No association between body mass index and sperm DNA integrity

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STUDY QUESTION: Is overweight associated with impaired sperm DNA integrity?

SUMMARY ANSWER: High body mass index (BMI) is not associated with impaired sperm DNA integrity as assessed by the DNA Fragmentation Index (DFI).

WHAT IS KNOWN ALREADY: Previous studies, based on fewer subjects and including mainly subfertile men, have shown conflicting results regarding the influence of overweight and obesity on sperm DNA integrity.

STUDY DESIGN, SIZE, DURATION: This cross-sectional study was based on semen samples from 1503 men from the general population.

PARTICIPANTS/MATERIALS, SETTING, METHODS: We included two cohorts (cohort A and B) of military recruits (n = 275, n = 304, respectively), one group (cohort C) of fertile men and men without known fertility problems (n = 724), and one group (cohort D) of men between 19 and 40 years without known fertility problems (n = 200). In all cohorts, data were available on BMI, DFI as measured by the sperm chromatin structure assay (SCSA), standard semen characteristics, and potential confounders (age, abstinence time, smoking habits). The subjects were categorized according to BMI into four groups: underweight (< 18.5 kg/m²), normal weight (18.5–24.9 kg/m²), overweight (25.0–29.9 kg/m²) and obese (≥ 30.0 kg/m²). Using a linear regression model, the inter-group differences in DFI were calculated. Furthermore with the normal-weight group as the reference, the odds ratios (ORs) for DFI ≥ 20% and DFI ≥ 30%, were calculated for the other groups. Calculations were made for the material as a whole and after exclusion of cohort C which included proven fertile men.

MAIN RESULTS AND THE ROLE OF CHANCE: We found that normal-weight men had significantly higher DFI than overweight men, with a mean difference of 1.13% (95% CI: 1.05–1.22%; P = 0.001). Overweight men had a reduced risk of having DFI ≥ 20% and DFI ≥ 30%, compared with normal-weight men; adjusted odds ratio (OR) = 0.61 (95% CI: 0.42–0.88; P < 0.01) and adjusted OR = 0.48 (95% CI: 0.28–0.84; P < 0.01), respectively. When excluding cohort C, the statistical significance was lost. Regarding standard semen parameters, we found that obese men had a higher percentage of progressive motile spermatozoa than normal-weight men; mean difference 1.15% (95% CI: 1.02–1.30%, P < 0.05) but the significance was lost when excluding cohort C. All other standard semen parameters were unaffected by BMI.

LIMITATIONS, REASONS FOR CAUTION: A main limitation might be the cross-sectional nature of the data. Furthermore our study included a significant proportion of men with proven fertility (75% of cohort C, n = 550), and could therefore be biased toward fertility.

WIDER IMPLICATIONS OF THE FINDINGS: Our study indicates that overweight per se is not associated with a higher level of sperm DNA damage.
Introduction

Increasing attention has been given to the impact of environmental and lifestyle factors on male reproductive function during the past two decades. Although some studies show no decline (Axelsson et al., 2011), there are indications of a time-related negative trend in semen quality (Carlsen, 2011), there are indications of a time-related negative trend in semen quality (Carlsen, 2011). There have been increased reports of infertility in the general population, which may partly be due to an increased exposure to pollutants acting as endocrine disruptors (Spano et al., 2005; Elzanaty et al., 2006). Others suggest a negative impact of certain lifestyle-related factors (Sharpe, 2010). A high percentage of body fat, most commonly approximated by the body mass index (BMI), has been associated with hormonal alterations in men (Jensen et al., 2004; Fejes et al., 2006; Kort et al., 2006), and high BMI in both the male and the female partner has been suggested to reduce couple fertility (Sallmén et al., 2006; Ramlau-Hansen et al., 2007). A possible impact on semen parameters is however more disputed. While several studies have indicated negative associations between high BMI and standard semen parameters, such as sperm concentration, motility, total sperm count and morphology (Jensen et al., 2004; Koloszár et al., 2005; Kort et al., 2006; Qin et al., 2007; Chavarro et al., 2010; Macdonald et al., 2013; Belloc et al., 2014), others have not (Aggerholm et al., 2008; Thomsen et al., 2014). Two meta-analyses have recently been published. While one that was performed in 2010 did not find evidence of association between increased BMI and impaired semen parameters (MacDonald et al., 2010), the other more recently published study claimed an increased prevalence of azoospermia and oligozoospermia in overweight and obese men (Sermondade et al., 2013). The reason behind these contradictory results may be that the majority of available data are based on subjects recruited from fertility clinics, raising the issue of potential selection bias toward overweight and impaired semen parameters.

Some standard semen parameters such as volume and motility have limited predictive power in relation to fertility (Bonde et al., 1998), and all standard parameters are dependent on the individual skill of the laboratory technician performing the analysis. Sperm DNA integrity has recently been suggested as a more objective, clinically relevant complement to routine assessment of semen quality (Bungum et al., 2011).

Recent studies have examined associations between body weight and sperm DNA integrity (Kort et al., 2006; Chavarro et al., 2010; Hakonen et al., 2011, 2012; Hammiche et al., 2011; Rybar et al., 2011; Tunc et al., 2011; Fariello et al., 2012; La Vignera et al., 2012; Dupont et al., 2013; Eisenberg et al., 2014; Faure et al., 2014; Thomsen et al., 2014). The majority indicate a negative impact of increasing weight (Kort et al., 2006; Chavarro et al., 2010; Fariello et al., 2012; La Vignera et al., 2012; Dupont et al., 2013; Faure et al., 2014), whereas two further studies suggest a trend that failed to reach statistical significance (Hakonen et al., 2011, 2012). However, the study populations range from 43 to 612 participants, and only four studies (Hakonen et al., 2011, 2012; La Vignera et al., 2012; Eisenberg et al., 2014) are based on men from the general population.

The origin and mechanism of sperm DNA damage is not yet fully understood, but is likely multi-factorial (Sakkas and Alvarez, 2010). Possible causes may include defects during spermatogenesis, oxidative damage and abortive apoptosis (Erenpreiss et al., 2006). High BMI is associated with the metabolic syndrome (MetS) which in turn is associated with a systemic proinflammatory state and increased oxidative stress, which at the level of the testicular micro-environment may result in sperm DNA damage (Kasturi et al., 2008).

One of the techniques available to assess sperm DNA damage, for which a clear clinical cutoff level for impairment of male fertility potential has been established, is the sperm chromatin structure assay (SCSA) (Erenpreiss et al., 2006; Bungum et al., 2011). The cutoff level for DNA fragmentation index (DFI) has been demonstrated to be a strong, independent predictor of human fertility in vivo (Evenson et al., 1999; Spanò et al., 2000; Giwercman et al., 2010) and to some degree even in vitro (Bungum et al., 2007). Studies have indicated that the chance of spontaneous pregnancy is not affected for a DFI < 20% but starts to decrease when the DFI exceeds this level (Spanò et al., 2000; Giwercman et al., 2010), being close to zero when the DFI reaches >30% (Spanò et al., 2000; Erenpreiss et al., 2006). The association between sperm DNA damage and the standard semen parameters is moderate since 20–40% of infertile men who show normal standard semen parameters have a DFI of >20% using SCSA (Erenpreiss et al., 2008; Giwercman et al., 2010).

In order to provide more robust data regarding an association between BMI and sperm DNA integrity, we have retrieved and analyzed data from four cohorts of men recruited from the general population, analyzing the association between BMI and the SCSA parameter DFI. Additionally, we investigated the relation between BMI and standard semen characteristics.

Materials and Methods

Study populations

A database was created and composed of 1560 men who had previously been enrolled in one of four cohorts:

Cohort A: Swedish military conscripts 2000–2001

In 2000–2001 about 95% of all 18-year-old Swedish men underwent medical health examination at the National Service Administration in Sweden.
Regardless of fertility status, were included (Richthoff et al., 2002).

Cohort A: Swedish military conscripts 2008–2010
Due to restrictions on the military budget, only 25% of all Swedish 18-year-old men underwent medical examination at NSAS during December 2008 to May 2010. All 1681 men who underwent the examination, lived within a 60 km radius of the city of Malmö, and were born of and raised by mothers born and raised in Sweden, were asked to participate, and 241 (14%) accepted. Another 73 men of the same age and raised by mothers born and raised in Sweden, where the semen samples were collected at home. In part, this material was utilized. BMI was calculated, and then categorized in three of the samples and four times in one of the samples. In these cases the data were utilized. The laboratory at the Reproductive Medicine Centre, Skåne University Hospital. In each of the four regions of cohort C, the Polish and Ukrainian samples were analyzed at one central hospital in each region. Greenlandic samples were analyzed at the local hospital or nursing station, and Swedish samples were analyzed by a mobile laboratory at the participants’ residences. For cohort D, analyses were performed at laboratories in Tromsø and Oslo, depending on where the sample was obtained. The laboratory at the Reproductive Medicine Centre, Skåne University Hospital, participated in the external quality control program of the European Society of Human Reproduction and Embryology in collaboration with Nordic Association of Andrology, and therefore supervised semen analyses in studies C and D.

Sperm Chromatin Structure Assay
SCSA is a flow cytometric technique (Evenson et al., 2002), which measures the susceptibility of sperm DNA to acid induced DNA denaturation in situ, followed by staining with the fluorescent dye acridine orange (AO). When excited by a blue light, AO intercalated in double-stranded DNA emits green fluorescence, whereas AO associated with single-stranded DNA emits red fluorescence. The percentage of defective sperm with detectable DFI (DNA Fragmentation Index) was calculated from the DFI frequency histogram obtained from the ratio between the red and total (red plus green) fluorescence intensity with the help of dedicated software. By using a flow cytometer, 5000–10 000 spermatozoa can be analyzed within a short time, providing objective, repeatable and robust results.

Cohort C: Inuendo—a multicenter study of Inuit and European men
As a part of an EU FP5 granted study, men at four different locations were recruited to investigate the impact of exposure to persistent organochlorine pollutants on reproductive function. Between May 2002 and February 2004, spouses of pregnant women were recruited in different regions of Greenland (n = 200), in Kharkiv, Ukraine (n = 207) and in Warsaw, Poland (n = 143). Recruitment was when the pregnancy was recorded at the participating hospitals. Additionally, fishermen from the west and east coast of Sweden, regardless of fertility status, were included (n = 184), constituting the final 734. The participants’ ages ranged from 18 to 69 years. In part, this material has been used and described in previous publications. The overall participation rate was 18% among the originally invited men (Spanò et al., 2005; Elzanaty et al., 2006).

Cohort D: Norwegian men 2001–2002
In order to assess possible impact of seasonal variation in male reproductive function, men living in Tromsø or Oslo, Norway were recruited through advertisements on the radio and in local newspapers during the period 2001–2002. Eligible were men aged 19–40 years, who had lived in the area for at least 1 year and who would remain in the area for at least another year. A total of 207 men were recruited. The data on standard sperm parameters have been previously published (Malm et al., 2004).

Ethical approval
In each of the above studies, the subjects received compensation for their participation (55–120 Euro). All of the studies were approved by the local ethical committees and participants signed a written informed consent.

Physical examination and questionnaire
In cohorts A and B, subjects underwent physical examination, including assessment of weight and height, whereas for cohorts C and D self-reported data were utilized. BMI was calculated as the weight in kilograms divided by the square of height in meters. Participants were also asked to fill in a questionnaire concerning their lifestyle including current cigarette smoking habits.

Semen analysis
All participants delivered one (cohort A, B and C) or two (cohort D) semen samples. In cohorts A, B and D subjects were asked to keep 48–72 h of abstinence. In cohort C a minimum of 48 h was requested, but no upper limit was established. In each case, the actual abstinence time was recorded.

All semen samples were obtained by masturbation in the laboratory at the participating hospital for cohorts A, B, D, and all regions of cohort C except Sweden, where the semen samples were collected at home.

The methodologies used in the different cohorts for determining the standard semen characteristics are described in detail in previous publications (Richthoff et al., 2002; Malm et al., 2004; Toft et al., 2005, 2006; Axelsson et al., 2011). In brief, following liquefaction, all samples were analyzed according to WHO guidelines and theESHRE manual on basic semen analysis. Semen volume was estimated by weighing. Duplicate assessments of sperm concentration were made, by use of an improved Neubauer haemocytometer, with positive displacements pipettes used for proper dilution of the ejaculate. Motility was determined by placing a drop of semen on a slide, mounted on a heated stage. The proportions with rapid or slow progressive motility (category A and B, respectively), non-progressive motility (category C) or immotility (category D) were then determined within each of two drops of semen.

For cohorts A and B, standard semen analysis was performed at the Reproductive Medicine Centre, Skåne University Hospital. In each of the four regions of cohort C, the Polish and Ukrainian samples were analyzed at one central hospital in each region. Greenlandic samples were analyzed at the local hospital or nursing station, and Swedish samples were analyzed by a mobile laboratory at the participants’ residences. For cohort D, analyses were performed at laboratories in Tromsø and Oslo, depending on where the sample was obtained. The laboratory at the Reproductive Medicine Centre, Skåne University Hospital, participated in the external quality control program of the European Society of Human Reproduction and Embryology in collaboration with Nordic Association of Andrology, and therefore supervised semen analyses in studies C and D.

Statistical methods
Of the total 1560 men, 1503 subjects remained after excluding cases where height, weight and/or DFI were missing. The population comprised 275 from cohort A, 304 from cohort B, 724 from cohort C and 200 from cohort D.

In cohort A, DFI was analyzed two times in 30 of the samples, three times in three of the samples and four times in one of the samples. In these cases the mean values of the assessments were used. In cohort D, the men delivered two semen samples at ~6 monthly intervals, the means of which were included in this study. In cohorts B and C, participants delivered one sample which was analyzed only once.

In cohorts A and B height and weight were measured, whereas cohorts C and D used self-reported data. BMI was calculated, and then categorized based on the WHO cutoff values of: overweight: <18.5 kg/m²; normal-
weight, 18.5–24.9 kg/m²; overweight 25.0–29.9 kg/m² and obesity ≥30.0 kg/m². We chose not to include further sub-classifications of obesity in our main analyses, as only 17 men had BMI 35 kg/m² or above.

An interaction analysis revealed that study cohort did not modify the association between BMI and DFI (P = 0.2), and the study populations were merged in order to obtain a higher statistical power. The semen parameters were transformed using the natural logarithm in order to obtain a normal distribution of residuals. Results presented are back-transformed values of these results, and the mean differences and 95% confidence intervals thus correspond to the ratios and not differences between the means.

The association between the BMI category and standard semen parameters as well as DFI was analyzed using linear regression analysis. Adjustments were made for cohort, age, abstinence time (hours) and whether or not the subject currently smoked cigarettes regularly. When including an interaction term (Smoking*BMI) in the regression model, we did not find any statistically significant interaction between those two exposures in relation to DFI (P = 0.6). No adjustment for whether or not the subjects presented a varicocele was made, since this information was only available for cohorts A and B. In these two groups the presence of varicocele was not associated with BMI (P = 0.5).

With the group of normal-weight men as reference, logistic binary regression was used to calculate the odds ratios (ORs) for DFI over 20 and 30% in relation to BMI category.

Since cohort C included proven fertile men, two sub-analyses were performed; one in which this cohort was excluded, and one where it was analyzed separately using examination center (country), instead of cohort as a potential confounder.

Finally for exploratory purposes, we investigated a possible nonlinear relationship between BMI and DFI as continuous variables by applying a nonlinear smoothed regression model, with and without adjustments for potential confounders, using the gam package in R (R Development Core Team 2010, R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL: http://www.R-project.org/). All other data analyses were performed using SPSS (IBM SPSS Statistics, version 21).

**Results**

**Background characteristics**

The median (range) age of the men in the total study population was 26.0 (17.5–69.0) years, BMI was 24.0 (11.5–57.8) kg/m² and DFI was 10.3% (1.30–88.2%). Median abstinence time was 72 h, with a great range from 0 h to 240 days. A high proportion (45%; n = 672) of the men were smokers. Detailed background characteristics of the four cohorts are given in Table I, and stratified by WHO BMI cutoff levels in Table II.

**BMI and DFI**

Geometric mean lnDFIs with 95% confidence intervals in the different weight categories, stratified by cohort and the entire study population are given in Figs 1 and 2.

Both unadjusted and adjusted analysis showed that normal-weight men had statistically significantly higher DFI than overweight men, with a mean difference of 1.12% (95% CI: 1.04–1.20%, P < 0.005) and 1.13% (95% CI: 1.05–1.22%, P = 0.001), respectively. Adjusted average DFI values were also higher in normal-weight men compared with obese men, with mean difference 1.14% (95% CI: 1.00–1.29%, P < 0.05). There was no significant difference between the DFI of normal-weight men compared with underweight men.

If adjusted for country instead of cohort, adjusted average DFI values were still higher among normal-weight men than overweight and obese men, although statistical significance was only seen for the comparison with the overweight men, with a mean difference of 1.14% (95% CI: 1.06–1.23%).

When removing cohort C from the analysis, the unadjusted mean DFI of normal-weight men remained higher than that of overweight and obese men. However, statistical significance was seen only between the normal- and overweight men, with a mean difference of 1.13% (95% CI: 1.01–1.26%). Without cohort C, the adjusted mean DFI was also higher among normal-weight men, than among the overweight and obese men, but without statistical significance.

In a separate analysis using cohort C only, the average DFI of normal-weight men was higher than that of overweight and obese men, but statistical significance only appeared in the adjusted analysis, with a mean difference 1.22% (95% CI: 1.10–1.35%, P < 0.001) and 1.19% (95% CI: 1.02–1.39%, P < 0.05), respectively. No other statistically significant results were found.

The DFI values were above 20% in 253 men (17%), among whom 110 men (7%) had a DFI above 30%. Using normal-weight men as reference, we found that overweight men had a reduced risk of having DFI ≥ 20% and DFI ≥ 30%, adjusted OR = 0.61 (95% CI: 0.42–0.88; P < 0.01) and adjusted OR = 0.48 (95% CI: 0.28–0.84; P = 0.01), respectively. The underweight or obese men did not differ statistically significantly from the reference group regarding ORs of DFI ≥ 20% or ≥30% (Table III). However, when removing cohort C, the statistical significance was lost. Stratified ORs are given in Table III.

When comparing all men with BMI ≥ 25.0 kg/m² to those with a normal weight, we found an adjusted OR of 0.61 (95% CI: 0.43–0.86; P < 0.005) of having DFI ≥ 20% and an adjusted OR of 0.48 (95% CI: 0.29–0.81; P < 0.01) of having DFI ≥ 30% (Table IV).

Both adjusted and unadjusted non-linear smoothed regression analysis showed that increasing BMI decreased DFI, with P < 0.01.

**BMI and WHO semen parameters**

The four BMI groups did not differ with statistical significance in sperm concentration.

Normal-weight and overweight men had significantly higher ejaculate volumes than underweight men, mean difference 1.25% (95% CI: 1.03–1.51%, P < 0.05) and 1.26% (1.04–1.54%, P < 0.05). However no significant difference was found after adjusting for age, smoking, and abstinence time.

Adjusted values showed a significantly higher proportion of progressive motile spermatozoa in obese compared with normal-weight subjects (mean difference 1.15% [95% CI: 1.02–1.30%, P < 0.05]). Statistical significance did not appear when comparing other BMI groups. All significance was lost when removing cohort C (data not shown).

**Discussion**

In a cohort of >1500 men in which the majority were recruited from the general population, we did not find that high BMI was associated with increased levels of sperm DNA damage.

To our knowledge, only four studies have previously been published, which investigate the relationship between BMI and sperm DNA damage.
### Table I  Characteristics of the study population, stratified by cohorts A–D as well as the database as a whole (Total).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Total (N = 1503)</th>
<th>A (N = 275)</th>
<th>B (N = 304)</th>
<th>C (n = 724)</th>
<th>D (n = 200)</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>1503</td>
<td>275</td>
<td>304</td>
<td>724</td>
<td>200</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>27.9 (10.9)</td>
<td>26.0 (17.5–69.0)</td>
<td>18.2 (0.40)</td>
<td>18.0 (18.0–21.0)</td>
<td>18.4 (0.36)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>24.5 (3.67)</td>
<td>24.0 (11.5–57.8)</td>
<td>22.6 (3.11)</td>
<td>22.2 (14.9–41.7)</td>
<td>23.1 (3.08)</td>
</tr>
<tr>
<td>Duration of abstinence (h)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>90.7 (182)</td>
<td>72.0 (0–5760)</td>
<td>85.1 (53.7)</td>
<td>67.0 (12.0–500)</td>
<td>60.3 (34.0)</td>
</tr>
<tr>
<td>DFI (%)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>13.5 (10.2)</td>
<td>10.3 (1.30–88.2)</td>
<td>18.3 (14.3)</td>
<td>14.0 (1.66–86.5)</td>
<td>11.0 (6.09)</td>
</tr>
<tr>
<td>Sperm concentration (10⁹/ml)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>71.6 (61.3)</td>
<td>55.0 (0.08–419.0)</td>
<td>70.9 (65.6)</td>
<td>51.5 (0.48–391.9)</td>
<td>70.8 (59.6)</td>
</tr>
<tr>
<td>Semen volume (ml)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>3.42 (1.62)</td>
<td>3.20 (0.30–13.8)</td>
<td>3.31 (1.25)</td>
<td>3.20 (0.42–8.40)</td>
<td>2.93 (1.51)</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>54.0 (18.2)</td>
<td>56.0 (0–93.0)</td>
<td>55.0 (15.5)</td>
<td>57.0 (0.00–85.0)</td>
<td>53.2 (16.7)</td>
</tr>
<tr>
<td>Smokers, N (%)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td></td>
<td>672 (45)</td>
<td>78 (28)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects in BMI categories, N (%)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>&lt;18.5 kg/m²</td>
<td>27 (2)</td>
<td>7 (3)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18.5–24.9 kg/m²</td>
<td>905 (60)</td>
<td>233 (85)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>25.0–29.9 kg/m²</td>
<td>456 (30)</td>
<td>24 (9)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥30 kg/m²</td>
<td>115 (8)</td>
<td>11 (4)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All results are presented with arithmetic mean, standard deviation (SD), median and range.
in men without prior knowledge of fertility issues. Eisenberg et al. (2014) published a report on 501 couples attempting to conceive and did not indicate any significant relationship between BMI and DFI. This cohort was somewhat biased by overrepresentation of subjects being overweight and sedentary. In agreement with this, Hakonsen et al. (2011), did not find any significant improvement in DFI upon weight loss in a cohort of 43 obese men. In 2012, another study by Hakonsen et al. (2012) indicated that DFI was higher in overweight compared with normal-weight men. However, the results did not reach statistical significance, possibly explained by the fact that only 4% had a DFI above 20. A significant result was however found by La Vignera et al. (2012), who observed higher DNA damage in obese but not overweight men. The results are contradictory, perhaps due to differences in population size (43–501) and in the methods of assessing DFI. Although none of them fully supports our results, the only study reporting increased DFI in obese men was based on use of the TUNEL assay (La Vignera et al., 2012). Also, the choice of potential confounders differs between the individual studies. Only two studies recorded and adjusted for abstinence time (Hakonsen et al., 2011, 2012), whereas the others requested 2–4 days of abstinence, with no guarantees that this requirement was fulfilled by the participants. In the study that found significantly higher levels of fragmented DNA in obese men, no adjustments for any confounders were made.

Our cohort differs in some aspects from the ones on which the four above mentioned studies are based. Three of the four sub-cohorts included in our study truly represent the general population, as cohort C partly included proven fertile men. Also, adjustment for time of abstinence was included in our statistical analysis.

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### Table II

The distribution of age, smoking, abstinence hours, DFI and semen quality parameters stratified by WHO BMI cutoff levels.

<table>
<thead>
<tr>
<th>WHO BMI cutoff levels (kg/m²)</th>
<th>&lt;18.5 kg/m²</th>
<th>18.5–24.9 kg/m²</th>
<th>25.0–29.9 kg/m²</th>
<th>&gt;30 kg/m²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
<td>Median (range)</td>
<td>Mean (SD)</td>
</tr>
<tr>
<td>Age (years)</td>
<td>23 (7.5)</td>
<td>19 (18–43)</td>
<td>25 (9.3)</td>
<td>20 (18–67)</td>
</tr>
<tr>
<td>Abstinence time (h)</td>
<td>100 (99)</td>
<td>80 (10–500)</td>
<td>94 (230)</td>
<td>72 (0–5760)</td>
</tr>
<tr>
<td>DFI (%)</td>
<td>13 (9.6)</td>
<td>11 (1.3–42)</td>
<td>14 (11)</td>
<td>11 (1.4–88)</td>
</tr>
<tr>
<td>Sperm concentration (10⁶/µl)</td>
<td>76 (55)</td>
<td>66 (1.0–220)</td>
<td>72 (63)</td>
<td>54 (0.5–420)</td>
</tr>
<tr>
<td>Semen volume (ml)</td>
<td>2.8 (1.5)</td>
<td>2.5 (1.0–6.2)</td>
<td>3.5 (1.6)</td>
<td>3.2 (0.3–14)</td>
</tr>
<tr>
<td>Progressive motility (%)</td>
<td>52 (17)</td>
<td>55 (4.0–72)</td>
<td>53 (18)</td>
<td>56 (0–93)</td>
</tr>
<tr>
<td>Current smokers (N (%))</td>
<td>15 (56)</td>
<td>358 (40)</td>
<td>230 (50)</td>
<td>69 (60)</td>
</tr>
</tbody>
</table>

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**Figure 1** Boxplot showing unadjusted natural logarithm of DNA Fragmentation index (lnDFI) in relation to body mass index (BMI) in a cohort of 1503 non-infertile men. Outliers have been omitted.

**Figure 2** Boxplot showing unadjusted natural logarithm of DNA Fragmentation index (lnDFI) in relation to body mass index (BMI) for the four sub-cohorts. For each BMI category the boxplots represent (from left): (white with vertical line) first cohort of military conscripts; (white) second cohort of military conscripts; (black) proven fertile men; (gray) men from general Norwegian population.
Studies associating a high BMI with impaired semen parameters have suggested several possible mechanisms. One explanation could be that excess weight increases the conversion of testosterone to estrogen in adipose tissue leading to secondary hypogonadism through inhibition of the hypothalamic-pituitary-gonadal axis (Kasturi et al., 2008). Another explanation is that deposition of fat around scrotal blood vessels prevents heat loss, and therefore induces scrotal hyperthermia which is suggested to disturb spermatogenesis (Sharpe, 2010). Finally, oxidative stress at the testicular level may result in decreased spermatozoa DNA integrity was found (Tunc et al., 2012). Additionally, it has been claimed that rather than necessarily have been associated with a proinflammatory state, and therefore oxidative stress. Associated with a high DFI in obese men. MetS and several of its components, abdominal obesity, insulin resistance and dyslipidemia, are associated with systemic proinflammatory state and increased oxidative stress.

Having these hypotheses in mind, it seems plausible that recruitment of the study population may have an impact on the association between BMI and sperm DNA fragmentation. Thus, among overweight subfertile men, those having both subnormal testosterone levels and associated low grade systemic inflammation (Bobjér et al., 2013), due to their overweight, MetS or both, might be overrepresented. Consequently, this could lead to increased DFI in this subgroup. As the present study included men from the general population, an increased BMI may not necessarily have been associated with a proinflammatory state, and therefore not linked to DFI. This assumption is supported by the lack of impairment of standard semen parameters in men with BMI of 25 kg/m² or more.

A puzzling finding is the lower risk of increased DFI in overweight men. No plausible biological explanation can be given to this finding, which might be spurious. Nevertheless, a previous study has reported an inverse correlation between serum estradiol levels and DFI (Meeker et al., 2008). Thus, one might speculate about whether an increased BMI in men with normal testosterone levels leads to a higher concentration of estradiol (Chavarro et al., 2010), protecting sperm DNA.
However, the slight difference in DFI between normal-weight and overweight men, if present, is of limited clinical significance.

Furthermore, due to the cross-sectional design of this study, a lowering of DFI related to changes in lifestyle due to being overweight or obese, cannot be excluded. However, so far there are no reliable data showing that lifestyle changes in otherwise healthy men leads to significant alteration in the DFI.

The major strength of this study is the large sample size and inclusion of men from the general population. Thus, our data allow for assessing the possible link between high BMI and sperm DNA integrity, not blurred by the fertility status of the participants.

Some weaknesses should be addressed. The possibility of selection bias needs to be considered, since the participation rates were rather low for all of the cohorts. The group of conscripts, in general, can be considered as representative for the adolescent Swedish general population and although < 20% signed up for the study, it is unlikely that the choice of participation was driven by fear of reduced fertility in a group that young. In a Danish study of conscripts with a similar participation rate, the levels of reproductive hormones did not differ between those delivering semen sample and those who did not (Andersen et al., 2000). Regarding cohort C, men with high fertility is overrepresented relative to the general population since a large part of the cohort consisted of partners of pregnant women. However, our main findings were robust to excluding this cohort from the statistical analysis. Finally, in cohort D, the prevalence of infertility problems were at the same level as reported for Western countries, and sperm counts were comparable with those of the general Norwegian population.

Unlike the other subgroups, for cohort D an average of two DFI measurements was used for statistical analysis. Using this approach may introduce a lower variability for this group of subjects. However, previously we reported a 0.73 correlation coefficient between the summer and winter DFI (Rylander et al., 2009) values which indicates that our approach will hardly affect the final conclusion of our study.

Our BMI values are based on both objective measurements (cohorts A and B) and on self-reported data (cohort C and D). As with all self-reported data, there will be inaccuracies with regard to their accuracy, and in that respect, only the BMI values of cohorts A and B are truly reliable. However, it is hardly likely that self-reported data should lead to a systematic BMI category misclassification of the subjects. Also, although BMI may not be the ideal marker for overweight, there is no indication that men with excessive muscle mass, which may cause increased BMI without being associated with an increased percentage of body fat, were overrepresented in our cohorts.

Our study contained few participants with very low or very high BMI, which means that we cannot evaluate whether extreme deviations in BMI have an impact on DFI.

SCSA was applied to assess the level of sperm DNA damage. Since the correlation between the three major methods for assessment of sperm DNA integrity, SCSA, TUNEL and COMET, may vary from high to only moderate, we cannot exclude that even in our cohort, some adverse associations between sperm DNA integrity and BMI could exist. However, from a clinical point of view, SCSA is the most widely used technique and the method with mostly validated clinical relevant cutoff levels.

In conclusion, this study did not confirm the positive association between high BMI and sperm DNA damage proposed by previous studies in infertile men. One could speculate that such a link might not be significant in the general population, but more pronounced in subfertile men, in whom decreased fertility, and testosterone deficiency leading to overweight and DNA strand breaks, are parts of a common pathogenic mechanism.

Authors’ roles
I.B. and A.G. are responsible for the study conception and design. I.B. performed the statistical analyses and wrote the first version of the manuscript. I.B. and A.G. interpreted the data. M.B., J.R., J.M., J.A., H.S.P., J.K.L., K.C., A.H., G.T., J.P.B., M.S., G.M., T.B.H. and A.G. contributed to collection of data and critical revising of the manuscript. All authors approved of the final version to be published.

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Conflict of interest
The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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