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RESEARCH ARTICLE

Lifestyle and semen quality: role of modifiable risk factors

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Abstract

The relationship between exposure to lifestyle factors and adverse effects on human reproductive health is debated in the scientific literature and these controversies have increased public and regulatory attention. The aim of the study was to examine the association between modifiable lifestyle factors and main semen parameters, sperm morphology, and sperm chromatin structure. The study population consisted of 344 men who were attending an infertility clinic for diagnostic purposes with normal semen concentration of 20–300 M/ml or with slight oligozoospermia (semen total concentration of 15–20 M/ml) [WHO 1999]. Participants were interviewed and provided semen samples. The interview included questions about demographics, socio-economic status, medical history, lifestyle factors (consumption of alcohol, tobacco, coffee intake, cell phone and sauna usage), and physical activity. The results of the study suggest that lifestyle factors may affect semen quality. A negative association was found between increased body mass index (BMI) and semen volume ($p=0.03$). Leisure time activity was positively associated with sperm concentration ($p=0.04$) and coffee drinking with the percentage of motile sperm cells, and the percentage of sperm head and neck abnormalities ($p=0.01$, $p=0.05$, and $p=0.03$, respectively). Drinking red wine 1–3 times per week was negatively related to sperm neck abnormalities ($p=0.01$). Additionally, using a cell phone more than 10 years decreased the percentage of motile sperm cells ($p=0.02$). Men who wore boxer shorts had a lower percentage of sperm neck abnormalities ($p=0.002$) and percentage of sperm with DNA damage ($p=0.02$). These findings may have important implications for semen quality and lifestyle.

Keywords

Lifestyle, modifiable factors, semen quality

History

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Introduction

Semen quality in the adult male can be affected by a number of environmental and lifestyle factors. An increasing trend in male infertility observed in recent years may be associated at least in part with these factors, which are compounded by a change in lifestyle. Lifestyle associated exposures including cigarette smoke, alcohol, caffeine, use of mobile phones, and body mass index (BMI) have been studied in relation to male semen quality [Fejes et al. 2005a; Jensen et al. 2010; Magnusdottir et al. 2005; Ramlau-Hansen et al. 2007; Li et al. 2009]. An important lifestyle-dependent factor that adversely affects spermatogenesis is obesity. Several studies have shown up to a threefold higher incidence of obesity in infertile men than in those with normal semen quality [Hammoud et al. 2008; Magnusdottir et al. 2005]. A BMI of more than 25 kg/m² was associated with an average 25 percent reduction in sperm count and sperm motility [Jensen et al. 2004a;

Jensen et al. 2004b; Kort et al. 2006]. Meta-analysis of 31 studies showed a negative relationship for testosterone, sex hormone binding globin (SHBG), and free testosterone with increased BMI, however no evidence was found of an association between increased BMI and semen parameters [MacDonald et al. 2010]. Also studies on caffeine intake and semen quality have shown contradictory results. Some suggested no associations [Oldereid et al. 1992; Ramlau-Hansen 2008], whereas others found reduced sperm concentration as well as reduced total sperm count and motility [Jensen et al. 2010; Sobreiro et al. 2005]. Global System for Mobile Communications (GSM) phone usage in 5 days for 6 hours per day decreased the rapid progressive motility of sperm [Davoudi et al. 2002] and duration of mobile phone use was negatively correlated with sperm motility and positively with the proportion of slow progressive motile sperm [Fejes et al. 2005a]. Several studies also examined the effect of smoking and alcohol drinking on sperm parameters, but their results are inconsistent. Some studies [Marinelli et al. 2004; Povey et al. 2012] but not all [Li et al. 2011; Ramlau-Hansen et al. 2007; Vine 1996] suggested that smoking and alcohol has a limited effect on semen quality. However, recent reviews of male subfertility highlight how aspects of adult male

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lifestyle may significantly increase their risk of subfertility [Li et al. 2011; Sadeu et al. 2010]. Those reviews further suggested that higher age, smoking, alcohol consumption, and psychological stress were risk factors for poor semen quality [Li et al. 2011; Sadeu et al. 2010].

Nevertheless, our understanding of how the lifestyle choices of adult men themselves might impact their fertility remains uncertain and often contradictory. The high variability in semen parameters makes it difficult to obtain sufficient power in studies of semen quality. Also, differences in data analysis (use of different confounding factors), small number of examined subjects, and significant heterogeneity in the sample populations (fertile or infertile men) makes comparisons across studies difficult.

Research into potential modifiable risk factors may ultimately lead to more satisfactory and cost-effective preventive and curative treatments and may have important implications for preventing or modifying poor semen quality by changing lifestyle. To increase our knowledge in this field we examined the association between the modifiable lifestyle factors and main semen parameters, sperm morphology, and sperm chromatin structure. Our objective was to examine semen parameters in relation to: BMI, smoking, alcohol consumption, wearing loose underwear (boxer shorts), coffee intake, leisure time physical activity, and using cell phone and sauna.

Results

Study population

The study population consisted of 344 men who were attending infertility clinics for diagnostic purposes. The mean age of the men participating in this study was 32.5 years old. Most of them had higher (40.7%) or secondary (37.2%) education, while only about 22% had a vocational education; 41.6% of study participants were non smokers (Table 1). Past diseases which may have impact on semen quality (e.g., mumps, cryptorchidism, testes surgery, testes trauma) were reported by 14.5% of participants. The abstinence before the semen analysis was 3–7 days (86.2%) in most of the men examined (Table 1).

Lifestyle factors among study participants

Most of the study participants were overweight (BMI 25–29.9 kg/m²) 46.5% and 21% were obese (BMI 30–40 kg/m²), whereas 33% had normal weight (BMI 18–24.9 kg/m²; Table 1). The leisure time physical activity was reported by 69.2% of the study population (Table 1) of which 37% of the men studied had a leisure time physical activity of the Metabolic Equivalent Task (MET) indicator <24 whereas 33% had a leisure time physical activity MET indicator ≥24. The use of the cell phone from 6 to 10 years was documented among 52% of the study subjects. A total of 24% of the men used the cell phone more than 10 years (Table 1). Of the study population, 41% of the men wore loose underwear (boxer shorts) and 16% used the sauna in 3 months prior to the semen collection (Table 1). Everyday coffee drinking was reported by 52% of the study participants. Most of the men drank beer 1–3 times per week (46%) and white wine,

Table 1. Characteristics of the study population.

Characteristics	n (%)
Education	
Primary and vocational	76 (22.09)
Secondary	128 (37.21)
Higher	140 (40.70)
Smoking determined by cotinine level	
No	143 (41.57)
Ex-smoker	91 (26.45)
Smoker	110 (31.98)
*Past diseases, which may have impact on semen quality	
No	294 (85.47)
Yes	50 (14.53)
Abstinence [days]	
<3	18 (6.21)
3–7	250 (86.21)
>7	22 (7.58)
Missing data	54 (15.70)
BMI [kg/m ²]	
18–24.9	113 (32.85)
25–29.9	160 (46.51)
30–40	71 (20.64)
Mean (SD)	27.2 (3.80)
Median (min–max)	27.4 (18.5–39.5)
Age [years]	
22–30	113 (32.85)
31–40	217 (63.08)
41–57	14 (4.07)
Mean (SD)	32.5 (4.80)
Median (min–max)	32.2 (22.0–56.8)
Beer [days/week]	
<1/week	137 (39.83)
1–3/week	159 (46.22)
4–7/week	48 (13.95)
White wine [days/week]	
<1/week	329 (95.64)
1–3/week	15 (4.36)
4–7/week	0
Red wine [days/week]	
<1/week	306 (88.95)
1–3/week	31 (9.01)
4–7/week	7 (2.03)
Vodka [days/week]	
<1/week	313 (90.99)
1–3/week	30 (8.72)
4–7/week	1 (0.29)
Coffee [days/week]	
<1/week	83 (24.13)
1–6/week	81 (23.55)
Everyday	180 (52.33)
Leisure time activity (MET)	
No	106 (30.81)
<24	126 (36.63)
≥24	112 (32.56)
Cell phone [years]	
0–5	55 (15.99)
6–10	179 (52.03)
11–25	82 (23.84)
Missing data	28 (8.14)
Boxer shorts	
No	203 (59.01)
Yes	141 (40.99)
Sauna	
No	194 (83.98)
Yes	37 (16.01)
Missing data	113 (32.88)

BMI: body mass index; *Past diseases which may have impact on semen quality: mumps, cryptorchidism, testes surgery, testes trauma.

Table 2. Distribution of semen parameters.

Semen parameters	Mean (sd)	Median (min–max)	n (%)
Volume [ml]	3.43 (1.38)	3.0 (1.7–12.3)	342 (99.42)
Total concentration [M/ml]	50.71 (52.46)	32.60 (15.0–360.0)	342 (99.42)
Motility [%]	55.7 (19.91)	55.0 (4.0–99.0)	338 (98.26)
Atypical [%]	53.70 (23.85)	50.0 (11.0–98.0)	340 (98.84)
Sperm head abnormalities [%]	36.63 (25.88)	28.0 (0.0–98.0)	341 (99.13)
Sperm neck abnormalities [%]	13.82 (8.21)	12.0 (0.0–44.0)	341 (99.13)
Sperm tail abnormalities [%]	5.92 (5.92)	5.0 (0.0–42.0)	341 (99.13)
DFI [%]	15.99 (10.83)	13.16 (2.7–71.23)	285 (82.85)

DFI: DNA fragmentation index

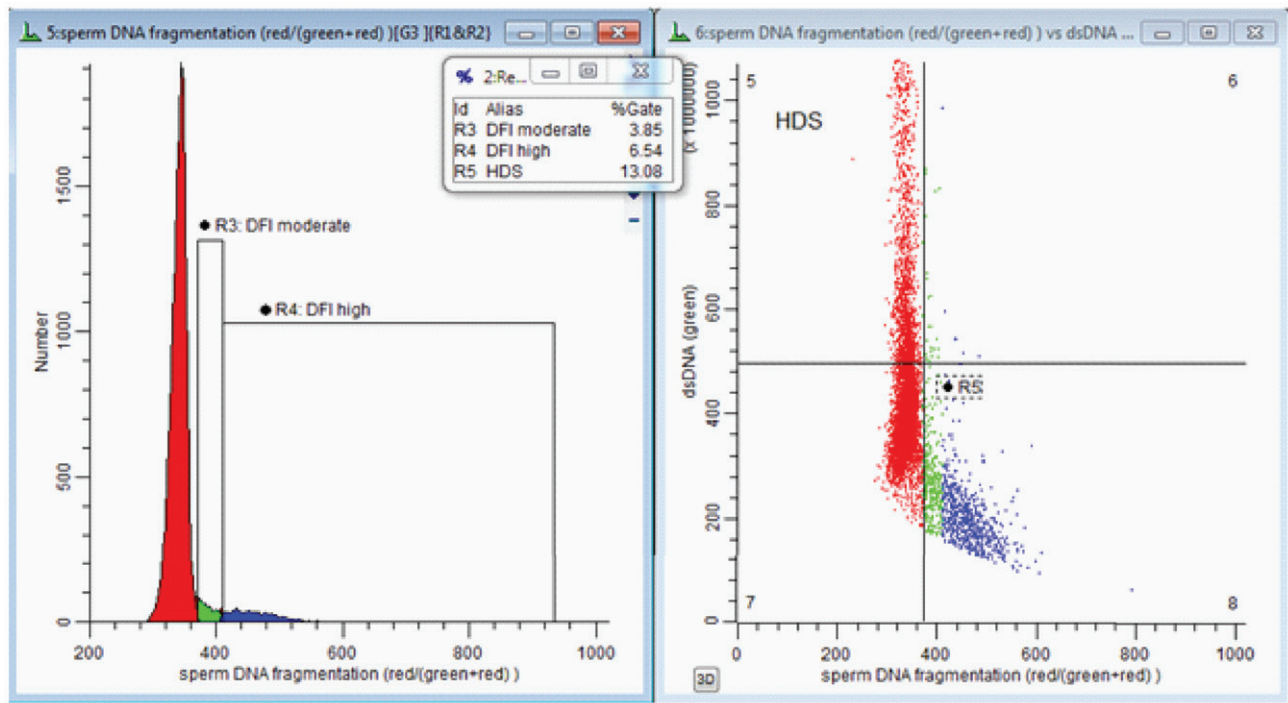


Figure 1. Representative flow cytometric figure. Sperm DNA fragmentation examined by Sperm Chromatin Structure Assay (SCSA) method. Green (dsDNA) and red (ssDNA) fluorescence were collected by flow cytometry after sample staining with acridine orange. The fluorescence bands corresponded to intact double stranded DNA (green fluorescence) and fragmented, single stranded (red fluorescence) sperm DNA. Moderate (DFI moderate) to high (DFI high) DNA fragmentation levels were found within these red fluorescing sperm cells. The DNA Fragmentation Index (DFI) was calculated according to the formula: $DFI = (\text{cells with shift of } \alpha\text{-t parameter/all cells}) \times 100$ each analyzed sperm cell and was shown on a histogram. Cells with an abnormal chromatin structure showed a distinct shift of the $\alpha\text{ t}$ parameter value. High DNA stainability (%HDS) was calculated on the basis of the percentage of sperm with high levels of green fluorescence, which are thought to represent immature spermatozoa with incomplete chromatin condensation. The histogram on the left: DFI:DNA Fragmentation Index; DFI moderate and DFI high: percentage of spermatozoa with moderate and high DNA fragmentation, respectively. The dotplot on the right: HDS: High DNA stainability – percentage of spermatozoa with poorly condensed (immature) chromatin.

red wine, and vodka less than once a week (96%, 89%, and 91%, respectively) (Table 1).

Semen quality among study participants

The semen quality among the study participants was in the normal range of the WHO reference value [WHO 1999] for semen analysis. The mean semen volume was 3.43 (range: 1.7–12.3) ml and the concentration was 50.71 M/ml (range: 15–360 M/ml) (Table 2). The percentage of motile sperm cells was about 56 %. The percentage of atypical sperm was 54% (Table 2). In the case of semen morphology, head abnormalities constituted about 37%, neck abnormalities 14%, and tail abnormalities 6% (Table 2). The percentage of sperm cells with DNA damage was 16 % (Table 2) as measured by flow cytometry (Figure 1).

The association between lifestyle factors and main semen parameters, sperm morphology, and sperm chromatin structure

The overweight men (BMI 25–29.9 kg/m²) had decreased semen volume ($p = 0.01$). Obesity (BMI 30–40 kg/m²) was negatively related to semen volume and the percentage of motile sperm cells ($p = 0.01$ and 0.02, respectively) compared to men with normal weight (Table 3). Drinking red wine 1–3 times per week was associated with lower semen volume ($p = 0.04$) and the percentage of sperm neck abnormalities ($p = 0.04$), whereas drinking coffee 1–6 times per week was related to an increase in the percentage of motile sperm cells ($p = 0.05$) and in sperm head abnormalities ($p = 0.04$) (reference category- drinking <1/week). Also, everyday coffee drinking was associated with an increase in

Table 3. The association between semen parameters and modifiable lifestyle factors.

Variable	Volume b (p)	Conc. b (p)	Motility b (p)	Atypical b (p)	Sperm head abnormalities (%) b (p)	Sperm neck abnormalities (%) b (p)	Sperm tail abnormalities (%) b (p)	DFI (%) b (p)
BMI (kg/m ²)								
(18–24.9)	1.25 (ref.)	3.39 (ref.)	53.96 (ref.)	53.74 (ref.)	36.56 (ref.)	14.09 (ref.)	2.09 (ref.)	2.60 (ref.)
(25–29.9)	–0.10 (0.01)*	–0.04 (0.78)	0.73 (0.77)	0.05 (0.99)	0.13 (0.97)	–0.28 (0.78)	0.05 (0.73)	0.00 (0.95)
(30–40)	–0.13 (0.01)*	0.10 (0.57)	–7.12 (0.02)*	–0.31 (0.93)	0.05 (0.99)	–0.67 (0.59)	0.15 (0.39)	–0.11 (0.30)
Smoking								
No	1.17 (ref.)	3.40 (ref.)	54.58 (ref.)	54.43 (ref.)	37.34 (ref.)	14.16 (ref.)	2.15 (ref.)	2.59 (ref.)
Ex-smoker	–0.05 (0.22)	0.04 (0.79)	0.79 (0.77)	–1.97 (0.5)	–3.59 (0.30)	–0.22 (0.84)	0.14 (0.36)	–0.08 (0.40)
Smoker	0.05 (0.26)	–0.05 (0.74)	3.04 (0.23)	–0.64 (0.83)	0.74 (0.82)	–0.89 (0.40)	–0.11 (0.45)	0.04 (0.69)
Beer (days/week)								
(<1/week)	1.17 (ref.)	3.37 (ref.)	54.17 (ref.)	53.80 (ref.)	36.80 (ref.)	13.07 (ref.)	2.19 (ref.)	2.59 (ref.)
(1–3/week)	–0.01 (0.89)	0.09 (0.51)	2.71 (0.25)	0.31 (0.91)	–0.26 (0.93)	1.06 (0.27)	–0.04 (0.74)	–0.02 (0.84)
(4–7/week)	0.05 (0.33)	–0.16 (0.41)	2.60 (0.44)	–1.72 (0.67)	–0.39 (0.93)	1.86 (0.18)	–0.18 (0.34)	–0.02 (0.86)
White wine (days/week)								
(<1/week)	1.17 (ref.)	3.39 (ref.)	55.54 (ref.)	53.99 (ref.)	36.98 (ref.)	13.76 (ref.)	2.16 (ref.)	2.58 (ref.)
(1–3/week)	0.01 (0.93)	–0.01 (0.99)	5.76 (0.31)	–7.07 (0.28)	–8.48 (0.23)	1.60 (0.48)	–0.27 (0.39)	–0.02 (0.91)
Red wine (days/week)								
(<1/week)	1.19 (ref.)	3.37 (ref.)	55.94 (ref.)	53.85 (ref.)	36.05 (ref.)	14.07 (ref.)	2.17 (ref.)	2.58 (ref.)
(1–3/week)	–0.12 (0.04)	0.31 (0.14)	–2.62 (0.49)	3.56 (0.43)	6.18 (0.21)	–3.27 (0.04)	–0.09 (0.67)	–0.03 (0.82)
(4–7/week)	–0.20 (0.15)	–0.45 (0.34)	3.73 (0.65)	0.12 (0.99)	0.95 (0.93)	2.59 (0.44)	–0.66 (0.16)	0.07 (0.78)
Vodka (days/week)								
(<1/week)	1.17 (ref.)	3.38 (ref.)	55.58 (ref.)	54.36 (ref.)	36.77 (ref.)	13.93 (ref.)	2.17 (ref.)	2.59 (ref.)
(1–3/week)	0.02 (0.81)	0.08 (0.70)	2.06 (0.59)	–6.95 (0.13)	–1.11 (0.83)	–1.17 (0.46)	–0.24 (0.28)	–0.07 (0.55)
(4–7/week)	–0.04 (0.90)	1.07 (0.34)	2.42 (0.90)	–23.36 (0.33)	–15.77 (0.54)	–2.93 (0.72)	–2.17 (0.06)	–0.43 (0.50)
Coffee (days/week)								
(<1/week)	1.19 (ref.)	3.30 (ref.)	52.01 (ref.)	55.61 (ref.)	41.57 (ref.)	12.22 (ref.)	2.32 (ref.)	2.56 (ref.)
(1–6/week)	–0.01 (0.87)	0.19 (0.27)	6.26 (0.05)	–4.81 (0.20)	8.22 (0.04)	1.26 (0.33)	0.19 (0.29)	0.03 (0.78)
(Everyday)	–0.03 (0.43)	0.09 (0.56)	4.41 (0.10)	–1.48 (0.64)	5.75 (0.10)	2.49 (0.02)	0.24 (0.12)	0.02 (0.82)
Leisure Time Activity (MET indicators)								
No	1.19 (ref.)	3.19 (ref.)	56.05 (ref.)	51.16 (ref.)	34.00 (ref.)	13.77 (ref.)	2.06 (ref.)	2.63 (ref.)
(<24)	–0.01 (0.86)	0.34 (0.02)*	0.10 (0.97)	1.67 (0.60)	3.48 (0.31)	–0.09 (0.94)	0.06 (0.67)	–0.06 (0.51)
(≥24)	–0.04 (0.34)	0.25 (0.10)	–0.98 (0.72)	5.94 (0.07)	4.18 (0.24)	0.24 (0.83)	0.20 (0.20)	–0.10 (0.28)
Cell phone (years)								
(0–5)	1.16 (ref.)	3.03 (ref.)	60.77 (ref.)	45.73 (ref.)	32.42 (ref.)	12.04 (ref.)	2.02 (ref.)	2.52 (ref.)
(6–10)	–0.06 (0.32)	0.29 (0.22)	–4.13 (0.30)	4.44 (0.42)	2.28 (0.69)	–0.25 (0.86)	–0.01 (0.96)	0.01 (0.97)
(11–25)	–0.01 (0.84)	0.42 (0.13)	–11.27 (0.01)	19.00 (0.002)*	17.58 (0.01)*	0.12 (0.94)	–0.02 (0.93)	0.20 (0.22)
Boxer shorts								
No	1.21 (ref.)	3.36 (ref.)	55.11 (ref.)	55.47 (ref.)	36.61 (ref.)	15.66 (ref.)	2.28 (ref.)	2.65 (ref.)
Yes	0.10 (0.01)*	0.08 (0.51)	1.58 (0.47)	–4.33 (0.10)	0.05 (0.10)	–4.48 (0.001)*	–0.32 (0.01)	–0.18 (0.02)*
Sauna								
No	1.12 (ref.)	3.30 (ref.)	55.83 (ref.)	51.43 (ref.)	36.49 (ref.)	11.75 (ref.)	1.95 (ref.)	2.55 (ref.)
Yes	–0.02 (0.75)	0.16 (0.43)	–2.74 (0.44)	10.20 (0.04)	9.85 (0.05)	0.56 (0.64)	0.36 (0.09)	0.08 (0.50)

Conc.: semen concentration; DFI: DNA fragmentation index; BMI: body mass index; MET: Metabolic Equivalent Task; *significant associations after the control for multiple comparisons

Bold Values: Significance level 0.05 was used for statistical interference.

sperm neck abnormalities ($p=0.02$; Table 4). Leisure time physical activity (MET indicator <24) was positively associated with sperm concentration ($p=0.02$) compared to men who did not report leisure time physical activity. Comparatively, cell phone use of more than 11 years was negatively related to the percentage of motile sperm cells ($p=0.01$) and positively with the percentage of atypical sperm ($p=0.002$) and the percentage of sperm head abnormalities ($p=0.01$) (references- men using cell phones 0–5 years; Table 3,4).

Wearing boxer shorts among study participants was positively associated with the semen volume ($p=0.01$) and negatively with the percentage of neck, tail abnormalities and percentage of sperm cells with DNA damage ($p=0.001$,

$p=0.01$, and $p=0.02$, respectively). Also men who used the sauna had a higher percentage of atypical sperm ($p=0.04$) (Table 3).

After applying the correction for multiple comparisons, a negative association between BMI and semen volume and percentage of motile sperm was observed. Leisure time physical activity was positively associated with sperm concentration and using cell phone more than 10 years was positively related to the percentage of atypical sperm and the percentage of sperm head abnormalities. Additionally wearing boxer shorts was positively associated with the semen volume and negatively with the percentage of sperm neck abnormalities and the percentage of sperm cells with DNA damage.

Table 4. The association between modifiable lifestyle factors and semen parameters- multivariate analysis.

Main semen parameters	Coef (p value)
Volume (ml)	
BMI (kg/m ²) (25–30)	–0.09 (0.03)
BMI (kg/m ²) (30–40)	–0.12 (0.03)*
Red wine (days/week) (1–3/week)	–0.09 (0.15)
Red wine (days/week) (4–7/week)	–0.15 (0.25)
Boxer shorts yes	–0.06 (0.12)
Semen concentration (M/ml)	
Leisure time activity (MET indicator) (<24)	0.33 (0.04)*
Leisure time activity (MET indicator) (≥24)	0.34 (0.04)
Red wine (days/week) (1–3/week)	0.39 (0.09)
Cell phone (years) (11–25)	0.33 (0.14)
Motility (%)	
BMI (kg/m ²) (30–40)	3.25 (0.36)
Coffee (days/week) (1–6/week)	8.69 (0.02)
Coffee (days/week) (everyday)	7.55 (0.01)
Cell phone (years) (11–25)	–11.08 (0.02)*
Atypical (%)	
Cell phone (years) (11–25)	12.26 (0.06)
Boxer shorts (Yes)	–2.84 (0.42)
Leisure time activity (MET indicator) (≥24)	7.84 (0.07)
Sauna Yes	3.85 (0.45)
Vodka (days/week) (1–3/week)	1.82 (0.74)
Coffee (days/week) (1–6/week)	2.52 (0.78)
Sperm heads abnormalities (%)	
Coffee (days/week) (everyday)	8.47 (0.05)
Coffee (days/week) (1–6/week)	8.30 (0.12)
Cell phone (years) (11–25)	12.08 (0.07)
Sauna (Yes)	3.67 (0.48)
Sperm neck abnormalities (%)	
Red wine (days/week) (1–3/week)	–3.72 (0.01)
Coffee (days/week) (everyday)	2.34 (0.03)
Beer (days/week) (4–7/week)	0.87 (0.79)
Boxer shorts (Yes)	–4.35 (0.002)*
Sperm tail abnormalities (%)	
Boxer shorts (Yes)	0.05 (0.75)
Red wine (days/week) (4–7/week)	–0.14 (0.60)
Vodka (days/week) (4–7/week)	–1.77 (0.12)
Coffee (days/week) (everyday)	–1.55 (0.23)
Leisure time activity (MET indicator) (≥24)	0.55 (0.66)
Sauna (Yes)	0.37 (0.09)
DFI (%)	
Boxer shorts (Yes)	–0.22 (0.02)*

Adjusted for: age, time of sexual abstinence, past diseases; *significant associations after the control for multiple comparisons; References categories: BMI (kg/m²) (25,30], BMI (kg/m²) (30,40]- ref. BMI (kg/m²) [18–24.9], Red wine (days/week) 1–3/week, Red wine (days/week) 4–7/week- ref. Red wine (days/week) <1/week, Boxer shorts yes – ref. Boxer shorts no; Leisure time activity (MET) (0,24], Leisure time activity (MET) (25,185]- ref. No leisure time activity; Coffee (days/week) 1–6/week, Coffee (days/week) everyday- ref. Coffee (days/week) <1/week; Cell phone (years) (11,25]- ref. Cell phone (years) (0,5]; Sauna Yes- ref. no sauna; Vodka(days/week) (1–3/week)-ref. Vodka(days/week) <1/week; Beer (days/week) 1–3/week- ref. Beer days/week) <1/week; BMI: body mass index; DFI: DNA fragmentation index

Bold Values: Significance level 0.05 was used for statistical interference.

Relationship between the lifestyle factors and main semen parameters, sperm morphology, and sperm chromatin structure- multivariate analysis

All variables at the level of significance of 0.2 were included into multivariate models (Table 4). All presented associations were adjusted for potential confounders (time of sexual

abstinence, age, past diseases). A negative association between being overweight and obesity and semen volume ($p=0.03$ and $p=0.03$, respectively) compared to men with normal weight was found. Leisure time activity, MET indicators <24 and ≥24 were positively associated with sperm concentration ($p=0.04$, $p=0.04$, respectively) compared to participants that did not report leisure time physical activity. Also coffee drinking 1–6 days per week ($p=0.02$) and everyday ($p=0.01$) was positively associated with the percentage of motile sperm cells (reference category <1/week; Table 4). Everyday coffee drinking increased the percentage of sperm head and neck abnormalities ($p=0.05$ and $p=0.03$, respectively; Table 4). Whereas using the cell phone more than 10 years was negatively associated with the percentage of motile sperm cells ($p=0.02$; Table 4).

Men who wore boxer shorts had a lower percentage of sperm neck abnormalities ($p=0.002$) and percentage of sperm with DNA damage ($p=0.02$) compared to those who did not wear such underwear. Drinking red wine (1–3/week) was negatively associated with the percentage of sperm neck abnormalities ($p=0.01$; Table 4).

After applying the correction for multiple comparisons, a positive association between leisure time activity and sperm concentration was observed. Using a cell phone for more than 10 years was negatively associated with the percentage of motile sperm cells, and increased BMI was negatively related to semen volume. Additionally wearing boxer shorts decreased the percentage of sperm neck abnormalities and the percentage of sperm with DNA damage.

Discussion

Data presented in this study showed a significant negative association between increased BMI and semen volume. Leisure time activity was positively associated with sperm concentration. Also coffee drinking was positively associated with the percentage of motile sperm, and the percentage of sperm head and neck abnormalities and drinking red wine (1–3/week) was negatively associated with sperm neck abnormalities. In comparison, the use of a cell phone for more than 10 years decreased the percentage of motile sperm cells. Men who wore boxer shorts had a lower percentage of sperm neck abnormalities and the percentage of sperm with DNA damage.

After applying the correction for multiple comparisons, positive association between leisure time activity and sperm concentration was observed. The use of a cell phone for more than 10 years was negatively associated with the percentage of motile sperm cells and increase in BMI was negatively related to semen volume. Additionally, wearing boxer shorts decreased the percentage of sperm neck abnormalities and the percentage of sperm with DNA damage.

The results are in agreement with previous studies which suggested that lifestyle factors may affect semen quality [Jensen et al. 2004a; Povey et al. 2012; Sallmen et al. 2006]. In the present study increased BMI was significantly associated with a decreased semen volume.

Excess and lower body weight are both observed as risk factors for low sperm quality in many studies [Fejes et al. 2005b; Jensen et al. 2004a; Kort et al. 2006;

Sallmen et al. 2006]. A dose-response relationship between BMI and male infertility was observed in the study performed in Finland [Sallmen et al. 2006]. In Hungary significant correlations were found between waist and hip circumferences and semen characteristics [Fejes et al. 2005b]. Jensen et al. [2004a] noticed a reduction in sperm concentration and total sperm count among overweight, obese, and underweight men. Men presenting with a BMI greater than 25 kg/m² have fewer chromatin-intact normal-motile sperm cells per ejaculate [Kort et al. 2006] and a lower total sperm count and sperm motility [Sekhavat and Moein 2010]. Whereas meta-analysis of 31 studies found no evidence of an association between increased BMI and semen parameters [MacDonald et al. 2010].

Our findings suggest that leisure time physical activity was significantly associated with increased sperm concentration. The same positive relationship between leisure time physical activity and semen quality was observed in the study performed in Spain. Physically active men had higher sperm motility, sperm concentration, semen volume, elevated levels of FSH, LH, testosterone, and an increased testosterone/cortisol ratio [Vaamonde et al. 2012]. Whereas in the study in Boston, bicycling ≥ 5 h/wk was associated with low sperm concentration and low total motile sperm compared with no regular exercise [Wise et al. 2011].

Measuring leisure time physical activity by MET in the above study represents a simple, practical, and easily understood procedure for expressing the energy cost of physical activities as a multiple of the resting metabolic rate [Jette et al. 1990]. But it also has some limitations. These include its variance according to body size, level of fitness, skill, and whether or not the activity is performed in a competitive situation.

In the present study a negative relationship between the use of a cell phone (more than 10 years) and sperm motility was observed. This is in line with some studies [Agarwal et al. 2008; Davoudi et al. 2002; Kilgallon and Simmons 2005] showing that GSM phone usage of 5 days for 6 hours per day decreased the rapid progressive motility of sperm [Davoudi et al. 2002] and cell phones used for longer durations adversely affected the quality of semen by decreasing the sperm counts, motility, viability, and morphology [Agarwal et al. 2008; Fejes et al. 2005a]. Also men who carried their mobile phone in their hip pocket or on their belt had lower sperm motility [Kilgallon and Simmons 2005].

Wearing boxers was positively associated with the percentage of sperm neck abnormalities and sperm with DNA damage compared to men who do not use such underwear. The lower risk of poorer semen quality among men who usually wear boxer shorts is consistent with some [Povey et al. 2012; Tiemessen et al. 1996] but not with all previous studies [Munkelwitz and Gilbert 1998].

Studies on caffeine intake and semen quality have shown contradictory results. Some suggested no association [Oldereid et al. 1992; Ramlau-Hansen 2008], whereas others found reduced sperm concentration, total sperm count, and motility [Jensen et al. 2010; Sobreiro et al. 2005; Vine 1996]. In the present study, coffee consumption increased the percentage of motile sperm cells and the percentage of sperm head and neck abnormalities. However, the estimation

of caffeine intake based on self-report can be a problem because cups of coffee vary in strength related to brewing and brand (caffeine content) and caffeine is present in many products that would not necessarily be recognized and reported during the interview.

In the review of the studies of the effect of alcohol drinking and semen quality the data showed an apparent protective effect of moderate alcohol drinking on sperm parameters [Marinelli et al. 2004] which is in line with our results suggesting a relationship between drinking red wine 1–3 times per week and a decreased percentage of sperm neck abnormalities. In comparison, the recent study performed by Povey and colleagues [2012] no reduction in low-motile sperm concentration was found and no protective effect was observed. Although we have not validated reports of exposure to alcohol (for example by measuring fatty acids in hair) and we must assume that some degree of misclassification exists, we can reasonably conclude that our findings do not result from differential misclassification.

The absence of an effect of tobacco smoke on semen quality in the present study is consistent with some [Li et al. 2009; Trummer et al. 2002] but not all [Vine 1996] previous studies. Evidence that intensive and frequent use of saunas has a negative influence on semen quality is scarce [Brown-Woodman et al. 1984; Saikhun et al. 1998] and this hypothesis has not been confirmed in the present study. A significant decrease in average CASA parameters were found after exposure to a sauna during a 2 week period [Saikhun et al. 1998]. Also the total sperm count decreased 1–5 weeks after exposure to 60–70% [Brown-Woodman et al. 1984].

On one hand, our study had several strengths. The results were adjusted for many factors that may be associated with semen quality. Additionally the smoking status was verified using the level of cotinine in the saliva. A third strength arises from the fact that many different semen parameters were included in the analysis: main semen parameters, morphology, and sperm chromatin structure.

On the other hand, the study does have weaknesses. First, we were not able to examine a representative sample of the general male population. We tried to overcome this disadvantage by selecting only men with normal semen parameters or with slight oligozoospermia according to the WHO [1999] classification among the male infertility patients. Further, although the power of the study was sufficient to draw conclusions about common lifestyle choices, in cases of some exposures the number of exposed man was rather small, e.g., the case of using a sauna.

In conclusion, data from the present study showed a significant effect of modifiable lifestyle factors (BMI, physical activity, alcohol drinking, coffee consumption, cell phone use, and wearing boxer shorts) on semen quality: main semen parameters, sperm morphology, and sperm chromatin structure. The findings are important in the prevention of the poorer semen quality and highlight the need for lifestyle modification. It is an important premise to expand the treatment of infertility with sensible health advice. It is necessary to watch weight, to drink alcohol within mild to moderate levels, and to avoid tight-fitting underwear.

Materials and Methods

Study population

The study population consisted of 344 men who were attending an infertility clinic for diagnostic purposes with a normal semen concentration of 20–300 M/ml or with slight oligozoospermia (semen concentration of 15–20 M/ml) [WHO 1999] from the study entitled “Environmental factors and male infertility” which is part of the “Epidemiology of reproductive hazards in Poland — multicentre study in Poland” project supported by the National Center for Research and Development in Poland, grant no. PBZ-MEiN-/8/2//2006. Out of 580 men who fulfill the inclusion criteria (based on semen analysis) 344 (59%) men agreed to participate in the study and signed the written agreement. The Nofer Institute of Occupational Medicine Bioethical Committee Board approved the study (Resolution No 9/2007 (04.06.2007) and written informed consent was obtained from all subjects before their participation.

Participants were all healthy and under 45 years of age. The men received a physical examination along with height and weight measurements. All participants were interviewed and provided a semen sample. The interview included questions about demographics, socio-economic status, medical history related to past diseases which may have had an impact on semen quality, lifestyle factors (consumption of alcohol, tobacco, coffee intake, wearing loose underwear, and cell phone and sauna usage), physical activity, and occupational information.

Information about lifestyle factors

A detailed questionnaire about the lifestyle factors was performed. The lifestyle factors were coded positively if they occurred during the 3-month window prior to semen collection. Height and weight were collected during the physical examination. The BMI was calculated according to WHO [2012].

Information regarding smoking was collected from the study participants. Additionally the smoking status was verified by measuring the cotinine level in saliva in a laboratory in the Nofer Institute of Occupational Medicine. The saliva cotinine level was measured using high performance liquid chromatography coupled with tandem mass spectrometry/positive electrospray ionization (LC-ESI+MS/MS) and the isotope dilution method. This procedure has been validated under ISO 17025 criteria and accredited by the Polish Center of Accreditation (Certificate AB215). Men were identified as smokers when their cotinine level in saliva was higher than 10 ng/ml. If self-reported smoking and cotinine measurements were not in agreement, the information about smoking status was based on the level of cotinine in saliva.

The Metabolic Equivalent Task (MET) method was used to calculate leisure time activity. A MET is defined as the resting metabolic rate, that is, the amount of oxygen consumed at rest, sitting quietly in a chair, approximately 3.5 ml O₂/kg/min (1.2 kcal/min for a 70-kg person) [Ainsworth et al. 1993; Ainsworth et al. 2000; Jette et al. 1990] and three METs (three times resting energy expenditure) is the approximate intensity of a brisk walk.

Moderate- or vigorous-intensity activities are those with an intensity level of at least three metabolic equivalents (METs) according to the Compendium of Physical Activities [Ainsworth et al. 1993; Ainsworth et al. 2000]. Leisure time physical activity was divided into three categories based on the intensity of activity: light activity (i.e., walking), moderate activity (i.e., biking), and vigorous activity (i.e., swimming). To each category of leisure time physical activity ranges were attributed: 3.3, 4, and 8, respectively [Ainsworth et al. 1993; Ainsworth et al. 2000]. The MET indicator was calculated as a sum of products, appropriate ranks, and time (in hours) spent on each type of intensity of leisure time physical activity weekly. Two categories of MET indicator categories were chosen: <24 and ≥24 based on the MET indicators distribution in the study population.

Study subjects were grouped according to their stated drinking behavior: type of alcohol drinking (beer, white and red wine, vodka) and the frequency of drinking (<1/week, 1–3/week, and 4–7/week). Also the consumption of coffee was based on the frequency of drinking per week (<1/week, 1–6/week, and everyday). Cell phone use was based on the years of usage of this equipment (0–5 years, 6–10 years, and 11–25 years). Also, information about type of underwear used by study participants and their sauna usage were collected.

Semen collection and analysis

Participants provided a semen sample for diagnostic analysis as a part of a fertility investigation, which was used for this study. The semen sample was collected into a standard plastic container and analyzed according to a protocol based upon the technique outlined by WHO [1999]. The specific length of abstinence was obtained. The semen quality parameters – liquefaction time, agglutination volume, pH, sperm concentration, and motility were determined.

Percentage motile sperm was determined with the use of computer-aided semen analysis (CASA) (Hamilton-Thorne Version 10HTM-IVOS, Hamilton-Thorne Research, Danvers, MA) using 2 Chamber Leja slides (Leja, The Netherlands). Simultaneously to the CASA analysis, sperm concentration was performed manually by counting a minimum of 200 fixed sperm on an improved Neubauer hemocytometer. Each manual count was performed twice. To validate the accuracy of the CASA analysis each day a count of standard quality-control suspensions of latex beads in liquid medium was performed (Accu Beads, Hamilton-Thorne Research). Two standard suspensions of 18 × 10⁶/mL (range, 17.5–20.5 × 10⁶/mL) and 35 × 10⁶/mL (range, 30–40 × 10⁶/mL) in liquid medium were routinely used.

Sperm morphology was quantified using strict Kruger criteria [Kruger et al. 1988] the semen smears were air-dried, fixed, and stained according to Papanicolaou then analysis was performed on an Nikon Eclipse 200 microscope with the magnification ×1000 (with oil immersion). A total of 200 sperm per sample were analyzed.

Assessment of the sperm chromatin structure assay (SCSA) was performed using flow cytometry [ASRM 2006]. Fluorescence of green (515–530 nm) and red (>630 nm) bands was measured by a DAKO Galaxy flow cytometer.

The green fluorescence band corresponded to intact double stranded DNA and the red fluorescence to fragmented, single stranded sperm DNA. Approximately 15 000–25 000 spermatozoa were acquired for each sample at a flow rate of 400–500 events/s. An artificial parameter alpha t (alpha t = red/(green + red) fluorescence), was created for the calculations. Cells with an abnormal chromatin structure (i.e., fragmented DNA) showed a distinct shift of the alpha t parameter value. The DNA Fragmentation Index (DFI) was calculated according to the formula: $DFI = (\text{cells with shift of alpha-t parameter} / \text{all cells}) \times 100$ each analyzed sperm cell and was shown on a histogram. Cells with an abnormal chromatin structure showed a distinct shift of the alpha t parameter value.

Statistical analysis

Linear regression was used to analyze relationships between semen quality and lifestyle factors. Some dependent variables were transformed if they were not a normal distribution. Volume, semen concentration, and DFI were log transformed. Sperm tail abnormalities were square root transformed. Apart from the univariate, multivariate analysis was also performed. All variables significant at the 0.2 level were included in multivariate models. The observations with missing data were excluded based on variables used in model. False Discover Rate (FDR) method was used to correct for multiple comparisons. The R version 2.15.1 was used for all statistical analyses (<http://www.R-project.org> [R Core Team]) [R Core Team 2012]. A significance level 0.05 was used for statistical inference.

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The study was performed at the Nofer Institute of Occupational Medicine, Department of Environmental Epidemiology, Lodz, Poland.

Declaration of interest

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Author contributions

Involved in study concept and design: JJ, WH, MR; Data analysis: WS; Drafted the manuscript: JJ; Determination of cotinine level in saliva: DL; Responsible for recruitment of men to the study and analysis of main semen parameters: MR, PR. Responsible for analysis of sperm chromatin structure: MB; All authors provided substantial intellectual contributions and approved the final version of manuscript.

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