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## ARTICLE

# Physical activity, fatness, educational level and snuff consumption as determinants of semen quality: findings of the ActiART study

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**Abstract** In this study, the association between physical activity and other potential determinants, objectively measured by accelerometry, was examined. Sixty-two men attending an infertility clinic participated in the study. Obese men (body mass index  $\geq 30$ ) and those with a waist circumference 102 cm or more had lower semen volume than the other men ( $P < 0.05$ ). Higher values in sperm parameters were observed in participants who completed university studies and those who did not consume snuff, compared with the other participants ( $P < 0.05$ ). Finally, men who spent an average number of 10 min-bouts of moderate-to-vigorous physical activity had significantly better semen quality than those who engaged in low or high numbers of bouts of activity ( $P < 0.05$ ). No associations were found for sedentary or moderate-to-vigorous physical activity time when it was not sustained over 10 min, i.e. not in bouts. Men who have average levels of physical activity over sustained periods of 10 min are likely to have better semen quality

<http://dx.doi.org/10.1016/j.rbmo.2015.03.004>

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than men who engage in low or high levels of such activity. Similarly, high levels of total and central adiposity, low educational level and snuff consumption are negatively related to semen quality. 

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**KEYWORDS:** accelerometry, adiposity, education, physical activity, semen, snuff

## Introduction

Infertility is an increasing public health problem, affecting about 15% of couples of fertile age (Boivin et al., 2007). Although reproductive abnormalities in the male partner are identified in about one-half of these cases, few risk factors for abnormal semen quality have been identified (Juul et al., 1999; Kovac et al., 2013). Emerging evidence suggests that lifestyle factors such as smoking (Ramlau-Hansen et al., 2007; Wegner et al., 2010), diet (Afeiche et al., 2014), physical activity (Hajizadeh Maleki et al., 2013; Vaamonde et al., 2006; Wise et al., 2011), overweight and obesity (Crujeiras and Casanueva, 2014; Sermondade et al., 2013), stress (Janevic et al., 2014), and sexual activity habits among others seem to have a cumulative effect on sperm quality (Wogatzky et al., 2012).

Physical activity is an efficient method for preventing weight gain, and has a broad and positive systemic influence, such as reducing the risk for cardiovascular diseases, stroke, type 2-diabetes, cancer and metabolic syndrome (US department of Hyealth and Human Services, 2008), and could therefore be potentially related to sperm quality. Closely related to physical activity is physical fitness, and particularly cardiorespiratory and muscular strength are considered powerful markers of health, as well as strong predictors of morbidity and mortality for cardiovascular disease and for all causes (Kodama et al., 2009; Ortega et al., 2012; Ruiz et al., 2008). Little information is available on whether fitness levels, e.g. cardiorespiratory fitness and muscular strength, are related to sperm quality.

As often happens in human physiology, too much or too little of something is not good. This is the case for body mass index (BMI) and semen quality, for which an inverted U-shape relationship has been proposed, i.e. too low or too high values of BMI are related to a poorer sperm quality (Jensen et al., 2004). This could also be the case for physical activity, as inactivity and sedentary lifestyle have a well-known harmful effect on many health outcomes (Pate et al., 2008), whereas too much physical activity has been negatively associated with semen quality (De Souza et al., 1994; Safarinejad et al., 2009; Vaamonde et al., 2006). In this context, recent studies support that recreationally active men have a healthier semen production than non-active men, but also than elite athletes, suggesting a U-shape relationship (Hajizadeh Maleki et al., 2013; Vaamonde et al., 2012).

The few studies analysing the association between regular physical activity and semen quality have shown inconclusive results (Braga et al., 2012; De Souza et al., 1994; Hajizadeh Maleki et al., 2013; Safarinejad et al., 2009; Vaamonde et al., 2006, 2012; Wise et al., 2011; Wogatzky et al., 2012). Most of these studies have used self-report methods to assess physical activity, i.e. questionnaires. Therefore, new empirical and objective data about physical activity and sperm quality are needed to better understand the role of physical activity in male reproductive health.

In the present study, the association of objectively measured physical activity was analysed using accelerometry, with semen quality indicators among men attending an infertility clinic. In addition, the effect of other potentially related factors, such as fatness markers, educational level, television watching, consumption of smokeless tobacco (snuff, which is highly consumed in Nordic countries), handgrip strength and self-reported fitness were studied.

## Materials and methods

### Study sample and design

Male participants from the Physical Activity and Assisted Reproductive Technology (ActiART) project were enrolled in the present study. Data were collected from the Centre for Reproduction at Uppsala University Hospital, Sweden between February 2011 and January 2014. As couples undergoing infertility treatment might change their everyday physical activity levels during the treatment period (Kucuk et al., 2010), only participants who were attending the clinic for the first time were recruited, before they received any testing (including semen collection) or treatment. In total, 72 men agreed to participate. Participants with azoospermia ( $n = 3$ ) were not excluded from the study. After excluding the participants with missing values in semen parameters ( $n = 7$ ), the final sample size for the present study remained 62 men. Most of the participants did not have children when enrolled in the study, i.e. 86% with no previous child. The ActiART project was approved by the Regional Ethical Review Board of Uppsala on 1 July 2009 (reference number: 2009/084); written informed consent was obtained before participation.

### Semen analysis

Semen samples were collected and analysed at the Centre for Reproduction, at Uppsala University Hospital during routine fertility evaluation. The accuracy of the semen analysis was guaranteed by a handful of highly trained embryologists, all of whom had several or many years of experience, and who participated regularly in both internal and external (inter-laboratory) controls to ensure accuracy and reproducibility of the results.

Each participant provided one semen sample as a routine fertility work up at the clinic. Participants were asked for 3–5 days of sexual abstinence before the sample collection. Samples were collected by masturbation at the clinic or at the participants' home. In the latter case, the sample had to be kept at body temperature and brought to the laboratory within 1.5 h after the collection. On arrival at the laboratory, the abstinence period and occurrence of any loss of ejaculate were recorded.

Ejaculates were analysed according to the the World Health Organization (WHO) manual for examining and processing human semen (WHO, 2010). Concentration of sperm and concentration of motile sperm were determined by a Makler counting chamber (Sefi Medical Instruments, Israel) using standard clinical procedures. Visually assessed sperm motility, which indicates the percentage of sperm with progressive and non-progressive movement (WHO, 2010), was calculated by using concentration values for sperm and motile sperm. Total number of sperm and total number of motile sperm were estimated by multiplying the respective concentrations with semen volume. The latter one was measured by pipette. The percentage of participants above recommended levels of the WHO for sperm parameters was calculated (Cooper et al., 2010).

### Assessment of fatness

Double measurement (mean used for analyses) of weight, height and waist circumference was obtained by an assistant nurse. The BMI was calculated as weight (kg)/height<sup>2</sup> (m). On the basis of international BMI cut-off values for adults (24), participants were grouped into weight status categories. Waist circumference data were dichotomized according to the healthy waist circumference recommendation for men (<102 cm) (WHO, 2011).

### Assessment of socioeconomic factors and lifestyle factors by questionnaire

All participants were asked to fill out a questionnaire so that information about their education, occupation, smoking and consumption of snuff and TV watching could be collected. Educational level was recorded as primary or secondary level or university or college level. Occupation was recorded as employed, unemployed or studying.

### Assessment of fitness

Physical fitness was objectively measured by the handgrip strength test, and self-reported by the International Fitness Scale (IFIS). The handgrip strength test was measured in both hands (average level was used for analyses) using the TTK digital hand dynamometer (Takei, Japan), which has shown to be valid and reliable (España-Romero et al., 2010), following the procedures previously described by our group (Ruiz-Ruiz et al., 2002).

The IFIS provides information on individual's general physical fitness, cardiovascular fitness, muscular strength, speed-agility and flexibility (Ortega et al., 2011, 2013b). This self-reported scale has shown a good agreement with measured fitness (validity), and to be reliable (test-retest) and to relate with health outcomes such as adiposity and metabolic syndrome in adults (Ortega et al., 2013b). A question about participants' overall health was added for ActiART study. Participants were asked to rate each fitness components, their overall fitness and overall health perceived on a five-level Likert scale (1 = very poor, 5 = very good). Because of the

small sample size, few participants fell into the extreme categories (very poor and very good). Consequently, for statistical analysis, the category 'very poor' was merged with 'poor' and 'very good' with 'good'. Fairly equal distribution of participants between the groups was obtained.

### Assessment of physical activity

Uniaxial ActiGraph accelerometer GT1M (ActiGraph LLC, Florida, USA) was used to objectively assess the duration and intensity of physical activity. During the participants' first visit to the clinic, a device was distributed together with accelerometer wearing information sheet and a diary to document the wear- and non-wear time. Participants were asked to wear an accelerometer for the following 7 days, starting from the subsequent morning and to remove it only while swimming, showering or sleeping. The accelerometer was attached to elasticized band, and participants were instructed to wear it around their waist. Epochs were set at 5 s.

Actilife software version 6.10.2 (ActiGraph LLC, FL, USA) was used to analyse accelerometer data. Data with zero counts lasting for 60 min pt,ptr, with allowance for 1–2 min of counts between 0 and 100, were considered as non-wear time (Troiano et al., 2008), and were therefore excluded. According to previous studies, only days with 10 h or more of registration time (Troiano et al., 2008) were included in the analysis. As 3–4 days of valid data are needed to be representative for individual's habitual physical activity level (Trost et al., 2005), only men with 3 or more days of valid accelerometer data ( $n = 57$ ) were included into the physical activity analysis. Compliance to wearing the accelerometer was high, i.e. 78.9% of the participants had valid accelerometer data for 7 days ( $n = 45$ ) and 98.2% for 5 or more days ( $n = 56$ ).

Time in minutes per day spent at different intensities of physical activity was calculated using Freedson's cut-points, as follows: vigorous physical activity if 5725 counts or more per min (cpm), which refers to activities of high energy expenditure ( $\geq 6$  metabolic equivalents [MET]); moderate physical activity if 1952–5724 cpm (3–5.9 MET) and sedentary time if less than 100 cpm (about 1.5 MET) (Freedson et al., 1998). Moderate-to-vigorous physical activity (MVPA) was calculated by summing the time spent at moderate and vigorous physical activity. Average physical activity expressed as mean counts per minute, was computed as the sum of counts per all valid days divided by the total wear time in minutes in these days.

As accumulation of activity might be important for health outcomes (US department of Hyealth and Human Services, 2008), the number of bouts was computed (i.e. blocks of sustained activity at a certain intensity) of MVPA, defined as 10 or more consecutive minutes above the moderate physical activity threshold (i.e. 1952 cpm), with allowance for interruption of 1 or 2 min below threshold (Hagströmer et al., 2010). A bout was terminated by 3 min below threshold. The mean numbers of bouts per day were calculated and used in the analyses.

### Statistical analysis

SPSS 20 for Windows (IBM, Chicago, IL, USA) was used for analysis. For descriptive purposes, mean and standard deviation



were used for normally distributed variables, whereas median and percentile 25th and 75th were used for non-normally distributed variables. Similarly, Pearson's correlation was used to analyse the association of age and lifestyle factors, i.e. physical activity levels, sedentary time, hand grip strength, weight and height, BMI, waist circumference, with semen quality indicators, except in categorical variables or those non-normally distributed (education, bouts of MVPA, IFIS and snuff) for which Spearman's correlation was used. Semen parameters were used as a continuous variable for all the analysis.

One-way analysis of variance (ANOVA) was used to explore the differences in the distribution of semen quality values across groups of the predictors included in this study, e.g. educational level, snuff consumption and physical activity variables (categorized as tertiles). Pair-wise comparisons were conducted using Bonferroni's adjustment. Physical activity levels were analysed as continuous variable for correlation analyses, but were categorized as tertiles for the ANOVA to test the inverted U-shaped hypothesis. Analysis of covariance was conducted to adjust the models for potential confounders, i.e. those variables studied who showed to be significantly correlated with semen parameters. Statistical significance level was set at 5%.

## Results

### Characteristics

Characteristics of the study sample are shown in Table 1. A total of 53.2% of participants were overweight or obese (12.9% of them obese) and 18% had a waist circumference above the recommended limit of 102 cm. Mean time spent in MVPA was 53.2 min/d and in sedentary time was 11.7 h/d. The mean value of each semen parameter was above the WHO reference value for semen characteristics (36), whereas 27.4% ( $n = 17$ ) of the participants had one parameter or more below these reference values.

### Relationships between lifestyle factors and indicators of semen quality

Bivariate correlations between the study exposures and the semen quality parameters are shown in Table 2. Weight, BMI and waist circumference showed negative correlation with ejaculate volume ( $r = -0.304$ ,  $P = 0.02$ ;  $r = -0.326$ ,  $P = 0.01$ ; and  $r = -0.334$ ,  $P = 0.009$ , respectively) and total number of sperm ( $r = -0.306$ ,  $P = 0.01$ ,  $r = -0.258$ ,  $P = 0.04$ ;  $r = -0.286$ ,  $P = 0.03$  respectively).

Educational levels showed positive correlation with sperm concentration ( $r = 0.347$ ,  $P = 0.006$ ), total number of sperm ( $r = 0.384$ ,  $P = 0.002$ ), motile sperm concentration ( $r = 0.264$ ,  $P = 0.04$ ) and total motile sperm ( $r = 0.293$ ,  $P = 0.02$ ). Consumption of snuff was negatively correlated with sperm concentration ( $r = -0.314$ ,  $P = 0.02$ ), total number of sperm ( $r = -0.299$ ,  $P = 0.02$ ), motile sperm concentration ( $r = -0.375$ ,  $P = 0.003$ ), total motile sperm ( $r = -0.349$ ,  $P = 0.006$ ) and total motility percentage ( $r = -0.299$ ,  $P = 0.02$ ). A borderline significant and positive association was also found between the number of 10-min bouts of MVPA and sperm concentration

( $r = 0.261$ ,  $P = 0.05$ ). No significant correlations were found between the other physical activity variables, television watching, handgrip or self-reported fitness and semen parameters (Table 2).

The ANOVA analyses showed a significantly higher ejaculate volume in men with normal weight compared with obese men ( $3.41 \pm 1.5$  versus  $2.07 \pm 0.9$  ml,  $P = 0.04$ ; no differences observed between normoweight and overweight men); and borderline significant difference in men who met the recommendation for waist circumference control compared with men who did not ( $3.23 \pm 1.4$  versus  $2.33 \pm 1$  ml,  $P = 0.05$ ). Men who attended university level studies showed higher sperm concentration ( $61.8 \pm 46.3$  versus  $33.3 \pm 37.0$  M/ml,  $P = 0.006$ , total number of sperm ( $209.81 \pm 176.6$  versus  $89.47 \pm 92.7$ ,  $P = 0.004$ ), motile sperm concentration ( $38.3 \pm 33.7$  versus  $21.2 \pm 22.4$  M/ml,  $P = 0.03$ ) and total motile sperm ( $133.8 \pm 135.5$  versus  $59.9 \pm 67.2$  M,  $P = 0.02$ ) compared with those men who only attended primary or secondary school (Figure 1). Men who did not consume snuff had better sperm quality compared with those men who consumed snuff, particularly in sperm concentration ( $56.7 \pm 44.0$  versus  $33.2 \pm 45.0$  M/ml,  $P = 0.02$ ), motile sperm concentration ( $36.0 \pm 29.6$  versus  $17.7 \pm 30.1$  M/ml,  $P = 0.007$ ), total motile sperm ( $117.1 \pm 109.5$  versus  $62.4 \pm 120.5$  M,  $P = 0.008$ ) and total motile percentage ( $59.51 \pm 18.3$  versus  $45.15 \pm 19.5$  %,  $P < 0.009$ ) (Figure 2). Please, note that the Figures show the information as entered in the final models, i.e. transformed data as appropriate, whereas, in the text above, the raw means are presented as complementary information to the Figures.

Semen parameters were compared with ANOVA according to tertiles (T) of 10-min bouts of MVPA, and an inverted U-shape association was observed for most of the semen quality indicators studied (Figure 3). Significant differences were found in sperm concentration (overall  $P = 0.02$ , T1 versus T2  $P = 0.01$ ), total sperm number (overall  $P = 0.03$ , T1 versus T2  $P = 0.03$ ), motile sperm concentration (overall  $P = 0.01$ , T1 versus T2  $P = 0.01$ ) and total motile sperm (overall  $P = 0.01$ , T1 versus T2  $P = 0.01$ ). After adjusting the model for educational level and snuff consumption, the U-shape trend persisted (Figure 3). Similar U-shape pattern was observed for the rest of variables studied (sedentary time, MVPA and average physical activity), yet differences between groups were not significant (data not shown).

## Discussion

This study analysed the relationship of objectively measured physical activity, muscular strength, self-reported fitness, total and central adiposity and lifestyle variables such as educational level, employment status, snuff consumption, and television-watching, with semen quality parameters among men attending an infertility clinic. Several major findings were obtained: (i) higher BMI and waist circumference were related with semen volume, but not with the rest of semen parameters studied; (ii) lower educational levels and snuff consumption were associated with poorer semen quality; (iii) an inverted U-shape association was observed between the number of bouts of MVPA, i.e. blocks of 10-min of sustained activity, and most of semen parameters studied, yet no association was observed in the rest of physical activity or sedentary variables studied.

Table 1 Characteristics of the study sample.<sup>a</sup>

	N	
Age (years) (mean $\pm$ SD)	62	35.2 $\pm$ 5.7
Weight (kg) (mean $\pm$ SD)	62	85 $\pm$ 12.7
Height (cm) (mean $\pm$ SD)	62	181.5 $\pm$ 8.1
BMI (kg/m <sup>2</sup> ) (mean $\pm$ SD)	62	25.8 $\pm$ 4
BMI categories		
Underweight (%)	0	0
Normal weight (%)	29	46.8
Overweight (%)	25	40.3
Obese (%)	8	12.9
Waist circumference (cm) (mean $\pm$ SD)	61	90.3 $\pm$ 10.7
Waist circumference $\geq$ 102 cm (%)	11	18.0
Education		
Primary or secondary education (%)	23	37.1
University or college education (%)	39	62.9
Occupation		
Employed (%)	56	93.3
Unemployed (%)	1	1.7
Studying (%)	3	5
Smoking		
Never (%)	40	67.8
Yes, before, but not now (%)	15	25.4
Yes, more than one cigarette per day (%)	1	1.7
Yes, less than cigarette per day (%)	3	5.1
Snuff: Yes (%)	17	28.3
Television watching (h/day) (mean $\pm$ SD)	62	1.9 $\pm$ 1.3
International Fitness Scale (IFIS):		
General physical condition		
Poor/average/good (%)	4/24/34	6.5/38.7/54.8
Cardiovascular fitness		
Poor/average/good (%)	10/24/28	16.1/38.7/45.2
Muscular strength		
Poor/average/good (%)	3/12/47	4.8/19.4/75.8
Speed-agility		
Poor/average/good (%)	9/15/38	14.5/24.2/61.3
Flexibility		
Poor/average/good (%)	10/26/26	16.1/41.9/41.9
Overall health		
Poor/average/good (%)	05/9/48	8.1/14.5/77.4
Handgrip (kg) (mean $\pm$ SD)	62	53.6 $\pm$ 7.4
Physical activity levels (measured by accelerometry):		
Sedentary time (min/da) (mean $\pm$ SD)	57	701.2 $\pm$ 70.4
MVPA (min/day) (mean $\pm$ SD)	57	53.2 $\pm$ 19.4
Number of bouts (10 min) of MVPA (median (p25th, p75th))	57	0.5(0.2, 1.1)
Average physical activity (counts/min) (mean $\pm$ SD)	57	321.8 $\pm$ 100.9
Semen parameters		
Volume (ml) (mean $\pm$ SD)	62	3.1 $\pm$ 1.4
Sperm concentration (M/ml) (mean $\pm$ SD)	62	51.2 $\pm$ 45.0
Total number (M) (mean $\pm$ SD)	62	165.2 $\pm$ 161.2
Motile concentration (M/ml) (mean $\pm$ SD)	62	31.9 $\pm$ 31.0
Total motile sperm (M) (mean $\pm$ SD)	62	106.4 $\pm$ 120.0
Total motility percentage (mean $\pm$ SD)	62	56 $\pm$ 19.6
Semen parameters below WHO reference values:		
Volume (%)	5	8.1
Sperm concentration (%)	15	24.2
Total number (%)	17	27.4
Motile concentration	15	24.2
Total motile sperm (%)	15	24.2
Total motility percentage (%)	9	14.5

MVPA, moderate to vigorous physical activity.

<sup>a</sup>WHO reference values (2010) for semen parameters: volume 1.5 ml or greater; sperm concentration 15 M/ml (million/ml) or greater; total number 39 M or greater; motile concentration 6 M/ml or greater; total motile sperm 15 M or greater; total motility 40% or greater (35).

**Table 2** Correlations between physical activity and sedentary time, muscular strength, anthropometric and lifestyle variables with semen parameters.

	N	Volume	Sperm concentration <sup>a</sup>	Total number	Motile concentration <sup>a</sup>	Total motile sperm <sup>a</sup>	Total motility
		<i>r</i> ( <i>p</i> )	<i>r</i> ( <i>p</i> )	<i>r</i> ( <i>p</i> )	<i>r</i> ( <i>p</i> )	<i>r</i> ( <i>p</i> )	<i>r</i> ( <i>p</i> )
Age	62	0.022 (NS)	−0.045 (NS)	−0.045 (NS)	−0.020 (NS)	−0.022 (NS)	0.082 (NS)
Body mass index	62	−0.326 <sup>c</sup> (0.010)	−0.107 (NS)	−0.258 <sup>c</sup> (0.043)	−0.173 (NS)	−0.204 (NS)	−0.235 (NS)
Waist circumference	61	−0.334 <sup>c</sup> (0.009)	−0.112 (NS)	−0.286 <sup>c</sup> (0.026)	−0.158 (NS)	−0.184 (NS)	−0.168 (NS)
Education <sup>b</sup>	62	0.167 (NS)	0.347 <sup>c</sup> (0.006)	0.384 <sup>c</sup> (0.002)	0.264 <sup>c</sup> (0.038)	0.293 <sup>c</sup> (0.021)	−0.004 (NS)
Snuff <sup>b</sup>	60	−0.048 (NS)	−0.314 <sup>c</sup> (0.015)	−0.299 <sup>c</sup> (0.020)	−0.375 <sup>c</sup> (0.003)	−0.349 <sup>c</sup> (0.006)	−0.299 <sup>c</sup> (0.020)
Television (h/day)	62	−0.046 (NS)	0.041 (NS)	−0.007 (NS)	0.018 (NS)	0.025 (NS)	−0.010 (NS)
International Fitness Scale:							
General physical condition <sup>b</sup>	62	0.103 (NS)	0.096 (NS)	0.105 (NS)	0.039 (NS)	0.056 (NS)	−0.037 (NS)
Cardiovascular fitness <sup>b</sup>	62	0.226 (NS)	0.086 (NS)	0.149 (NS)	0.078 (NS)	0.123 (NS)	0.038 (NS)
Muscular strength <sup>b</sup>	62	−0.150 (NS)	−0.071 (NS)	−0.149 (NS)	−0.068 (NS)	−0.128 (NS)	−0.055 (NS)
Speed- agility <sup>b</sup>	62	0.071 (NS)	−0.077 (NS)	−0.046 (NS)	−0.071 (NS)	−0.040 (NS)	0.012 (NS)
Flexibility <sup>b</sup>	62	−0.021 (NS)	−0.015 (NS)	−0.014 (NS)	−0.022 (NS)	−0.018 (NS)	0.030 (NS)
Overall health <sup>b</sup>	62	0.231 (NS)	0.110 (NS)	0.170 (NS)	0.046 (NS)	0.116 (NS)	−0.048 (NS)
Handgrip	62	0.068 (NS)	0.096 (NS)	0.050 (NS)	0.108 (NS)	0.135 (NS)	0.072 (NS)
Physical activity levels							
Sedentary time	57	0.221 (NS)	−0.039 (NS)	0.130 (NS)	0.005 (NS)	0.028 (NS)	0.098 (NS)
MVPA	57	−0.133 (NS)	−0.084 (NS)	−0.127 (NS)	−0.123 (NS)	−0.223 (NS)	−0.177 (NS)
Bouts of MVPA <sup>b</sup>	57	0.021 (NS)	0.261 <sup>d</sup> (NS)	0.220 (NS)	0.168 (NS)	0.155 (NS)	0.002 (NS)
Average physical activity	57	−0.126 (NS)	−0.044 (NS)	−0.106 (NS)	−0.083 (NS)	−0.156 (NS)	−0.146 (NS)

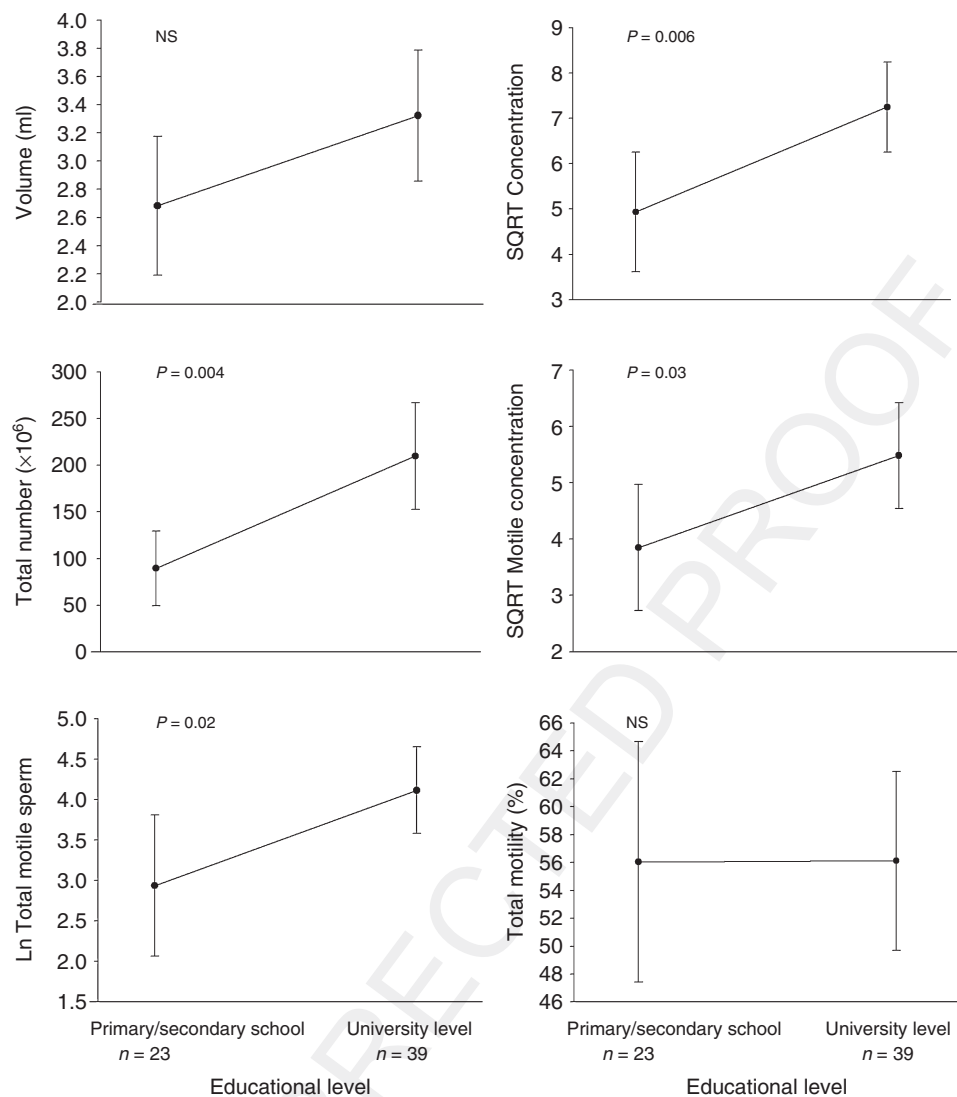
NS, non-significant *P*-values ; MVPA indicates moderate to vigorous physical activity.

<sup>a</sup>The variables 'sperm concentration' and 'motile concentration' were squared-root formed and 'total motile sperm' was Ln transformed to obtain a normal distribution. The variables smoking and occupation were not included for correlation analyses since there were too few subjects in some of the categories.

<sup>b</sup>Pearson correlation was used in all variables, except in categorical variables or those not normally distributed, in which Spearman correlation was used. The variable 'education' was coded as primary = 1, secondary = 2, university less than 3 years = 3 and Univ more than 3 years = 4; the subcategories for variable IFIS were coded as poor = 1, average = 2, good = 3; the variable snuff was coded as yes = 1, no = 0.

<sup>c</sup>Significant correlation, *P* < 0.05.

<sup>d</sup>Borderline significant correlation, *P* = 0.05.

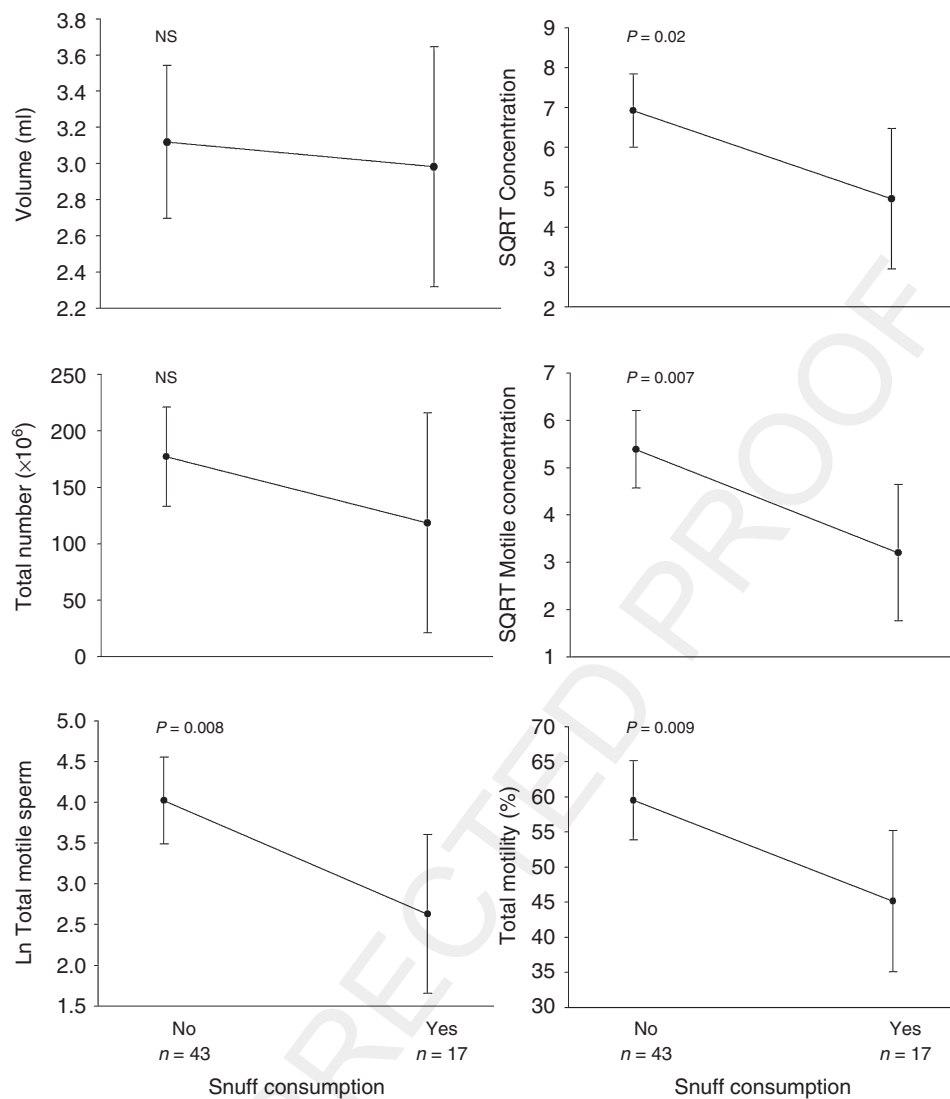


**Figure 1** Indicators of semen quality according to dichotomous educational level categories. Dots in the graph represent group means; vertical lines mark the error bars (95% confidence intervals). Semen concentration and motile concentration were square-root transformed (SQRT), and for total motile sperm logarithmic transformation was applied (Ln) to obtain a normal distribution. One way analysis of variance test was used to identify the differences between the groups and *P* refers to the significance level of the differences between these groups. Significant *P*-values are presented in numbers, and non-significant *P*-values are presented as NS.

### Body mass index, waist circumference and semen quality

Widespread evidence suggests that fatness has a harmful effect on general health and reproductive health (Crujeiras and Casanueva, 2014). Several studies have shown that a BMI over 25, i.e. overweight, but also a BMI less than 20, i.e. underweight, is associated with a lower ejaculate volume, sperm concentration, motility and total sperm count (Hammiche et al., 2012; Hammoud et al., 2008; Jensen et al., 2004; Sermondade et al., 2013; Wogatzky et al., 2012). Furthermore, the Norwegian Mother and Child Cohort study of 26,300 couples showed that higher BMI is associated with increased risk of infertility (Nguyen et al., 2007). These findings refer to U-shaped relationships, where low or high levels of BMI is associated with poorer sperm quality (Jensen et al., 2004;

Sermondade et al., 2013) and increased infertility (Nguyen et al., 2007) than a medium BMI (normoweight). In addition, a waist circumference greater than 102 cm has also been related with a poorer semen quality (ejaculate volume, sperm concentration, sperm number, motile sperm number and some reproductive hormones) (Fejes et al., 2005; Hammiche et al., 2012). In the present study sample, no underweight men were included; therefore, we could not test this U-shape hypothesis. Normoweight men, however, were compared with overweight or obese men, and a significant decrease in ejaculate volume was found in obese men and those with a waist circumference equal or higher than 102 cm. This is consistent with a previous study (Hammiche et al., 2012), which found that BMI and waist circumference were related to ejaculate volume, sperm concentration and sperm motility. A negative relationship between overweight and central adiposity and semen quality is an important finding, as it is preventable



**Figure 2** Indicators of semen quality according to snuff consumption. Dots in the graph represent group means; vertical lines mark the error bars (95% confidence intervals). Semen concentration and motile concentration were square-root transformed (SQRT), and for total motile sperm logarithmic transformation was applied (Ln) to obtain a normal distribution. One way analysis of variance test was used to identify the differences between the groups and *P* refers to the significance level of the differences between these groups. Significant *P*-values are presented in numbers, and non-significant *P*-values are presented as NS.

by regular physical activity. Indeed, a study conducted in obese men showed that a median weight loss of 15% resulted in a significant increase of total sperm count, semen volume and serum testosterone (Håkonsen et al., 2011). The relationship between BMI, waist circumference and semen quality indicators could be explained by genetic, hormonal and environmental factors, adipokines and physical mechanisms (Cabler et al., 2010). It could be also linked to oxidative stress, which has been reported to be increased in overweight men (Tunc et al., 2011) and, as previously indicated, is harmful for semen (Saleh and Agarwal, 2002).

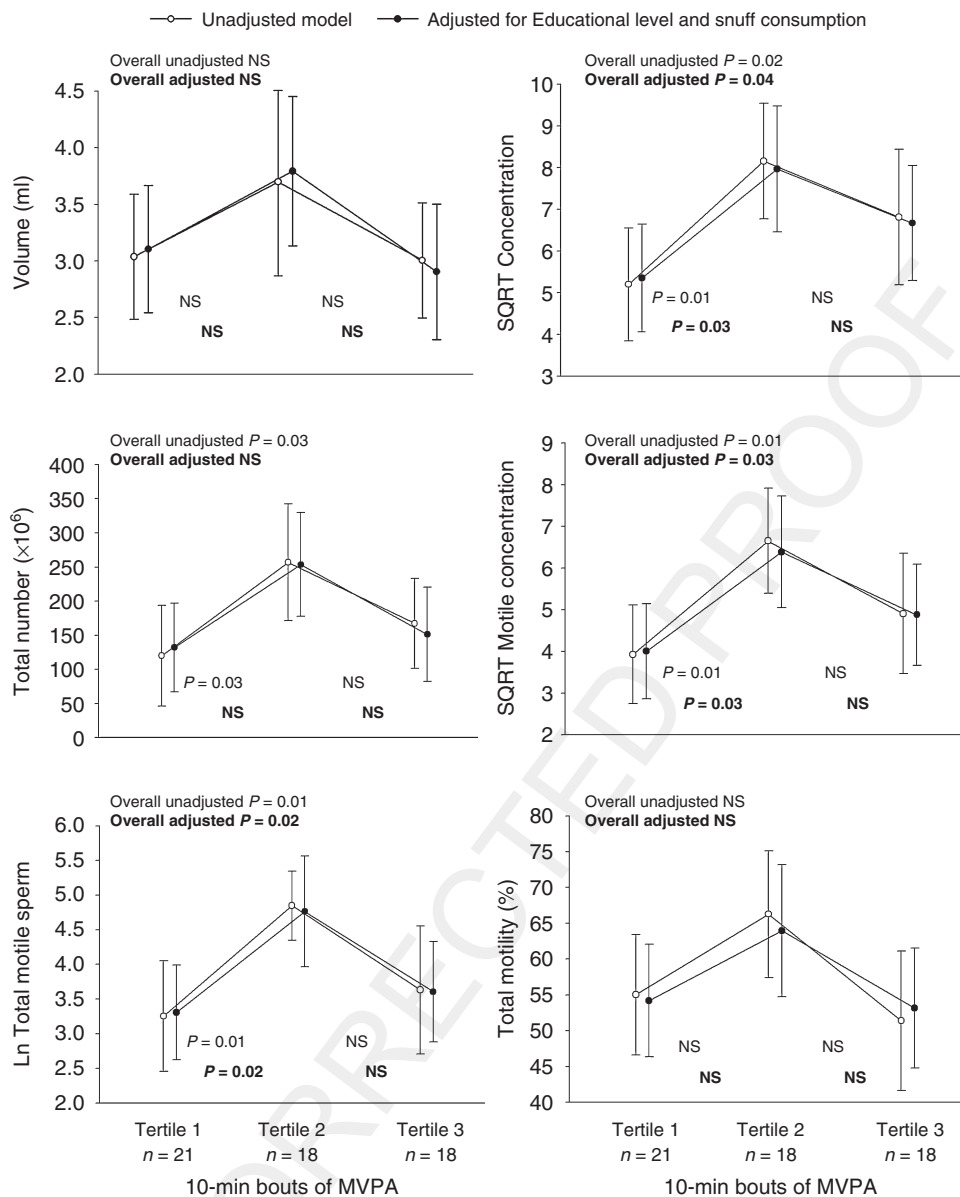
### Educational level, snuff consumption and semen quality

Educational level has shown to be a lifestyle factor related to different health outcomes (Cleland et al., 2009; Kaplan and

Keil, 1993; Ortega et al., 2013a). The few studies investigating the influence of educational level on male fertility have reported that the higher the educational level the lower the semen quality (Lund and Sørensen, 1995), and the higher the percentage of couples not achieving pregnancy (Zinaman et al., 2000). In contrast, higher levels of education were observed to be related to a better semen profile. Indeed, a recent multi-centre prospective cohort study on over 500,000 individuals showed that higher educational levels were related to lower BMI and waist circumference (Hermann et al., 2011). As evidence of the deleterious effect of BMI and waist circumference in male fertility is lacking, this could partially explain the relationship found in the present sample between educational level and semen quality.

Previous studies have shown the harmful effect of tobacco (smoked or chewed) on semen quality (Ramlaau-Hansen et al., 2007; Said et al., 2005). The reason why smokeless tobacco could cause harm to semen is probably due to the increased





**Figure 3** Indicators of semen quality according to tertiles of number of 10-min bouts of moderate to vigorous physical activity. Dots in the graph represent group means; vertical lines mark the error bars (95% confidence intervals). Semen concentration and Motile concentration were square-root transformed (SQRT), and for total motile sperm logarithmic transformation was applied (Ln) to obtain a normal distribution. One-way analysis of the variance test was used to identify the differences between the groups and analysis of the covariance test was used to adjust the model for educational level and snuff consumption. Bold font for *P* values refers to the unadjusted model, whereas the non-bold font refers to the adjusted model. Overall *P* refers to the significance level of the differences between groups generically in each model. When a *P*-value is among two groups, means the significance level of the differences found for that specific comparison. Significant *P*-values are presented in numbers, and non-significant *P*-values are presented as NS. Number of 10-min bouts per week and total time accumulated in repeated 10-min bouts per week expressed as Median (IQR) was: for tertile 1, one bouts (0-2) and 10.3 min (0-29.7); for tertile 2, 4 bouts (3-5) and 66.1 min (45.3-80.2); and for tertile 3, 10 bouts (8-11) and 146.7 min (121.6-178.6).

oxidative stress in snuff users (Samal et al., 2006). Snuff has proved to decrease sperm quality gradually (sperm counts, motility, morphology and viability) as the use increases (Said et al., 2005). Also, in our study, snuff users had significantly lower sperm concentration, motile sperm number, motile sperm concentration and motile sperm percentage. In Nordic countries, snuff is frequently used: about 20% of Swedish men use

snuff regularly, and its use is rapidly increasing worldwide (Willis et al., 2012). Among our study population, the prevalence of snuff users was even higher than in previous studies (28%). The results of the present study confirm the negative effects of smokeless tobacco on semen quality and, therefore, couples undergoing infertility treatment should be advised about the potential adverse effects of snuff on sperm quality.

## Physical activity and semen quality

Studies on the relationship between regular physical activity and semen quality are scarce and their results inconsistent. Some of these studies have observed no association between physical activity and sperm quality (Braga et al., 2012; Wise et al., 2011; Wogatzky et al., 2012). All these studies, however, used self-reported methods, i.e. questionnaires for assessing physical activities. Although questionnaires can provide important information, their limitations are well-known, including the complexity to accurately recall the amount of time spent in physical activity, the intensity and frequency of the activity.

Hajizadeh Maleki et al. (2013) compared several sperm quality indicators in three groups of men: non-active men, recreational active men and elite athletes. They concluded that the recreationally active men had healthier sperm values than both non-active men and elite athletes, supporting the notion that physical activity could relate to semen in a U-shape fashion. In line with these findings, some studies have found an association between high intensity physical activity (Safarinejad et al., 2009; Vaamonde et al., 2006) and high volumes of physical activity (De Souza et al., 1994) with poorer semen quality compared with men who engage in moderate recreational physical activity. Also supporting this U-shape hypothesis, Vaamonde et al. (2012) observed a poorer semen quality in sedentary men compared with recreationally physically active men (Vaamonde et al., 2012). Our results provide additional support to the U-shape association, yet it is important to mention that only the variable of number of bouts of 10-min of MVPA significantly showed this pattern. The variables time spent in sedentary activities, MVPA or average physical activity (cpm) were not significantly associated with sperm parameters, yet the U-shape trend was observed in most of the analyses. Although the U-shape association was clear and consistent for the variable number of bouts of MVPA, it is important to highlight that statistical significance was observed mainly between the low and middle physically active groups (i.e. tertile 1 versus tertile 2) in most of semen parameters studied. This indicates that the worst semen values are observed in participants with low number of bouts of physical activity being in line with previous studies (De Souza et al., 1994; Hajizadeh Maleki et al., 2013; Safarinejad et al., 2009; Vaamonde et al., 2006). Nevertheless, one should bear in mind when interpreting our findings that the present sample does not include elite athletes, so the high physical activity groups cannot be interpreted as extremely high physical activity levels.

Several mechanisms can explain why both activity extremes result in lower semen quality. First, high-intensity exercise training is associated with increase in oxidative stress (Suredu et al., 2009). A high level of reactive oxygen species, possibly toxic to sperm, could potentially reduce its quality (Hajizadeh Maleki et al., 2013; Saleh and Agarwal, 2002). Second, extreme physical activity affects body's homeostasis (Safarinejad et al., 2009). It has also been hypothesised that high intensity exercise could affect the hypothalamic-pituitary-gonadal axis and thus levels of testosterone in participants who do endurance training (Daly et al., 2005; De Souza and Miller, 1997). Safarinejad et al. (2009) reported the same effect, but also indicated that during recovery phase

testosterone level normalized. Testosterone is the major male reproductive hormone and alterations in its levels could influence semen quality. Furthermore, sperm quality is receptive to increases in scrotal temperature (Jung and Schuppe, 2007). Jung and Schuppe (2007) observed that, compared with walking, sitting position increased scrotal temperature up to 2°C. This might explain a negative effect of cycling to sperm quality, as lower sperm concentration and total motile sperm among men who cycle 5 h or more per week was observed, whereas, no association was found with regular physical activity (Wise et al., 2011). In the present study, however, the type of activity was not assessed; therefore it is a limitation that should be faced in future studies.

## Limitations and strengths

Some limitations should be addressed. The relatively small sample size should be acknowledged, yet the accurate and objective method used to assess physical activity counteracts this drawback. Collecting data about cycling habits would have provided meaningful information, as this specific type of physical activity has been related to lower semen quality (Wise et al., 2011). Further, as semen samples might have some intra-individual variability, double semen sample would have given more precise results. Also, sperm morphology was not measured in our study, yet it has been indicated to be a relevant parameter (Lewis, 2007) and negative association has been reported between sperm morphology and physical activity levels (MacDonald et al., 2010). Because of practical reasons, not all the main health-related components of physical fitness could be assessed. A validated scale (IFIS) was used as the only alternative available, by which the participants self-reported their fitness levels. Although this scale has high agreement with actual fitness levels measured by standardized fitness tests (Ortega et al., 2011, 2013b; Sánchez-López et al., 2014), this scale was originally designed to be used in large-scale and population-based studies, so that its validity is limited when used in relatively small sample sizes as it is the case in this study. In addition to self-reported fitness, handgrip strength test was used as an indicator of muscular strength, and no significant association with semen parameters was observed. It is important to bear in mind, however, that handgrip strength is higher in overweight and obese people, owing to their higher muscle mass (Artero et al., 2010) which could counteract a potential beneficial effect of muscular strength on semen quality. Moreover, the inclusion of only potentially infertile men, which limits generalizability and the multiple hypothesis testing, should also be acknowledged as a limitation. Finally, although this study examined a relatively large number of factors potentially related to male infertility, it is unknown whether other non-measured factors, such as diet or lipid profile could have influence the results. On the other hand, this study should be acknowledged as the first study, to the best of our knowledge, to relate sperm quality to objectively measured physical activity and sedentary time, using accelerometers instead of using only questionnaires.

The findings of this study provide evidence for an inverted U-shape association between physical activity and semen quality, suggesting that men who engage in an average

number of 10-min bouts of MVPA tend to have better semen quality compared with those with low or high number of bouts of MVPA. These findings, however, should be interpreted cautiously, as not all physical activity variables studied were significantly related to semen quality. Further, our findings support previous studies about the negative association of high levels of adiposity (both total and central, as measured by BMI and waist circumference) with sperm quality parameters, particularly semen volume. Finally, our data also suggest that more educated men and those who do not consume snuff might have better sperm quality.

## Acknowledgements

The authors thank all individuals for their participation. This work was supported by Karolinska Institutet Foundation grants [Refs: 2011FoBi1184]; Estonian National Kristjan Jaak scholarship program, implemented by the Archimedes Foundation [Refs: 16-3.5/1080]; Spanish Ministry of Economy and Competitiveness [RYC-2010-05957; RYC-2011-09011]; European Research Council, Marie Curie Actions, Intra-European Fellowships (IEF) [FP7-PEOPLE-2012-IEF, Ref. No. 329812]; grants from Uppsala University; the Family Planning Foundation, Uppsala; Estonian Ministry of Education and Research (grant SF0180044s09); Enterprise Estonia (grant EU30020).

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*Declaration: The authors report no financial or commercial conflicts of interest.*

Received 11 September 2014; refereed 6 March 2015; accepted 10 March 2015.