.107, 0.104)	-0.013 (-0.123, 0.097)	0.052 (-0.055, 0.160)	0.047 (-0.065, 0.159)	0.046 (-0.058, 0.151)	0.048 (-0.061, 0.157)

† hsCRP>10 are excluded (n=10); **effects significant at 0.05 level are shown in bold.**

Table 4. Associations between serum testicular hormones and gonadotrophins and metabolic parameters at 20/21 years of age summarised using standardized beta coefficients and their 95% confidence intervals (CI). All beta coefficients were adjusted for age at 20 years of age, history of cryptorchidism and varicocele were made in all analyses (β_1) and separate coefficients are shown with additional adjustment for BMI at 20 years of age (β_2). Unless otherwise specified, data were collected at 20/21 years of age.

	Testosterone		**Inh B**		**LH**		**FSH**	
	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)	β_1 (95% CI)	β_2 (95% CI)
Biochemistry								
Glucose	-0.038 (-0.119, 0.043)	0.002 (-0.077, 0.081)	-0.049 (-0.129, 0.032)	-0.014 (-0.093, 0.065)	-0.018 (-0.098, 0.063)	-0.009 (-0.090, 0.072)	0.003 (-0.077, 0.084)	0.006 (-0.076, 0.087)
Triglycerides	**-0.128 (-0.209, -0.048)**	-0.068 (-0.148, 0.012)	**-0.151 (-0.231, -0.072)***	**-0.101 (-0.181, -0.021)**	0.036 (-0.045, 0.116)	0.053 (-0.030, 0.135)	0.033 (-0.047, 0.114)	0.039 (-0.044, 0.121)
HDL cholesterol	**0.201 (0.122, 0.281) ***	**0.132 (0.051, 0.213) ***	**0.095 (0.015, 0.175)**	0.027 (-0.054, 0.109)	-0.007 (-0.087, 0.073)	-0.027 (-0.111, 0.057)	-0.024 (-0.104, 0.056)	-0.031 (-0.115, 0.053)
LDL cholesterol	-0.025 (-0.106, 0.057)	0.038 (-0.042, 0.119)	-0.055 (-0.136, 0.026)	-0.002 (-0.083, 0.078)	-0.057 (-0.138, 0.024)	-0.046 (-0.129, 0.037)	-0.020 (-0.101, 0.061)	-0.018 (-0.101, 0.065)
Iron	**0.173 (0.092, 0.253) ***	**0.166 (0.089, 0.243) ***	0.014 (-0.067, 0.094)	0.008 (-0.071, 0.086)	-0.045 (-0.126, 0.036)	-0.047 (-0.127, 0.034)	-0.056 (-0.136, 0.025)	-0.056 (0.137, 0.025)
Transferrin	-0.011 (-0.092, 0.070)	0.021 (-0.058, 0.099)	0.014 (-0.066, 0.095)	0.042 (-0.036, 0.121)	-0.031 (-0.111, 0.049)	-0.024 (-0.105, 0.057)	-0.035 (-0.116, 0.045)	-0.034 (-0.115, 0.047)
Transferrin saturation %	**0.167 (0.086, 0.247) ***	**0.149 (0.071, 0.227) ***	0.012 (-0.069, 0.093)	-0.004 (-0.083, 0.075)	-0.032 (-0.113, 0.049)	-0.036 (-0.118, 0.045)	-0.039 (-0.120, 0.042)	-0.040 (-0.121, 0.041)
Ferritin	**-0.095 (-0.176, -0.013)**	-0.048 (-0.128, 0.032)	-0.011 (-0.093, 0.070)	0.031 (-0.049, 0.111)	-0.030 (-0.111, 0.051)	-0.020 (-0.103, 0.062)	-0.025 (-0.106, 0.056)	-0.023 (-0.106, 0.059)
Insulin	**-0.241 (-0.320, -0.162)***	**-0.177 (-0.258, -0.097)***	**-0.211(-0.289, -0.132)***	**-0.155(-0.236, -0.074)***	-0.012 (-0.093, 0.069)	0.005 (-0.079, 0.090)	0.072 (-0.009, 0.152)	0.083 (-0.002, 0.167)
hsCRP[†]	**-0.249 (-0.329, -0.169)***	**-0.187 (-0.268, -0.106)***	-0.024 (-0.106, 0.058)	0.046 (-0.037, 0.129)	-0.073 (-0.155, 0.008)	-0.061 (-0.146, 0.024)	**-0.123 (-0.204, -0.042)**	**-0.129 (-0.214, -0.045)**
ALT	**-0.116 (-0.197, -0.035)**	-0.045 (-0.127, 0.036)	**-0.126 (-0.206, -0.045)**	-0.067 (-0.148, 0.015)	-0.016 (-0.097, 0.065)	-0.0001 (-0.084, 0.084)	-0.004 (-0.085, 0.077)	-0.001 (-0.085, 0.084)
AST	**-0.155 (-0.236, -0.074)**	-0.076 (-0.159, 0.008)	**-0.149 (-0.230, -0.068)**	-0.083 (-0.166, 0.001)	-0.017 (-0.099, 0.065)	0.002 (-0.084, 0.088)	0.038 (-0.044, 0.120)	0.047 (-0.039, 0.133)
GGT	-0.013 (-0.094, 0.068)	0.007 (-0.071, 0.085)	-0.006 (-0.087, 0.074)	0.011 (-0.067, 0.090)	-0.024 (-0.104, 0.057)	-0.028 (-0.135, 0.078)	-0.056 (-0.136, 0.024)	-0.055 (-0.136, 0.025)
Blood pressure								
Systolic	**-0.084 (-0.165, -0.004)**	0.012 (-0.071, 0.095)	**-0.129 (-0.208, -0.049)**	-0.053 (-0.136, 0.030)	0.002 (-0.079, 0.082)	0.026 (-0.060, 0.111)	0.037 (-0.043, 0.118)	0.048 (-0.038, 0.134)
Diastolic	**-0.086 (-0.167, -0.004)**	-0.047 (-0.127, 0.032)	-0.075 (-0.156, 0.006)	-0.042 (-0.122, 0.037)	0.050 (-0.032, 0.131)	0.059 (-0.022, 0.141)	0.062 (-0.019, 0.143)	0.065 (-0.017, 0.147)
Cytokines at 16/17 yrs								
IL18	-0.020 (-0.112, 0.072)	-0.033 (-0.121, 0.056)	0.020 (-0.071, 0.111)	0.009 (-0.079, 0.098)	0.066 (-0.025, 0.157)	0.063 (-0.028, 0.154)	-0.012 (-0.104, 0.079)	-0.013 (-0.104, 0.978)
sTNFR1	-0.027 (-0.119, 0.064)	-0.010 (-0.099, 0.078)	**-0.128 (-0.218, -0.038)**	**-0.114 (-0.202, -0.026)**	**0.108 (0.018, 0.198)**	**0.112 (0.022, 0.202)**	**0.153 (0.063, 0.242)***	**0.154 (0.064, 0.244)***
sTNFR2	-0.004 (-0.095, 0.088)	0.011 (-0.078, 0.099)	-0.011 (-0.102, 0.079)	-0.001 (-0.087, 0.090)	0.039 (-0.051, 0.130)	0.043 (-0.048, 0.134)	0.037 (-0.054, 0.128)	0.038 (-0.053, 0.129)
DEXA								
Total fat %	**-0.243 (-0.327, -0.159)***	-0.105 (-0.214, 0.005)	**-0.137 (-0.222, -0.052)**	0.036 (-0.074, 0.146)	**-0.120 (-0.205, -0.035)**	**-0.139 (-0.252, -0.027)**	-0.076 (-0.161, 0.010)	**-0.115 (-0.228, -0.002)**
Soft tissue fat %	**-0.241(-0.325, -0.157)***	-0.103 (-0.212, 0.006)	**-0.139 (-0.224, -0.054)**	0.031 (-0.079, 0.140)	**-0.121 (-0.206, -0.036)**	**-0.140 (-0.252, -0.028)**	-0.074 (-0.159, 0.012)	-0.111 (-0.223, 0.001)
Total fat mass	**-0.271 (-0.354, -0.188)***	**-0.143 (-0.268, -0.019)**	**-0.175 (-0.259, -0.091)***	0.010 (-0.114, 0.135)	**-0.112 (-0.197, -0.027)**	**-0.150 (-0.277, -0.022)**	-0.058 (-0.143, 0.028)	-0.106 (-0.234, 0.022)
Total lean mass	-0.038 (-0.122, 0.047)	**0.167 (0.070, 0.265)***	**-0.139 (-0.222, -0.055)***	-0.009 (-0.108, 0.089)	0.052 (-0.032, 0.136)	**0.124 (0.023, 0.224)**	**0.086 (0.002, 0.170)**	**0.136 (0.036, 0.237)**
Metabolic syndrome								
HOMA	**-0.237 (-0.341, -0.133)***	**-0.172 (-0.278, -0.066)**	**-0.203 (-0.282, -0.124)***	**-0.149(-0.230, -0.068)***	-0.016 (-0.097, 0.065)	0.001 (-0.083, 0.085)	0.069 (-0.011, 0.150)	0.080 (-0.004, 0.164)

[†] hsCRP>10 are excluded (n=10); **effects significant at 0.05 level are shown in bold. *p = or <0.001**

Table 5. Comparison of testicular volume, semen parameters and serum hormones by metabolic clusters, all assessments made at 20 years of age in the top part of the table, and metabolic cluster analysis and associations at 17 years of age are listed in the lower part of the table. Data is represented as Median (IQR, R) and Mean (SD) as appropriate.

Cluster parameters at 20 years of age	**N High**	**High risk at 20 years of age [Mean (SD)]**	**N_{Low}**	**Low risk at 20 years of age [Mean (SD)]**	**p-value**
Systolic blood pressure (mm/Hg)	43	130.8 (10.6)	342	121.8 (12.2)	**<0.001**
Insulin (µu/ml)	43	14.2 (12.8)	342	3.2 (2.2)	**<0.001**
Triglycerides (mmol/l)	43	1.8 (1.1)	342	1.0 (0.4)	**<0.001**
Waist circumference (cm)	43	100.2 (12.9)	342	80.2 (7.6)	**<0.001**
		Metabolic cluster at 20 years of age			
Testicular function assessment at 20 years		**High risk at 20 years of age [Median (IQR, R)]**		**Low risk at 20 years of age [Median (IQR, R)]**	
Testicular volume (ml)	42	14.7 (12.3-16.9, 9.0-23.8)	325	15.2 (13.0-17.4, 7.6-28.4)	0.574
Semen parameters					
Volume (ml)	34	2.7 (1.9-4.0, 0.9-7.2)	303	2.8 (1.9-3.7, 0.1-11.0)	0.979
Total sperm output (M)	34	115.3 (51.0-194.0, 0.0-551.8)	303	113.4 (50.6-207.0, 0.0-927.5)	0.738
Sperm concentration (M/mL)	34	42.5 (19.4-70.5, 0-142)	303	46 (23-73, 0-220)	0.663
SCSA (%)	32	2.5 (1.5-4.7, 0.6-10.8)	298	3.1 (1.9-5.2, 0.2-30.0)	0.106
Morphology (*N*, %)	32	5.5 (3.6-9.0, 3-17)	294	5 (3-7, 0-18)	0.144
Motility (a + b, %)	33	58.0 (43.5-70.5, 19-86)	300	58 (44-67, 1-88)	0.773
Serum hormones					
Testosterone (ng/ml)	75	3.6 (3.0-4.0, 1.1-6.5)	522	4.8 (3.8-5.9, 1.3-10.3)	**<0.001**
LH (IU/l)	76	6.7 (7.6-12.8, 5.2-19.3)	522	10.5 (8.3-13.1, 2.3-28.4)	0.097
FSH (IU/l)	76	4.4 (2.9-6.8, 0.8-25.8)	522	4.3 (3.0-6.1, 0.6-39.5)	0.492
InhB (pg/ml)	76	167.9 (132.1-217.0, 28.9-389.3)	523	223.7 (180.6-272.9, 4.5-543.9)	**<0.001**
		Metabolic cluster at 17 years of age			
Testicular function assessment at 20 years		**High risk at 16/17 yrs of age [Median (IQR, R)]**		**Low risk at 16/17 yrs of age [Median (IQR, R)]**	
Testicular volume (ml)	39	15.6 (13.3-17.5, 10.1-23.2)	249	14.7 (12.6-17.1, 8.0-28.4)	0.215
Semen parameters					
Volume (ml)	37	2.5 (1.6-3.6, 0.3-11.0)	227	2.8 (2.0-3.6, 0.7-7.5)	0.347
Total sperm output (M)	37	110.7 (52.2-288.9, 0.0-592.2)	227	122.2 (56.0-217.6, 0.0-927.5)	0.711
Sperm concentration (M/mL)	37	50 (26.5-88.5, 0-220)	227	47 (23-71, 0-210)	0.280
SCSA (%)	35	3.6 (1.8-6.5, 0.7-30)	222	3.3 (1.9-5.5, 0.2-19.0)	0.416
Morphology (*N*, %)	35	5.0 (3.0-7.0, 0.5-17)	219	4.5 (3.0-7.0, 0.5-18.0)	0.782
Motility (a + b, %)	36	51.0 (38.5-65.8, 7.0-88.0)	224	59.0 (43.3-68.0, 7.0-88.0)	0.170
Serum hormones					
Testosterone (ng/ml)	67	4.0 (3.2-4.9, 1.6-7.2)	356	4.9 (3.6-6.0, 1.8-9.9)	**<0.001**
LH (IU/l)	68	10.1 (7.8-13.9, 5.4-19.8)	357	10.6 (8.6-13.2, 4.3-28.4)	0.425
FSH (IU/l)	68	4.4 (3.4-6.8, 1.1-14.3)	357	4.3 (3.0-6.2, 0.8-39.5)	0.285
InhB (pg/ml)	68	193.2 (144.8-226.5, 48.7-389.3)	357	221.9 (180.3-269.0, 56.7-543.9)	**<0.001**

Table 6. Comparison of testicular volume, semen parameters and serum testicular hormones and gonadotrophins by HOMA-IR, all assessments made at 20 years of age. Data is represented as Median (IQR, R).

Testicular function assessment	**N_{IR}**	**IR (HOMA>4)**	**N_{Normal}**	**Normal (HOMA≤4)**	**p-value**
Testicular volume (ml)	14	12.8 (11.1-14.7, 10.0-16.9)	359	15.2 (13.0-17.4, 7.6-28.4)	**0.010**
Sperm parameters					
Semen volume (ml)	13	2.6 (1.5-3.6, 0.9-4.2)	326	2.8 (1.9-3.8, 0.1-11.0)	0.320
Total sperm output (M)	13	136.8 (81.0-253.9, 0.0-383.4)	326	110.6 (50.6-206.7, 0.0-927.5)	0.459
Sperm concentration (M/ml)	13	64.0 (30.0-88.5, 0-160)	326	44.5 (22.0-70.3, 0-220)	0.293
SCSA (%)	12	2.8 (1.5-5.4, 1.4-10.8)	320	3.1 (1.8-5.2, 0.2-30.0)	0.654
Sperm morphology (*N*, %)	12	5.5 (3.3-10.0, 3-17)	316	5 (3-7, 0-18)	0.402
Sperm motility (a + b, %)	12	63 (47.3-75.8, 26-79)	323	58 (43-67, 1-88)	0.330
Serum hormone concentrations					
Testosterone (ng/ml)	24	3.2 (2.6-4.0, 1.1-5.3)	583	4.6 (3.7-5.9, 1.3-10.3)	**<0.001**
LH (IU/l)	24	10.6 (8.2-13.3, 6.3-17.1)	584	10.5 (8.3-13.0, 2.3-28.4)	0.903
FSH (IU/l)	24	6.1 (3.4-7.9, 1.1-14.3)	584	4.3 (3.0-6.1, 0.6-39.5)	**0.046**
InhB (pg/ml)	24	172.4 (130.0-213.4, 54.8-389.3)	585	217.8 (174.0-267.6, 4.5-543.9)	**0.001**

HOMA = homeostasis model assessment insulin (Fasting insulin [μu/ml] × Fasting glucose [mM]/22.5).