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ORIGINAL ARTICLE *Andrology*

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

R.J. Hart[1,2,*], D.A. Doherty[1,3], T.A. Mori[4], L.A. Adams[5], R.-C. Huang[6], N. Minaee[3], D.J. Handelsman[7], R. McLachlan[8], R.J. Norman[9], J.E. Dickinson[1], J.K. Olynyk[10], and L.J. Beilin[4]

[1]Division of Obstetrics and Gynaecology, University of Western Australia (UWA), Perth, Western Australia, Australia [2]Fertility Specialists of Western Australia, Claremont, Western Australia, Australia [3]Women and Infants Research Foundation, Perth, Western Australia, Australia [4]Medical School, UWA, Royal Perth Hospital, Western Australia, Australia [5]Medical School, UWA, Sir Charles Gairdner Hospital, Nedlands, Western Australia, Australia [6]Telethon Kids Institute, UWA, Perth, Western Australia, Australia [7]ANZAC Research Institute, University of Sydney, Sydney, New South Wales, Australia [8]Hudson Institute, Monash University, Melbourne, Australia [9]Robinson Institute, Research Institute, School of Medicine, University of Adelaide & Fertility SA, Adelaide, Australia [10]Department of Gastroenterology, Fiona Stanley and Fremantle Hospital and School of Medical and Health Sciences, Edith Cowan University, Joondalup, Western Australia, Australia

*Correspondence address. Division of Obstetrics & Gynaecology, King Edward Memorial Hospital, 374 Bagot Road, Subiaco, Western Australia 6008, Australia. Tel: +61-89-340-1322; E-mail: roger.hart@uwa.edu.au

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STUDY QUESTION: Are early signs of metabolic disorder in late adolescence associated with features of impaired testicular function many years before the majority seek parenthood?

SUMMARY ANSWER: Adolescents with features of metabolic disorder at 17 years, or insulin resistance (IR) at 20 years of age, show impaired testicular function and altered hormone levels compared to those 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. Concomitant increases in obesity and diabetes suggest 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 merely 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 maybe a precursor of 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). At 17 years of age, 490 boys 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 at 20 years; 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 was performed according to World Health Organization guidelines. Concentrations of LH, FSH and inhibin B (inhB) in serum were measured by immunoassay and total testosterone by liquid chromatography-mass spectrometry.

At 17 years of age, a liver ultrasound examination was performed to determine the presence of non-alcoholic fatty liver disease (NAFLD), and serum analysed for the cytokines interleukin-18 and soluble tumour necrosis factor receptor 1 and 2 (sTNFR1, sTNFR2).

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 and low density lipoprotein cholesterol, high sensitivity C-reactive protein and uric acid. The homoeostatic model assessment (HOMA) was calculated and approximated IR was defined by a HOMA >4. Anthropometric data was collected and dual energy X-ray absorptiometry measurement performed for lean and total fat mass. As at this young age the prevalence of metabolic syndrome

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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 and increased cardiometabolic risk.

MAIN RESULTS AND THE ROLE OF CHANCE: Men at age 17 years with increased cardiometabolic risk had lower concentrations of serum testosterone (medians: 4.0 versus 4.9 ng/mL) and inhB (193.2 versus 221.9 pg/mL) ($P < 0.001$ for both) compared to those within the low risk metabolic cluster. Men with ultrasound evidence of NAFLD ($n = 45$, 9.8%) had reduced total sperm output (medians: 68.0 versus 126.00 million, $P = 0.044$), testosterone (4.0 versus 4.7 ng/mL, $P = 0.005$) and inhB (209.1 versus 218.4 pg/mL, $P = 0.032$) compared to men without NAFLD.

Men with higher concentrations of sTNFR1 at 17 years of age had a lower sperm output and serum concentration of inhB, with an increase in LH and FSH (all $P < 0.05$ after adjustment for age, BMI, abstinence and a history of cryptorchidism, varicocele, cigarette smoking, alcohol and drug use), compared to those without an elevated sTNFR1. Multivariable regression analysis, adjusting for confounders, demonstrated that men in the high-risk metabolic cluster at 20 years had a lower serum testosterone and inhB ($P = 0.003$ and $P = 0.001$, respectively). A HOMA-IR > 4 was associated with a lower serum testosterone ($P = <0.001$) and inhB ($P = 0.010$) and an increase in serum FSH ($P = 0.015$).

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 3-year interval between some metabolic assessments and assessment of testicular function, we cannot exclude the 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

Introduction

There is an ongoing debate as to whether there has been a general decline in sperm production in recent times, (Handelsman and Cooper, 2013; Skakkebaek *et al.*, 2016; Levine *et al.*, 2017). The parallel increase in the rates of lifestyle related disorders, such as obesity and diabetes (Finucane *et al.*, 2011), raises the possibility that lifestyle factors may contribute to any potential decline in sperm production. In populations of men seeking fertility treatment, the evidence supports an association of obesity (Kort *et al.*, 2006; Ramlau-Hansen *et al.*, 2007; Hammoud *et al.*, 2008; Belloc *et al.*, 2014; Ventimiglia *et al.*, 2016) and the metabolic syndrome with impaired testicular function (Eisenberg *et al.*, 2015; Ventimiglia *et al.*, 2016). However, it is unclear as to whether these disorders have a common origin in early life (Hart *et al.*, 2016), 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 (Huang *et al.*, 2009), many will have ultrasound evidence of a fatty liver (Ayonrinde *et al.*, 2011). Non-alcoholic fatty liver disease (NAFLD) is the most prevalent chronic liver disorder, affecting almost one in five adolescents (Schwimmer *et al.*, 2006), and is a recognized antecedent of progressive liver disease and cardiometabolic disorder (Fazel *et al.*, 2016). Hyperinsulinemia, or the presence of a fatty liver (Simo *et al.*, 2015), is associated with a reduction in hepatic synthesis of sex hormone-binding globulin, 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 (IL)-6, tumour necrosis factor-α (TNF-α), and its receptors 1 and 2 (TNFR1 and TNFR2), as well as the production of oxygen free radicals, all of which may impair sperm and testicular function (Bobjer *et al.*, 2013; Aitken, 2017). We therefore proposed that impaired testicular function may reflect or cause poor metabolic health.

Our study was driven by the question of whether or not, in a young adult population, representative of the Western Australian population (Straker *et al.*, 2017), the 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 markers of adverse cardiometabolic health, in adolescence and early

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adulthood, to markers of testicular function in 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 at around 18 weeks of gestation in 1989–1991 (Newnham *et al.*, 1993; Straker *et al.*, 2017). A total of 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 testicular assessment by ultrasound and/or semen examinations ($n = 423$) (Hart *et al.*, 2015; Straker *et al.*, 2017). 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.

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, LH, FSH and inhibin B (inhB) concentrations ($n = 609$). A testicular ultrasound examination was performed ($n = 404$), and a semen sample ($n = 365$) analysed at the Fertility Specialists of Western Australia, as previously reported (Hart *et al.*, 2015). Semen samples were analysed as per the World Health Organization semen manual guidelines (Cooper *et al.*, 2010) including sperm concentrations (million/mL), total sperm output (million per ejaculate), motility (%A grade + %B grade) and morphology. The sperm chromatin structural assay was performed (Evenson and Jost, 2000), 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, USA); LH and FSH were measured by ELISA (IBL International, Hamburg, Germany), and testosterone was measured by liquid chromatography–tandem mass spectrometry, as described (Harwood and Handelsman, 2009) (for further details refer to Supplementary Data) Testicular ultrasonography was performed as described (Hart *et al.*, 2015), and the volume of each testis calculated (Sakamoto *et al.*, 2007). Varicocele was defined as present when the maximal venous diameter was over 3 mm, and increased with the Valsalva manoeuver (Lenz *et al.*, 1993).

Metabolic assessment at 17 years of age

Hepatic ultrasound

The methods of hepatic ultrasound examinations conducted among 587 cohort members at age 17 years for diagnosing NAFL have been reported previously (Ayonrinde *et al.*, 2011), and the data was used in this study.

Cytokine assessment

The serum from 520 cohort members was stored at −80°C and the cytokine IL-18 and soluble TNF receptors (sTNFR1, sTNFR2) were measured using a commercially available ELISA and cytometric bead array, respectively. Individual cytokine concentrations were determined using FCAP Array software (BD Biosciences, San Jose, CA, USA) (see Supplementary Data).

Cardiometabolic assessment

Data from a previous publication (Huang *et al.*, 2009) 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, high density lipoprotein (HDL) and low density lipoprotein cholesterol, high sensitivity CRP (hsCRP), and uric acid, as previously described (Huang *et al.*, 2009; Black *et al.*, 2016), excluding serum hsCRP concentrations >10 mg/L (Huang *et al.*, 2009; Black *et al.*, 2016). Glucose, insulin, total cholesterol and TG were measured by automated analysers (Supplementary Data). Homoeostatic model assessment (HOMA) was calculated as fasting insulin (microunits per millilitre) × fasting glucose (millimoles per liter)/22.5, and insulin resistance (IR) was defined by a HOMA >4 (Matthews *et al.*, 1985). Resting blood pressure (BP) readings were taken (Supplementary Data). The cardiometabolic data was used to derive a 'high-risk metabolic cluster' as phenotyped previously in this cohort (Huang *et al.*, 2009), and described below.

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 protocols used at 17 years. To assess body fat distribution dual energy X-ray absorptiometry (DEXA) measurement was performed (Flegal *et al.*, 2009; Demmer *et al.*, 2016).

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) (Expert Panel on Detection, 2001), and the International Diabetes Federation (IDF) definitions (Alberti *et al.*, 2006), which differ significantly (Supplementary Data). 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, namely a two-step cluster analysis, was used (Huang *et al.*, 2009, 2012; Hart *et al.*, 2011). This is an effective tool to define groups by variables when 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 (Zhang and Livny, 1996), designed specifically to handle large data sets and has been used previously within this cohort (Huang *et al.*, 2009, 2012; Hart *et al.*, 2011). It preselects subjects into sub-clusters before further grouping into the desired number of clusters by use of log-likelihood distance. The cluster groups were formed by using waist circumference, TGs, insulin and systolic BP measured at 20 years of age to derive a 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 (Huang *et al.*, 2009).

Data analysis

Continuous data were summarized using medians and interquartile 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 standardized coefficients (β) and their 95% 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).

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Reproductive outcomes; semen parameters, serum hormones and testicular volume 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 the 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 (Lew, 2016; Wasserstein and Lazar, 2016). SPSS (version 22.0, IBM SPSS, St. Leonards, New South Wales, Australia) statistical software was used for data analysis.

Results

Demographics

Of the 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. A total of 404 underwent a testicular ultrasound and 609 had serum available for reproductive hormone assessment (Table I). Most (459) 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 I). Participants who took part in the testicular assessment (semen sample and/or testicular ultrasound) were similar clinically to those that declined participation (Table II). There was no difference between the participants and the non-participants with respect to markers of socio-economic status (data not shown). The prevalence of metabolic syndrome among males within the participants was 4.1% by the NCEP-ATPIII definition (Expert Panel on Detection, 2001) and 5.4% using the IDF definition (Alberti *et al.*, 2006).

Associations between markers of metabolic disorder and testicular parameters

Metabolic indices at 17 years

Multivariable linear regression analysis adjusting for current age, sexual abstinence, a history of cryptorchidism, presence of a varicocele, and

Table I Flow of study participants.

				N
Pregnant women enroled 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 17- and/or 20-year follow-up	Total participants underwent assessment	Testicular volume measurements available	Semen sample parameters available	Serum sample available for gonadotrophins and testosterone
17 years follow up				
Serum cytokines assessment	520	319	290	478
Liver ultrasound for NAFL presence (Ayonrinde *et al.*, 2011)	587	298	280	437
Serum available for full metabolic assessment (Huang *et al.*, 2009)	544	289	264	439
20 years follow up				
Contactable	913			
Participated	705			
Anthropometric examination	687			
Blood pressure measured	693	391	360	603
Serum available for biochemistry	620	374	340	609
Serum available for full metabolic assessment	608	367	337	599
Serum for HOMA calculation	618	373	339	609
DEXA scan performed	634	362	333	557

HOMA = homoeostatic model assessment (fasting insulin [μu/mL] × fasting glucose [mM]/22.5).
DEXA = Dual energy X-ray absorptiometry.
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, diagnosed on ultrasound) was derived from a previous study (Ayonrinde *et al.*, 2011) and the data identifying individuals within or without the high cardiometabolic risk cluster, derived using cluster analysis in a previous study (Huang *et al.*, 2009).

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Table II Participant characteristics at 20 years of age: a comparison between those who participated in at least one aspect of the testicular assessment and those who did not.

	Male participants		Male non-participants		*P*-value
	n = 648		*n* = 57		
	N	Median (IQR, *R*) or *N* (%)	*N*	Median (IQR, *R*) or *N* (%)	
Age at 17 years 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 years 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 20 years	606	76 (12.5%)	2	0	
High risk at 17 years	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)			

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Table II *Continued*

	Male participants		Male non-participants		*P*-value
	n = 648		*n* = 57		
	N	**Median (IQR, *R*) or *N* (%)**	***N***	**Median (IQR, *R*) or *N* (%)**	
Cytokines (at 17 years)					
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 years)					
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 × 100/(fat mass + lean mass), [b]total fat percentage = fat mass × 100/(fat mass + lean mass + bone mineral content), [c]high sensitivity C-reactive protein (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. *P*-values were obtained using Mann Whitney tests (continuous outcomes) and Chi-square tests (categorical outcomes). Unless otherwise specified, data were collected at 20 years of age.

ALT= alanine transaminase; AST = asparagine transaminase; CRP = C-reactive protein; DEXA = dual energy X-ray absorptiometry; GGT = gamma glutamine transaminase; HDL = high density lipoprotein (HDL); HOMA = homoeostasis model assessment; IL18 = interleukin-18; InhB = inhibin B; LDL = low density lipoprotein; sTNFR1 = soluble tumour necrosis factor receptor 1; sTNFR2 = soluble tumour necrosis factor receptor 1; TG = triglycerides.

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 IL-18 ($P = 0.025$), or sTNFR1 ($P = 0.036$) and their sperm concentration was negatively associated with their serum IL-18 concentration ($P = 0.020$) measured at 17 years (Tables III and IV). In addition, higher sTNFR1 was negatively associated with inhB ($P = 0.011$), and positively associated with serum LH and FSH ($P = 0.015$, and $P = 0.001$, respectively) 3 years later (Table V). 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 (Hart *et al.*, 2015), and that testicular volume was positively associated with height, and total soft and lean body mass (Hart *et al.*, 2016).

Associations between metabolic cluster analysis at 17 years and 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, men within the high-risk metabolic cluster had lower median testosterone and inhB concentrations than men within the low risk metabolic cluster ($P < 0.001$ for both) (Table VI).

Associations between markers of metabolic disorder at 20 years of age and testicular function

After adjustment for age, abstinence, a history of cryptorchidism, varicocele and BMI (and waist circumference—data not shown), 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) (Table III), and serum testosterone was positively associated with serum HDL, iron, transferrin saturation and lean mass and was negatively associated with hsCRP and serum insulin (all $P < 0.05$) (Table V).

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

Men within the high-risk metabolic cluster at 20 years of age, had lower median testosterone and inhB concentrations than men within the low risk metabolic cluster (Table VI, Supplementary Fig. S1A and B), after adjustment for age, cryptorchidism, presence of a varicocele and BMI (both $P = 0.003$ and $P = 0.001$, respectively) (Supplementary Table SIV).

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

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Table III Associations between reproductive and metabolic parameters at 20/21 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 years years								
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. *$P < 0.001$.

Data are summarized using standardized beta coefficients and their 95% 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_2 semen parameters were also adjusted for abstinence period. Unless otherwise specified, data were collected at 20 years of age.

hsCRP = high sensitivity CRP.

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Table IV Associations between semen parameters and metabolic parameters at 20/21 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 years						
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.

Data are summarized using standardized beta coefficients and their 95% 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_2 semen parameters were also adjusted for abstinence period. Unless otherwise specified, data were collected at 20 years of age. SCSA = sperm chromatin structural assay.

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Table V Associations between serum testicular hormones and gonadotrophins and metabolic parameters 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 years								
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. Data are summarized using standardized beta coefficients and their 95% 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.

Table VI Comparison of testicular volume, semen parameters and serum hormones by metabolic clusters.

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 years of age [Median (IQR, *R*)]**		**Low risk at 16/17 years 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**

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 are median (IQR, *R*) and mean (SD) as appropriate.
P-values were obtained using *t*-tests (individual metabolic cluster parameters) and Mann Whitney tests (reproductive outcomes).

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those participants who did not show IR, their median testicular volume was smaller, and median testosterone and inhB concentrations were lower, and median serum FSH concentration was higher (Table VII, Supplementary Fig. S2A–D). These altered serum concentrations remained after adjustment for age, cryptorchidism, presence of varicocele and BMI: testosterone ($P < 0.001$), inhB ($P = 0.01$) and FSH ($P = 0.015$) (sT4). Furthermore, the 51 men who had a fasting serum insulin greater than 10 μU/ml (91st centile) had lower median serum testosterone and inhB concentrations, and their FSH concentration was greater (Supplementary Table SI, Supplementary Fig. S3A–C).

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

Ultrasound evidence of NAFLD was present in 44 out of 458 men (9.6%) who subsequently underwent some assessment of testicular function. Compared to participants without NAFLD, there were reductions in median total sperm output, serum testosterone and inhB concentrations (Supplementary Table SII and Supplementary Fig. S4A–C), although not after adjustment (Supplementary Table SIV).

Associations between serum hsCRP at 20 years of age and testicular function

In an unadjusted analysis, men whose serum hsCRP was greater than the 75% centile (1.62 mg/L) at 20 years of age (after exclusion of concentrations >10 mg/L), in comparison to those below 1.62 mg/L, showed a reduction in median seminal volume, serum testosterone, LH and FSH concentrations (Supplementary Table SIII and Supplementary Fig. S5A–D).

Discussion

The findings of this observational study of adult men at 20 years of age showed that over one-third were already overweight or obese, and many displayed features of metabolic disturbance associated with adverse cardiovascular outcomes in later life. Men with features of the metabolic syndrome, or who had a high HOMA result at 20 years, or had NAFLD at 17 years displayed features consistent with a primary hypogonadism at 20 years; They had reductions in testicular volume, sperm output, and serum testosterone and inhB, with a reciprocal increase in serum FSH. All of these variables are well established as adverse markers of reproductive potential (Hart *et al.*, 2015; Skakkebaek *et al.*, 2016). In considering potential mechanisms for the observed finding it is possible there are contrasting influences of metabolic disorder with either a direct gonadotoxic or a central hypogonadal influence depending on the cause of the metabolic disturbance. 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 consistent with a direct gonadotoxic effect. 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 in inhB levels and testicular volume these could be chance associations.

It is interesting that already at 20 years of age, irrespective of BMI, the markers of cardiometabolic disorder (a higher fasting serum insulin, TGs, hsCRP and HOMA score) were negatively associated with the testicular hormones. This suggests a potential link between metabolic and reproductive health, in that these adverse metabolic features

Table VII Comparison of testicular volume, semen parameters and serum testicular hormones and gonadotrophins by HOMA-IR, with all assessments made at 20 years of age.

Testicular function assessment	N_{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**

Data are median (IQR, R).
HOMA-IR = homoeostasis model assessment of insulin resistance (Fasting insulin [μu/ml] × Fasting glucose [mM]/22.5).
P-values were obtained using Mann Whitney tests.

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recorded at 17 and 20 years of age may predispose a man to impaired testicular function, irrespective of adiposity. One can speculate that if the cardiometabolic picture deteriorates over time, then testicular function may worsen, adversely influencing reproductive potential. The direction of causality will require further investigation, as it is known that a low circulating testosterone is associated with cardiometabolic disorder (Ding *et al.*, 2006; Araujo *et al.*, 2011; Holmboe *et al.*, 2015). Due to a 3-year interval between some metabolic assessments and assessment of testicular function, we cannot exclude the 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 (Mengel *et al.*, 2017). Irrespective of a proven causal link, 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 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 was already showing some features of the metabolic syndrome.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

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Authors' roles

R.J.H. conceived the study, wrote the grant application for the testicular function study, sought ethical approval, coordinated the study, interpreted the data and had primary responsibility for article preparation. D.A.D. assisted with grant writing, had primary responsibility for data analysis and assisted with article preparation. T.M. had primary responsibility for the metabolic analyses and assisted with data interpretation and article preparation. L.A.A. had primary responsibility for coordinating the hepatic ultrasound assessments and assisted with article preparation. R.-C.H. assisted with data interpretation of the metabolic clustering and assisted with article preparation. N.M. assisted D.A.D. with the data analysis and article preparation. D.J.H. assisted with testicular function grant application, data interpretation and article preparation. R.Mc. assisted with testicular function grant application, data interpretation and article preparation. R.J.N. assisted with testicular function grant application, data interpretation and article preparation. J.D. was primarily responsible for the testicular ultrasound assessments and assisted with article preparation. J.K.O. assisted with the hepatic ultrasound data assessments and assisted with article preparation. L.B. was primarily responsible for grant applications to perform the metabolic assessments and assisted with data interpretation and article preparation.

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Conflict of interest

Authors disclosures; D.A.D., J.E.D., N.M., L.A.A., R.-C.H., T.A.M., J.K.O., L.J.B. have nothing to declare. R.J.H. is Medical Director of Fertility Specialists of Western Australia, has equity interests in Western IVF, and has received grant support from MSD, Merck-Serono and Ferring Pharmaceuticals. R.Mc.L. has equity interests in the Monash IVF Group. R.J.N. has equity interests in FertilitySA, and has received grant support from Merck Serono and Ferring Pharmaceuticals. D.J.H. has received institutional grant funding (but no personal income) for investigator-initiated testosterone pharmacology studies from Lawley and Besins Healthcare and has provided expert testimony to anti-doping tribunals and for testosterone litigation.

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