


Sperm count is increased by diet-induced weight loss and maintained by exercise or GLP-I analogue treatment: a randomized controlled trial

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STUDY QUESTION: Does diet-induced weight loss improve semen parameters, and are these possible improvements maintained with sustained weight loss?

SUMMARY ANSWER: An 8-week low-calorie diet-induced weight loss was associated with improved sperm concentration and sperm count, which were maintained after 1 year in men who maintained weight loss.

WHAT IS KNOWN ALREADY: Obesity is associated with impaired semen quality. Weight loss improves metabolic health in obesity, but there is a lack of knowledge on the acute and long-term effects of weight loss on semen parameters.

STUDY DESIGN, SIZE, DURATION: This is a substudy of men with obesity enrolled in a randomized, controlled, double-blinded trial (the S-LITE trial). The trial was conducted between August 2016 and November 2019. A total of 56 men were included in the study and assigned to an initial 8-week low-calorie diet (800 kcal/day) followed by randomization to 52 weeks of either: placebo and habitual activity (placebo), exercise training and placebo (exercise), the Glucagon Like Peptide I (GLP-I) analogue liraglutide and habitual activity (liraglutide) or liraglutide in combination with exercise training (combination).

PARTICIPANTS/MATERIALS, SETTING, METHODS: Inclusion criteria were men who delivered semen samples, 18 to 65 years of age, and a body mass index between 32 and 43 kg/m², but otherwise healthy. The study was carried out at Hvidovre Hospital and at the University of Copenhagen, and the participants were from the Greater Copenhagen Area. We assessed semen parameters and anthropometrics and collected blood samples before (T0), after the 8-week low-calorie dietary intervention (T1), and after 52 weeks (T2).

MAIN RESULTS AND THE ROLE OF CHANCE: The men lost on average 16.5 kg (95% CI: 15.2–17.8) body weight during the low-calorie diet, which increased sperm concentration 1.49-fold (95% CI: 1.18–1.88, $P < 0.01$) and sperm count 1.41-fold (95% CI: 1.07–1.87, $P < 0.01$). These improvements were maintained for 52 weeks in men who maintained the weight loss, but not in men who regained weight. Semen volume, sperm motility and motile sperm count did not change.

LIMITATIONS, REASONS FOR CAUTION: The S-LITE trial was a randomized controlled trial of weight loss maintenance. Analysis of semen was preregistered to explore the effects of weight loss and weight loss maintenance on semen parameters, but definite inferences cannot be made.

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WIDER IMPLICATIONS OF THE FINDINGS: This study shows that sperm concentration and sperm count were improved after a diet-induced weight loss in men with obesity. Our findings indicate that either or both liraglutide and exercise as weight maintenance strategies may be used to maintain the improvements in sperm concentration and count.

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TRIAL REGISTRATION NUMBER: The trial was approved by the Ethical Committee of the Capital Region of Denmark (H-16027082) and the Danish Medicines Agency (EudraCT Number: 2015-005585-32). ClinicalTrials.gov identifier (NCT number): NCT04122716.

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DATE OF FIRST PATIENT'S ENROLMENT: August 2016.

Key words: male reproduction / spermatozoa / obesity / weight loss / GLP-I agonist / liraglutide / semen quality

Introduction

The prevalence of obesity is increasing globally (Bentham *et al.*, 2017), while meta-analyses indicate declining sperm count over time (Carlsen *et al.*, 1992; Levine *et al.*, 2017). A decline in utero exposure to maternal smoking has not reflected an overall improvement of semen quality at the population level in Danish men, which suggests that other adverse factors may maintain the low semen quality (Priskorn *et al.*, 2018). Increasing BMI levels, overweight and obesity are associated with decreased sperm concentration, total sperm count, progressive sperm motility and sperm morphology (Andersen *et al.*, 2015; Salas-Huetos *et al.*, 2021). Increased BMI of both the woman and the male partner is also associated with subfertility (time-to-pregnancy > 12 months or use of assisted reproductive technology) (Hernández *et al.*, 2021). In line with these observations, results from a meta-analysis showed an increased risk of low (oligozoospermia) and very-low (azoospermia) sperm count in men with obesity compared to men with normal weight. Thus, compared to men with a BMI between 18.5 and 24.9 kg/m², the odds ratio for low sperm count increased to 1.28 for men with a BMI between 30.0 and 39.9 kg/m² and to 2.04 with a BMI above 40.0 kg/m² (Sermondade *et al.*, 2013). Obesity is therefore considered a risk factor for development of male infertility.

The S-LiTE study, a diet-induced weight loss intervention followed by exercise, GLP-I receptor agonist therapy or the combination to maintain weight loss, has shown that the detrimental metabolic effects of obesity are reversed, to a large extent, by weight loss (Lundgren *et al.*, 2021). While studies in women have shown that weight loss achieved by combining diet and exercise interventions was associated with increased pregnancy rates, only a limited number of studies have investigated the effect of weight loss on male fertility (Best *et al.*, 2017). Initial case reports of men with obesity who underwent bariatric surgery suggested that the dramatic weight loss was associated with an impairment of semen parameters (Lazaros *et al.*, 2012), while a more recent and larger study suggests a modest improvement

(Samavat *et al.*, 2018). Likewise, combined diet and exercise-induced weight loss interventions have shown promising effects on sperm count, semen volume, sperm morphology and decreased sperm DNA fragmentation (Håkonsen *et al.*, 2011; Faure *et al.*, 2014; Mir *et al.*, 2018). Higher self-reported moderate-to-vigorous physical activity was associated with higher levels of progressive and total motility in 746 potential sperm donors from China (Sun *et al.*, 2019). Furthermore, the frequency of 10 min bouts of moderate-to-vigorous-intensity physical activity performed and assessed by accelerometers was associated with higher sperm concentration, sperm count, motile concentration and total motile sperm in a cross-sectional study (Pärn *et al.*, 2015). However, it is unclear whether weight loss *per se* improves semen parameters and, if so, whether these improvements can be upheld with sustained weight loss.

Here, we investigated the effect of an 8-week diet-induced weight loss followed by randomization to one of four different 52 weeks weight maintenance strategies: placebo and habitual activity, placebo and exercise, liraglutide and habitual activity or liraglutide and exercise on semen parameters. The use of a GLP-I receptor agonist, which induces and maintains weight loss, is of particular interest since a case report suggested that liraglutide therapy was detrimental to sperm concentration and motility (Fontoura *et al.*, 2014).

Materials and methods

Study cohort

This study was performed as a substudy of the S-LiTE trial (Synergy effect of the appetite hormone GLP-I (Liraglutide) and Exercise on maintenance of weight loss and health after a low-calorie diet). The S-LiTE trial protocol with details of the design, a full list of inclusion and exclusion criteria and methods has been published (Jensen *et al.*, 2019) as well as the results of the primary endpoint, weight change

(Lundgren et al., 2021). In brief, the S-LiTE trial included 215 women and men (age 18–65 years) with obesity (BMI 32–43 kg/m²) but without diabetes. The participants were recruited to firstly adhere to a low-calorie diet (800 kcal/day) (Cambridge Weight-planner) for 8 weeks with the objective of losing $\geq 5\%$ body weight. The participants were then randomized in a 1:1:1:1 ratio to one of four groups for 52 weeks. Randomization was stratified by age (under or above 40 years of age) and sex and performed by blinded personnel not otherwise related to the study. The four intervention groups were: (i) placebo plus habitual activity (placebo group), (ii) exercise according to WHO guidelines (exercise group), (iii) liraglutide 3 mg/day plus habitual activity (liraglutide group) and (iv) exercise plus liraglutide (combination group). The participants allocated to the exercise intervention were strongly encouraged to fulfil the WHO recommendations of 150 min per week of moderate physical activity or 75 min per week of vigorous exercise or a combination of both by given the opportunity to participate in two 45 min group exercise sessions facilitated and supervised at Hvidovre Hospital (Denmark) and two 30 min individually exercise sessions. The aim for each session was an intensity of 80% of the maximum heart rate. The study personnel monitored all performed exercise with heart-rate monitors to ensure the fulfilment of the WHO recommendations of physical activity for health. The participants not allocated to exercise were asked to carry on their habitual activity. The participants injected the study medication, liraglutide or volume-matched placebo, subcutaneously with adjustable pens. The starting dose was 0.6 mg per day with a weekly increase of 0.6 mg per day until reaching the final dose of 3.0 mg per day or the highest tolerable dose. The trial included weekly visits during the 8-week low-calorie diet and approximately monthly visits during the 52 weeks randomized intervention. Three test days were performed before (T0) and after (T1) the 8-week low-calorie diet and at 52 weeks after randomization (T2). Changes in semen parameters were preregistered as explorative endpoints (Jensen et al., 2019) and were the aim of this substudy. The study was reported according to the CONSORT guideline. The trial was approved by the Ethical Committee of the Capital Region of Denmark (H-16027082) and the Danish Medicines Agency (EudraCT Number: 2015-005585-32) and was conducted according to the principles of the Declaration of Helsinki and Good Clinical Practice guidelines. All the participants provided written informed consent before the first trial visit.

Clinical parameters

All clinical parameters were measured after overnight fasting by the study personnel. Height was measured at study inclusion. Weight was measured wearing light clothes and to the nearest 0.1 kg (WB-110MA, Tanita, Tokyo, Japan), and a BMI (kg/m²) was calculated (weight (kg)/height (m)²). Total body-fat percentage was measured using the same Dual-energy X-ray Absorptiometry (DXA) scanner throughout the study (Discovery A, Hologic, Bedford, USA). Peak oxygen uptake (VO₂ peak) rates were assessed with a max cycle ergometer test.

Plasma C-reactive protein (CRP) was measured with a turbidimetric immunoassay, plasma cholesterol, low-density lipoprotein (LDL), high-density lipoprotein (HDL), very low-density lipoprotein (VLDL) and triglycerides with the enzymatic colourimetric method (COBAS 6000/8000, Roche, Basel, Switzerland). Glycated haemoglobin A1c (HbA1c) was measured with high-performance liquid chromatography (HLC

723 G8, TOSOH, Tokyo, Japan). Haemoglobin was measured with a photometric cyanide-free SLS method (XN 1000/9000, SYSMEX, Kobe, Japan). All measurements were analysed on the same day as the test visits at the Department of Clinical Biochemistry, Hvidovre Hospital, Hvidovre, Denmark.

Semen assessment

Single ejaculate semen samples were collected on-site in an examination room at the Faculty of Health and Medical Sciences, University of Copenhagen, Denmark. Participation in this substudy of the S-LiTE trial was encouraged and economically compensated, but participation was voluntary as part of the S-LiTE trial. All participants were controlled for testicular abnormalities by anamnesis. Each semen sample was delivered after 3 days of sexual abstinence directly into sterile polypropylene containers (Sarstedt, Nümbrecht, Germany) and stored at 37°C for 30 min for liquefaction of the sample. Semen volume (ml) was measured by using a wide-bore serological pipette, instead of weighing, which is the recommended measurement method by WHO (WHO, 2010). Basic sperm parameters were measured by inspection using phase-contrast microscopy according to WHO guidelines (WHO, 2010) at room temperature (22°C). Sperm concentration (million sperm/ml) was assessed using a Bürker-Türk haemocytometer (VWR, Søborg, Denmark) to count the amount of sperm of a diluted sample. At least 200 spermatozoa were counted in duplicate. Sperm count (million sperm/ejaculate) was calculated by multiplying sperm concentration by semen volume. Sperm motility was assessed on at least 200 spermatozoa in at least five microscope fields. Motile spermatozoa were defined according to the WHO three-category scheme: progressive motile, non-progressive motile and immotile. Sperm motility includes both progressive and non-progressive motile sperm. Motile sperm count (million motile sperm/ejaculate) was calculated by multiplying sperm count by the fraction of motile sperm.

Statistical analyses

Results are presented as mean \pm SD and changes are presented as mean (95% CI) if not otherwise stated. All analyses were performed as paired analyses based on the time points (T0, T1 and T2) of the individuals. Data were analysed for normality. For non-normal data, log transformation was used to normalize data. Paired t-test was carried out to evaluate the differences before and after the 8-week low-calorie diet, and Wilcoxon signed-rank test was used for non-normal data. The median weight loss from T0 to T2 was used to define two groups: good maintainers and poor maintainers. Data for the 52 weeks intervention were analysed with mixed-effects analysis, and Tukey's multiple comparisons test was used *post hoc* to investigate differences between time-points. A *P*-value below 0.05 was considered statistically significant. All statistical analyses were performed using GraphPad Prism Version 9.2.0.

Results

Change in semen parameters after an 8-week low-calorie diet-induced weight loss

A cohort of 47 men completed the 8-week low-calorie diet and had a semen sample collected before (T0) and after weight loss (T1) (Fig. 1

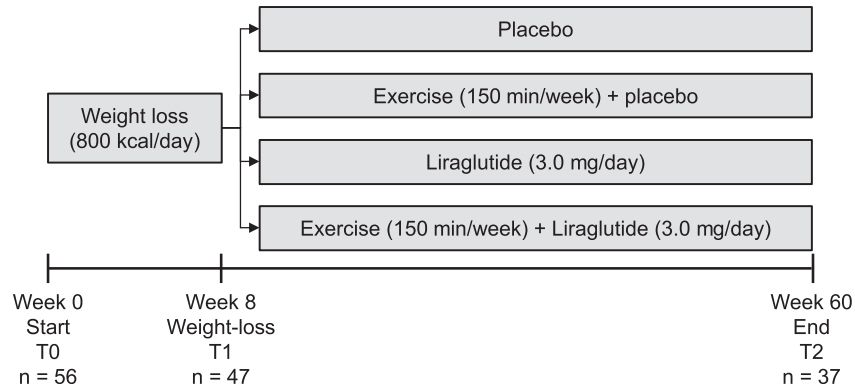


Figure 1. Schematic overview of the study design. The exercise program was designed to meet the World Health Organization (WHO) recommendations on physical activity for health of a minimum of 150 min per week of moderate-intensity aerobic physical activity or 75 min per week of vigorous-intensity aerobic physical activity, or an equivalent combination of both.

and Supplementary Fig. S1). The mean (range) age of the men at inclusion was 41 years (20–63 years), and the mean \pm SD BMI was $37.0 \pm 2.8 \text{ kg/m}^2$ (Table I). Participants lost an average of 16.5 kg (95% CI: 15.1–17.8 kg), and the mean BMI was decreased to $32.0 \pm 2.9 \text{ kg/m}^2$. The waist:hip ratio improved from 1.01 ± 0.07 to 0.96 ± 0.07 , as did the body-fat percentage (%), from 34.1 ± 3.9 to 31.0 ± 4.2 , while reductions in plasma CRP, HbA1c, plasma cholesterol and plasma triglycerides were also noted (Table I).

After the low-calorie diet, an improvement in sperm concentration of 1.49 fold (95% CI: 1.18–1.88, $P < 0.01$) (from 78.5 ± 74.9 to 91.7 ± 70.8 million sperm/ml) was observed, as well as an improvement in sperm count of 1.41 fold (95% CI: 1.07–1.87, $P < 0.01$) (from 191.2 ± 189.4 to 250.6 ± 319.4 million sperm/ejaculate) (Table I). The semen volume did not change. The number of individuals with oligospermia (sperm concentration below 15 million sperm per millilitre) (WHO, 2010) was 8 out of 47 before weight loss and 6 out of 47 after weight loss (Fig. 2). Although the change in motile sperm count did not reach statistical significance ($P = 0.1$), we observed that the number of individuals with a very low motile sperm count (< 1 million per ejaculate) was reduced from 6 out of 47 before the intervention to 1 out of 47 after the 8-week low-calorie diet ($P < 0.05$) (Fig. 2).

Change in semen parameters after 1 year of sustained weight loss

A total of 37 individuals delivered a semen sample 52 weeks after the initial weight loss with 8, 9, 7 and 13 individuals in the placebo, exercise, liraglutide or combination group, respectively (Fig. 1). Similar to the primary study (Lundgren et al., 2021), the combination of exercise and liraglutide was more efficient at maintaining weight loss than the two interventions alone, while the participants in the placebo group regained weight (Supplementary Table S1). In addition, compared to T1, HDL cholesterol increased in the three intervention groups but not in the placebo group. The participants in the exercise groups (exercise and combination group) increased VO_2 peak.

Individual weight loss from T0 to T2, irrespective of the intervention group, ranged from a weight gain of 5.7 kg to a weight loss of 50.7 kg,

with a median weight loss of 11.7 kg. Individual variation of semen parameters across the time points showed that weight loss affected sperm concentration and sperm count, but not semen volume, sperm motility and motile sperm count (Supplementary Fig. S2). Sperm concentration remained elevated at T2 in all intervention groups, while sperm count returned to baseline levels (Supplementary Fig. S2). To analyse the effect of sustained weight loss on semen parameters, we used the median weight loss from T0 to T2 (11.7 kg) to define good maintainers (mean weight loss of 21.4 kg (95% CI: 16.8–26.1 kg), $n = 18$) and poor maintainers (mean weight loss of 7.6 kg (95% CI: 5.8–9.4 kg), $n = 19$) (Table II). We found an increase in sperm concentration of 1.71-fold (95% CI: 1.15–2.55, $P < 0.05$) and sperm count of 1.97-fold (95% CI: 1.22–3.18, $P < 0.05$) at T2 compared to T0 for the group of men who maintained a weight loss of more than 11.7 kg after the 52 weeks, irrespective of the intervention group (Fig. 3). The semen volume and sperm motility did not change after 1 year of intervention in any group, while the motile sperm count was insignificantly increased by 1.50-fold (95% CI: 0.64–3.52, $P = 0.3$) in the group of good maintainers (Fig. 3). Analysis within the subgroups indicated a maintained increase in sperm concentration in the liraglutide group only (Supplementary Fig. S3). A total of 20 individuals received liraglutide for 52 weeks (7 received liraglutide alone and 13 in combination with exercise). Sperm concentration was increased after the low-calorie diet-induced weight loss, from T0 to T1 ($P = 0.04$), and was maintained after 52 weeks. No other semen parameters were changed. A similar analysis of the 21 individuals assigned to exercise (8 received exercise alone and 13 in combination with liraglutide) did not show changes in semen parameters during the study.

Discussion

In this study, we investigated the effect of weight loss and weight loss maintenance on semen parameters in men with obesity. We found that an 8-week diet-induced weight loss improved sperm concentration and sperm count. The improvements after the 8-week low-calorie diet were preserved after 52 weeks if a significant weight loss

Table 1 Clinical characteristics before (T0) and after an 8-week low-calorie diet-induced weight loss (T1) (n = 47).

Characteristic	Time of sampling	
	T0 (n = 47)	T1 (n = 47)
Age (years)	41 (range 20–63)	
Weight (kg)	122.3 (±13.7)	105.8 (±12.5)^a
BMI (kg/m ²)	37.0 (±2.8)	32.0 (±2.9)^a
Waist circumference (cm)	119.3 (±8.4)	106.1 (±9.0)^a
Hip circumference (cm)	118.5 (±6.8)	110.3 (±6.6)^a
Waist:hip ratio	1.01 (±0.07)	0.96 (±0.07)^a
Fat percentage (%) ^b	34.1 (±3.9)	31.0 (±4.2)^a
Plasma CRP (mg/l) ^b	2.4 (±2.3)	1.9 (±2.4)^a
HbA1c (mmol/mol) ^b	35.8 (±3.5)	33.0 (±3.2)^a
HbA1c (%) ^b	6.0 (±0.5)	5.6 (±0.5)^a
Plasma cholesterol (mmol/l)	4.8 (±0.9)	3.9 (±1.0)^a
Plasma cholesterol HDL (mmol/l)	1.1 (±0.2)	1.0 (±0.2)^a
Plasma cholesterol LDL (mmol/l)	3.0 (±0.9)	2.4 (±0.9)^a
Plasma cholesterol VLDL (mmol/l)	0.8 (±0.4)	0.5 (±0.2)^a
Plasma triglyceride (mmol/l)	1.7 (±0.8)	1.1 (±0.4)^a
Semen volume (ml)	2.5 (±1.3)	2.6 (±1.7)
< 1.5 ml, n [% of all]	11 [23]	14 [30]
Sperm concentration (million/ml)	78.5 (±74.9)	91.7 (±70.8)^a
< 15 million/ml, n [% of all]	8 [17]	6 [13]
Sperm count (million/ejaculate)	191.2 (±189.4)	250.6 (±319.4)^a
< 39 million/ejaculate, N [% of all]	11 [23]	9 [19]
Sperm motility (%)	33.3 (±20.2)	31.5 (±20.4)
< 40%, N [% of all]	30 [64]	32 [68]
Motile sperm count (million/ejaculate)	71.3 (±90.9)	83.7 (±115.7)
< 1 million/ejaculate, N [% of all]	6 [15]	1 [2]

Data are represented as mean (±SD) unless otherwise noted. Statistical values are highlighted in bold.
^aDifference versus T0 analysed with Student's t-test (Wilcoxon signed rank test where appropriate).
^bContains missing numbers.
CRP, C-reactive protein; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

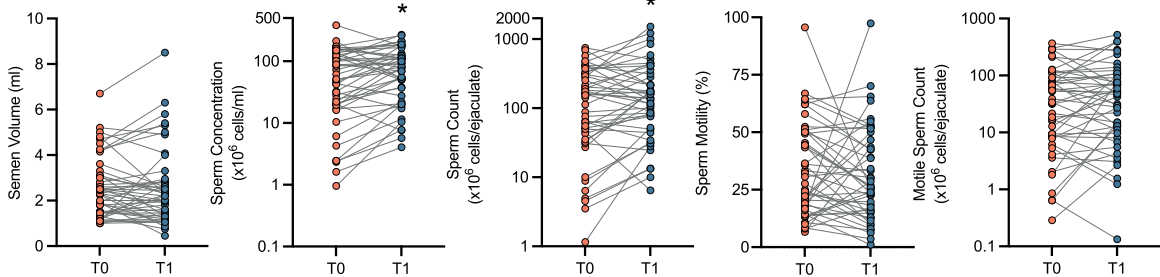


Figure 2. Effect of weight loss on semen parameters. Difference in semen parameters before (T0), and after an 8-week low-calorie diet-induced weight loss (T1) in men with obesity (n = 47). Differences between groups were calculated with Student's t-test. *P < 0.05 versus T0.

(>12 kg) was maintained. Obesity is associated with an increased prevalence of oligospermia (Sermondade et al., 2013). Here, we found a reduction in the percentage of men with oligospermia after an 8-week weight loss intervention from 17% to 13%, suggesting that obesity-associated oligospermia can be reduced with weight loss. Secondly, we report that a beneficial effect on sperm concentration

Table II Clinical characteristics of the two weight loss maintaining groups: poor maintainers (n = 19) and good maintainers (n = 18) before (T0) and after 52 weeks (T2).

Characteristic	Poor maintainers (n = 19)		Good maintainers (n = 18)	
	T0	T2	T0	T2
Age (years)	45 (range 28–63)		37 (range 19–60)	
Weight (kg)	121.3 (±12.9)	113.7 (±13.3)^a	124.3 (±15.5)	102.9 (±19.0)^a
BMI (kg/m ²)	37.4 (±3)	35.1 (±3.0)^a	36.2 (±2.1)	29.9 (±3.9)^a
Waist circumference (cm)	119.6 (±9.6)	113.0 (±8.9)^a	118.6 (±8.3)	98.8 (±11.9)^a
Hip circumference (cm)	116.9 (±6.8)	109.3 (±7.1)^a	113.8 (±6.3)	117.0 (±6.8)^a
Waist:hip ratio	1.02 (±0.08)	0.99 (±0.08)^a	0.99 (±0.08)	1.02 (±0.08)^a
Body-fat percentage (%)	33.5 (±3.7)	31.1 (±4.3)^a	34 (±3.8) ^b	27.2 (±6.3)^a
Plasma CRP (mg/l)	2.3 (±2.5)	3.6 (±8.9)	2.5 (±2.2)	1.0 (±0.9)^a
HbA1c (mmol/mol)	36.7 (±3.3)	34.4 (±2.5)^a	34.8 (±4.1) ^b	32.3 (±3.3)^a
HbA1c (%)	6.2 (±0.5)	5.8 (±0.4)^a	5.9 (±0.6) ^b	5.5 (±0.5)^a
Plasma cholesterol (mmol/l)	4.6 (±0.8)	4.6 (±0.8)	4.8 (±0.9)	4.3 (±0.8)^a
Plasma cholesterol HDL (mmol/l)	1.1 (±0.2)	1.2 (±0.2)^a	1.1 (±0.3)	1.3 (±0.3)^a
Plasma cholesterol LDL (mmol/l)	2.7 (±0.7)	2.7 (±0.7)	3.1 (±0.9)	2.5 (±0.8)^a
Plasma cholesterol VLDL (mmol/l)	0.9 (±0.4)	0.7 (±0.3)^a	0.6 (±0.2)	0.5 (±0.2)^a
Plasma triglyceride (mmol/l)	1.9 (±0.9)	1.4 (±0.6)^a	1.3 (±0.5)	1.0 (±0.5)^a
Semen volume (ml)	2.8 (±1.6)	2.2 (±1.1)	2.7 (±1.2)	3.0 (±1.2)
Sperm concentration (million/ml)	96.9 (±59.0)	90.6 (±78.6)	52.0 (±56.1)	63.0 (±52.3)^a
Sperm count (million/ejaculate)	235.2 (±160.4)	186.4 (±142.9)	154.3 (±191.1)	206.2 (±222.1)^a
Sperm motility (%)	39.9 (±16)	29.7 (±17.2) ^b	36.0 (±24.5)	22.3 (±14.9) ^b
Motile sperm count (million/ejaculate)	73.6 (±52.4)	47.9 (±44.4) ^b	65.2 (±100.3)	56.9 (±72.3) ^b

Data are represented as mean (±SD) unless otherwise noted. Statistical values are highlighted in bold.
^aDifference versus T0 analysed with Student's t-test (Wilcoxon signed rank test where appropriate).
^bContains missing numbers.
CRP, C-reactive protein; HbA1c, haemoglobin A1c; HDL, high-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein.

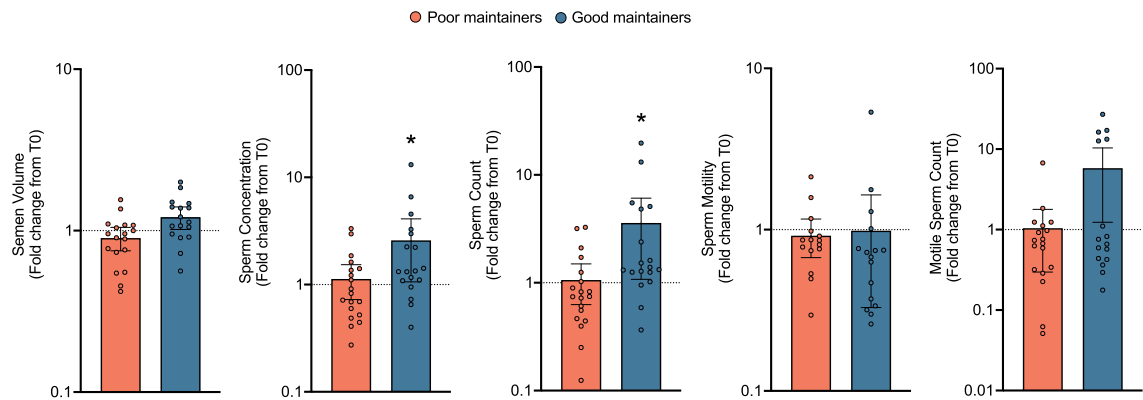


Figure 3. Substantial maintenance of weight loss after 52 weeks is associated with improved sperm concentration and sperm count. Difference in semen parameters after an 8-week low-calorie diet-induced weight loss followed by 52 weeks intervention (T2) compared to baseline (T0). Groups were based on weight loss after 52 weeks. Poor maintainers (red) had a weight loss of less than 11.7 kg after 52 weeks (n = 19). Good maintainers (blue) had a weight loss of more than 11.7 kg after 52 weeks (n = 18). Differences between groups were calculated with mixed-effects analysis using Turkey's multiple comparisons test. Black bars indicate 95% CIs. Dots indicate individual observations. *P = <0.05 versus T0.

and sperm count persists if a significant weight loss is maintained. Our results are in accordance with another study that showed an improvement in sperm concentration and sperm count in men with obesity after a 14-week weight loss program based on a healthy diet and daily exercise. In that study, the positive impact of diet on semen parameters was only seen in individuals with a weight loss of more than 12% body weight (Håkonsen et al., 2011). In two other weight loss studies using nutritional and exercise coaching, there was no improvement in sperm concentration or sperm count, but reductions in sperm DNA fragmentation (Faure et al., 2014; Mir et al., 2018). However, in the study by Mir et al. (2018) an improvement in progressive motility was observed. Compared to our study, there were fewer participants (6 versus 56) in the study by Faure et al. (2014), and participants lost considerably less weight (BMI -2.8 (kg/m^2) vs -6.4 (kg/m^2)) in the study by Mir et al. (2018).

We observed that sperm concentration and count were improved by weight loss while semen volume, motility and motile sperm count did not change. However, there was a trend towards improvement in motile sperm count after the 8-week low-calorie weight loss and after 52 weeks in the group of good maintainers compared to T0.

Exercise alone has previously been found to improve sperm parameters, including sperm concentration, sperm progressive motility and sperm morphology (Rosety et al., 2017). Our study showed that exercise helps maintain weight loss, but that semen parameters were not further improved with exercise beyond the rather significant effect of the initial 8-week diet-induced weight loss in men with obesity. Similarly, for liraglutide, we did not observe any additional effect beyond the initial improvements with diet-induced weight loss. In a previous case report, liraglutide treatment was associated with impairment of most semen parameters (Fontoura et al., 2014). Similar findings were reported in another case report of bariatric surgery, where an increase in endogenous GLP-1 is usually observed (Lazaros et al., 2012). However, in a more extensive study of 23 individuals undergoing bariatric surgery, such effects were not observed (Samavat et al., 2018). In the case report mentioned, the participant received a low dose of 0.6 mg liraglutide/day (Fontoura et al., 2014), in contrast to 3.0 mg liraglutide/day used in our study. Importantly, we did not observe any detrimental effects of liraglutide on semen parameters. In women treated with liraglutide, positive effects on both metabolic health and fertility have been observed. A low dose of liraglutide in combination with metformin was superior to metformin alone in improving metabolic health in women with obesity and polycystic ovarian syndrome, which also led to an increased IVF pregnancy rate compared to metformin alone (Salamun et al., 2018). Our data suggest that liraglutide treatment helps to maintain weight loss also without negatively impacting semen quality.

The strengths of the study lie in its randomized, controlled design, the duration of the study with a large diet-induced weight loss, the 1-year duration in the three arms (exercise, GLP-1 RA and the two combined) and the presence of a placebo group. This setup makes analysis of good versus poor weight loss maintainers possible on improvements of semen concentration and count. There were 56 men included in this study to investigate the effect of a low-calorie diet-induced weight loss. After the low-calorie diet, the men were randomized into different treatment arms to maintain weight loss, thereby lowering the number in each treatment arm which limits the statistical strength of each treatment arm. Another limitation is that the semen

volume was measured by a pipette and not weight as recommended from the fifth WHO laboratory manual for examination of human (WHO, 2010). As this method may lead to an underestimation of semen volume in the range of 0.3–0.9 ml (Brazil et al., 2004; Iwamoto et al., 2006; Cooper et al., 2007), the absolute values of volume should be compared with caution to other studies. Relative changes should not be influenced since volumetric assessment was used throughout the study, and it has been found to be a systematic error caused by residual semen left in the container (Woodward et al., 2016). The sixth WHO laboratory manual recommends haemocytometer chambers with improved Neubauer ruling for sperm counting. This study was performed before those guidelines and the Bürker-Türk haemocytometer used follows the fifth WHO laboratory manual which recommends 100- μm -deep haemocytometer chambers for counting.

In conclusion, our study shows that a short-term low-calorie weight loss intervention in men with obesity improves sperm concentration and sperm count. In addition, we provide evidence that liraglutide and/or exercise can be used to maintain the weight loss-induced improvements and may be used for preserving sperm quality in the long term. Improved sperm concentration and sperm count may be linked to a shorter time to pregnancy (Slama et al., 2002). The persistent improvement of sperm concentration and count after weight loss maintained through exercise and/or a GLP-1 receptor agonist may therefore also result in improved male fertility.

Supplementary data

Supplementary data are available at *Human Reproduction* online.

Data availability

De-identified data that underlie the results reported in this article can be made available upon reasonable request to the corresponding authors, and will require the completion of a data processing agreement.

Authors' roles

E.A. collected semen samples, analysed results and wrote the manuscript. C.R.J. analysed results and wrote the manuscript. E.T.K., Y.D. and M.S. collected semen samples. S.S.T., J.R.L., C.J., S.B.K.J., J.J.H., B.M.S. and S.M. designed the S-LiTE trial. C.R.J., J.R.L., C.J., O.M.D. and S.B.K.J. collected data in the S-LiTE trial. L.R.I. analysed the results. R.B. and S.S.T. designed the study, analysed results and wrote the manuscript. All authors provided critical revisions to the manuscript and approved the final version.

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Conflict of interest

E.A.: shareholder, employee of ExSeed Health Ltd. Grant Recipient from ExSeed Health Ltd and listed on Patents planned, issued or pending with ExSeed Health Ltd; J.J.H.: consultant for Eli Lilly A/S and Novo Nordisk A/S. Lecture fees for Novo Nordisk A/S. Listed on Patents planned, issued or pending with the University of Copenhagen, Advocacy group for Antag Therapeutics and Bainan Biotech; S.M.: lecture fees for Novo Nordisk A/S. Recipient of Support for attending meetings from Novo Nordisk A/S. Advisory boards of Novo Nordisk A/S; Sanofi Aventis and Merck Sharp & Dohme. S.S.T.: research grant recipient Novo Nordisk. The remaining authors have no conflicts of interest to declare.

References

- Andersen JM, Herning H, Aschim EL, Hjelmæsæth J, Mala T, Hanevik HI, Bungum M, Haugen TB, Witczak O. Body mass index is associated with impaired semen characteristics and reduced levels of anti-Müllerian hormone across a wide weight range. *PLoS One* 2015;**10**:e0130210.
- Bentham J, Cesare M, Di Bilano V, Bixby H, Zhou B, Stevens GA, Riley LM, Taddei C, Hajifathalian K, Lu Y et al. Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: a pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. *Lancet* 2017;**390**:2627–2642.
- Best D, Avenell A, Bhattacharya S. How effective are weight-loss interventions for improving fertility in women and men who are overweight or obese? A systematic review and meta-analysis of the evidence. *Hum Reprod Update* 2017;**23**:681–705.
- Brazil C, Swan SH, Tollner CR, Treece C, Drobnis EZ, Wang C, Redmon JB, Overstreet JW; Study for Future Families Research Group. Quality control of laboratory methods for semen evaluation in a multicenter research study. *J Androl* 2004;**25**:645–656.
- Carlsen E, Giwercman A, Keiding N, Skakkebaek NE. Evidence for decreasing quality of semen during past 50 years. *BMJ* 1992;**305**:609–613.
- Cooper TG, Brazil C, Swan SH, Overstreet JW. Ejaculate volume is seriously underestimated when semen is pipetted or decanted into cylinders from the collection vessel. *J Androl* 2007;**28**:1–4.
- Faure C, Dupont C, Baraibar MA, Ladouce R, Cedrin-Durnerin I, Wolf JP, Lévy R. In subfertile couple, abdominal fat loss in men is associated with improvement of sperm quality and pregnancy: a case-series. In Volle DH, editor. *PLoS One* 2014;**9**:e86300.
- Fontoura P, Cardoso M. C D A, Erthal-Martins MC, Werneck C, Sartorio C, Ramos CF. The effects of liraglutide on male fertility: a case report. *Reprod Biomed Online* 2014;**29**:644–646.
- Håkonsen LB, Thulstrup AM, Aggerholm AS, Olsen J, Bonde JP, Andersen CY, Bungum M, Ernst EH, Hansen ML, Ernst EH et al. Does weight loss improve semen quality and reproductive hormones? Results from a cohort of severely obese men. *Reprod Health* 2011;**8**:24.
- Hernández Á, Rogne T, Skåra KH, Håberg SE, Page CM, Fraser A, Burgess S, Lawlor DA, Magnus MC. Body mass index and subfertility: multivariable regression and Mendelian randomization analyses in the Norwegian Mother, Father and Child Cohort Study. *Hum Reprod* 2021;**36**:3141–3151.
- Iwamoto T, Nozawa S, Yoshiike M, Hoshino T, Baba K, Matsushita T, Tanaka SN, Naka M, Skakkebaek NE, Jørgensen N. Semen quality of 324 fertile Japanese men. *Hum Reprod* 2006;**21**:760–765.
- Jensen SBK, Lundgren JR, Janus C, Juhl CR, Olsen LM, Rosenkilde M, Holst JJ, Stallknecht BM, Madsbad S, Torekov SS. Protocol for a randomised controlled trial of the combined effects of the GLP-1 receptor agonist liraglutide and exercise on maintenance of weight loss and health after a very low-calorie diet. *BMJ Open* 2019;**9**:e031431.
- Lazaros L, Hatzis E, Markoula S, Takenaka A, Sofikitis N, Zikopoulos K, Georgiou I. Dramatic reduction in sperm parameters following bariatric surgery: report of two cases. *Andrologia* 2012;**44**:428–432.
- Levine H, Jørgensen N, Martino-Andrade A, Mendiola J, Weksler-Derri D, Mindlis I, Pinotti R, Swan SH. Temporal trends in sperm count: a systematic review and meta-regression analysis. *Hum Reprod Update* 2017;**23**:646–659.
- Lundgren JR, Janus C, Jensen SBK, Juhl CR, Olsen LM, Christensen RM, Svane MS, Bandholm T, Bojsen-Møller KN, Blond MB et al. Healthy weight loss maintenance with exercise, liraglutide, or both combined. *N Engl J Med* 2021;**384**:1719–1730.
- Mir J, Franken D, Andrabi SW, Ashraf M, Rao K. Impact of weight loss on sperm DNA integrity in obese men. *Andrologia* 2018;**50**:e12957.
- Pärn T, Ruiz RG, Kallak TK, Ruiz JR, Davey E, Hreinsson J, Wänggren K, Salumets A, Sjöström M, Stavreus-Evers A et al. Physical activity, fatness, educational level and snuff consumption as determinants of semen quality: findings of the ActiART study. *Reprod Biomed Online* 2015;**31**:108–119.
- Priskorn L, Nordkap L, Bang AK, Krause M, Holmboe SA, Egeberg Palme DL, Winge SB, Mørup N, Carlsen E, Joensen UN et al. Average sperm count remains unchanged despite reduction in maternal smoking: results from a large cross-sectional study with annual investigations over 21 years. *Hum Reprod* 2018;**33**:998–1008.
- Rosety MÁ, Díaz AJ, Rosety JM, Pery MT, Brenes-Martín F, Bernardi M, García N, Rosety-Rodríguez M, Ordoñez FJ, Rosety I. Exercise improved semen quality and reproductive hormone levels in sedentary obese adults. *Nutr Hosp* 2017;**34**:603–607.
- Salamun V, Jensterle M, Janez A, Vrtacnik Bokal E. Liraglutide increases IVF pregnancy rates in obese PCOS women with poor response to first-line reproductive treatments: a pilot randomized study. *Eur J Endocrinol* 2018;**179**:1–11.

- Salas-Huetos A, Maghsoumi-Norouzabad L, James ER, Carrell DT, Aston KI, Jenkins TG, Becerra-Tomás N, Javid AZ, Abed R, Torres PJ et al. Male adiposity, sperm parameters and reproductive hormones: An updated systematic review and collaborative meta-analysis. *Obes Rev* 2021;**22**:e13082.
- Samavat J, Cantini G, Lotti F, Franco A, Di Tamburrino L, Degl'Innocenti S, Maseroli E, Filimberti E, Facchiano E, Lucchese M et al. Massive weight loss obtained by bariatric surgery affects semen quality in morbid male obesity: a preliminary prospective double-armed study. *Obes Surg* 2018;**28**:69–76.
- Sermondade N, Faure C, Fezeu L, Shayeb AG, Bonde JP, Jensen TK, Wely M, Van Cao J, Martini AC, Eskandar M et al. BMI in relation to sperm count: an updated systematic review and collaborative meta-analysis. *Hum Reprod Update* 2013;**19**:221–231.
- Slama R, Eustache F, Ducot B, Jensen TK, Jørgensen N, Horte A, Irvine S, Suominen J, Andersen AG, Auger J et al. Time to pregnancy and semen parameters: a cross-sectional study among fertile couples from four European cities. *Hum Reprod* 2002;**17**:503–515.
- Sun B, Messerlian C, Sun Z-H, Duan P, Chen H-G, Chen Y-J, Wang P, Wang L, Meng T-Q, Wang Q et al. Physical activity and sedentary time in relation to semen quality in healthy men screened as potential sperm donors. *Hum Reprod* 2019;**34**:2330–2339.
- World Health Organization. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th edn. Geneva: World Health Organization Press, 2010.
- Woodward B, Gossen N, Meadows J, Tomlinson M. Uncertainty associated with assessing semen volume: are volumetric and gravimetric methods that different? *Hum Fertil (Camb)* 2016;**19**:249–253.