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Clinical Research Article

Improvements in Sperm Motility Following Low- or High-Intensity Dietary Interventions in Men With Obesity

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Abstract

Introduction: Obesity increases risks of male infertility, but bariatric surgery does not improve semen quality. Recent uncontrolled studies suggest that a low-energy diet (LED) improves semen quality. Further evaluation within a randomized, controlled setting is warranted.

Methods: Men with obesity (18-60 years) with normal sperm concentration (normal count) (n = 24) or oligozoospermia (n = 43) were randomized 1:1 to either 800 kcal/day LED for 16 weeks or control, brief dietary intervention (BDI) with 16 weeks' observation. Semen parameters were compared at baseline and 16 weeks.

Results: Mean age of men with normal count was 39.4 ± 6.4 in BDI and 40.2 ± 9.6 years in the LED group. Mean age of men with oligozoospermia was 39.5 ± 7.5 in BDI and 37.7 ± 6.6 years in the LED group. LED caused more weight loss than BDI in men with normal count (14.4 vs 6.3 kg; $P < .001$) and men with oligozoospermia (17.6 vs 1.8 kg; $P < .001$). Compared with baseline, in men with normal count total motility (TM) increased 48 ± 17% to 60 ± 10% ($P < .05$) after LED, and 52 ± 8% to 61 ± 6% ($P < .0001$) after BDI; progressive motility (PM) increased 41 ± 16% to 53 ± 10% ($P < .05$) after LED, and 45 ± 8% to 54 ± 65% ($P < .001$) after BDI. In men with oligozoospermia compared with baseline, TM increased 35% [26] to 52% [16] ($P < .05$) after LED, and 43% [28] to 50% [23] ($P = .0587$) after BDI; PM increased 29% [23] to 46% [18] ($P < .05$) after LED, and 33% [25] to 44% [25] ($P < .05$) after BDI. No differences in postintervention TM or PM were observed between LED and BDI groups in men with normal count or oligozoospermia.

Conclusion: LED or BDI may be sufficient to improve sperm motility in men with obesity. The effects of paternal dietary intervention on fertility outcomes requires investigation.

Key Words: male reproduction, male fertility, obesity, spermatogenesis, weight loss, sperm quality

Abbreviations: BDI, brief dietary intervention; BMI, body mass index; DFI, DNA fragmentation index; FSH, follicle-stimulating hormone; GnRH, gonadotrophin hormone-releasing hormone; LED, low-energy diet; LH, luteinizing hormone; NHS, National Health Service; PM, progressive motility; ROS, reactive oxygen species; SHBG, sex hormone–binding globulin; TM, total motility; TUNEL, terminal uridine nick-end labeling; UKNEQAS, UK National External Quality Accreditation Service.

Infertility is defined as the inability to conceive after 12 months of regular, unprotected intercourse (1). Poor sperm quality is the most common indication for assisted reproductive technologies such as in vitro fertilization, and global sperm counts are declining (2). In vitro fertilization is an effective yet prohibitively expensive treatment for male infertility that is unavailable to most couples and public health care systems worldwide (3). Gonadotrophin therapy can restore fertility in men with hypogonadotropic hypogonadism (4). However, there is currently no pharmacological therapy able to improve semen quality in the majority of men with poor semen quality without hypogonadotropic hypogonadism. Novel and affordable therapies are therefore needed to improve sperm quality in couples affected by male infertility.

Body mass index (BMI) is inversely correlated with sperm quality in men (5). Forty percent of all men investigated for infertility are reported to be overweight or obese (6). Bariatric surgery is the most efficacious treatment for obesity, but does not improve, and may even worsen, semen quality in men (7, 8). This suggests that extreme, rapid weight loss

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may have some negative effects on sperm quality (9), at least in the short-term. Recently published, uncontrolled interventional studies have reported improvements in sperm concentration during intensive programs of dietary weight loss in men with obesity (10, 11), suggesting that lifestyle interventions are a potential novel therapy for male infertility. However, the extent of dietary weight loss required to improve semen quality in men with obesity has not been investigated previously.

Male infertility is a marker of comorbidities including cardiovascular disease, diabetes mellitus, and osteoporosis (12, 13). Since available treatments for infertile men are limited, the possible contribution of obesity in men with no other risk factors for infertility requires investigation.

We conducted randomized, controlled studies comparing the effects of high- vs low-intensity dietary interventions on semen parameters in men with obesity. Men with obesity and either normal count (Study A) or oligozoospermia (Study B) were randomized to either a 16-week low-energy diet (LED), or a single, brief dietary intervention (BDI) according to National Health Service (NHS) guidelines with 16 weeks of observation.

Materials and Methods

Governance and Study Recruitment

The studies were granted ethics approval by the London-Queen Square Research Ethics Committee (Registration number 18/LO/0376) and conducted in accordance with the principles of the Declaration of Helsinki. Participants were recruited through local online and paper advertisements, clinics, Andrology Department at Hammersmith Hospital, Imperial College Healthcare NHS Trust, and primary care clinics within northwest London, UK. Participants were invited to a screening visit to ensure they were eligible based on the study's inclusion and exclusion criteria. Inclusion criteria consisted of men aged between 18 and 60 years with a BMI $\geq$30 kg/m^2 and normal sperm count or oligozoospermia (sperm concentration $<15 \times 10^6$/mL) for study A and B, respectively. Exclusion criteria were as follows: any medical condition likely to affect testicular function such as chronic or acute systemic illnesses, undescended testes, significant smoking history, medications with adverse effect on sperm, clinical evidence of varicocele, and azoospermia. All participants provided written informed consent prior to randomization. Twenty-four men completed study A and 43 men completed study B.

Protocol

The participants were assessed twice (screening visit and visit 1) before they commenced on the dietary intervention (Fig. S1 (14)). During the 2 visits, baseline measurements of body composition and semen analysis were performed. Eligibility for enrollment was assessed during the screening visit, based on the study's inclusion and exclusion criteria. Baseline semen parameters used later in data analysis were averaged from the 2 semen samples for each participant. During visit 1, all participants were randomized in a 1:1 ratio to either formula LED or BDI. LED products consisted of soups, shakes, and bars. In the first 12 weeks, participants randomized to LED were provided diet products providing 800 kcal/day (ie, 4 Cambridge Weight Plan products/day in study A, and 4 LighterLife products combined with 400 mL of semiskimmed milk/day in study B) to achieve weight loss (weight loss phase); in the remaining 4 weeks, participants were gradually reintroduced to food by replacing 1 LED product every 1 to 2 weeks with low-carbohydrate, high-protein meals. Participants randomized to BDI were provided a single, brief (10 minute) intervention consisting of NHS standard dietary recommendations based on "The Eatwell Guide" (NHS Eatwell) (15), which was then reinforced at every visit over the 16-week period. All participants attended either 4 (study A) or 5 (study B) subsequent visits until 16 weeks following randomization. All men were advised to limit physical activity to resistance training of 30 minutes 3 times a week, and to maintain an exercise log throughout the study.

Clinical and biochemical parameters

Body weight and composition (percentage and absolute values of fat mass, lean mass, and water) were measured using bioelectrical impedance analysis using the Tanita MC-780MA P (Tanita Corp., Tokyo, Japan). Height was recorded during the screening visit. BMI was calculated with the formula weight (kg)/height (m^2). Waist circumference was measured with a tape measure placed halfway between the end of ribs and superior iliac crests.

Morning fasting blood samples (up to 11 AM) were performed at screening and final visit for measurements of luteinizing hormone (LH), follicle-stimulating hormone (FSH), testosterone, and sex hormone–binding globulin (SHBG). These were analyzed in the clinical biochemistry department of Charing Cross Hospital using Abbott ARCHITECT, an automated immunoassay platform under UK National External Quality Accreditation Service (UKNEQAS) accreditation. Reference ranges for males were as follows: LH, 2 to 12 IU/L; FSH, 1.7 to 8 IU/L; testosterone, 10 to 30 nmol/L; SHBG, 15 to 55 nmol/L.

Semen samples were collected on site in a designated private room in the Andrology Department of Hammersmith Hospital. Semen handling and manual semen analysis were performed according to the 5th edition of the WHO Manual for the Laboratory Examination and Processing of Human Semen (16). All samples were analyzed by experienced biomedical scientists within a specialist hospital laboratory accredited by the UKNEQAS. We used manual semen analysis, which is the gold standard method recommended by WHO manual, 5th edition (16). For this reason, computer-assisted sperm analysis was not used for this study. Sperm motility was determined as the percentage of progressive motile, nonprogressive motile, and immotile spermatozoa by scoring at least 200 spermatozoa/slide (16). Normal sperm morphology was examined on Papanicolaou prestained slides using strict criteria (17). The total motile sperm count was calculated by multiplying the concentration by the volume and the fraction of motile sperm. The lower reference limit for sperm parameters according to WHO manual, 5th edition, was as follows: semen volume, 1.5 mL; total sperm count, 39 million/ejaculate; sperm concentration, 15 million/mL; total motility (TM), 40%; progressive motility (PM), 32%; morphology, 4% (16). The interobserver coefficients of variation are provided here: PM, 6.92%; nonprogressive motility, 23.95%; immotile, 6.67%; morphology, 17.43%.

Semen samples were also assessed for oxidative stress using 2 markers, namely sperm DNA fragmentation index (DFI) and reactive oxygen species (ROS). Sperm DFI was measured with an established TUNEL (terminal uridine nick-end labeling) assay using the Apo-Direct kit (Pharmingen, San Diego, CA, USA) according to previously described protocol (18).

Table 1. Baseline anthropometric, semen, and reproductive hormonal parameters of men with obesity and normal-count sperm (Study A) and oligozoospermia (Study B) according to diet groups (LED vs BDI)

Demographics/parameters	Dietary intervention groups		
Study A	**LED (n = 12)**	**BDI (n = 12)**	***P* value**
Anthropometric parameters			
Age (years)	40.2 ± 9.6	39.4 ± 6.4	.84
Weight (kg)	111.1 ± 12	114.1 ± 11.0	.53
BMI (kg/m^2)	35.3 ± 4.1	36.2 ± 2.4	.52
Semen parameters			
Sperm concentration (million/mL)	38.3 ± 15	54.2 ± 21.3	.05
Semen volume (mL)	3.3 ± 1.4	3.9 ± 1.1	.28
Total sperm count (million/ejaculate) median [IQR]	99.5 [76.3]	175.2 [72.3]	.008[c]
Progressive motility (%)	41 ± 16	45 ± 8	.50
Total motility (%)	48 ± 17	52 ± 8	.51
Total motile sperm count (million/ejaculate)[a] median [IQR]	50.4 [62.4]	91.3 [36.1]	.03[c]
Normal morphology (%)	2 ± 1	2 ± 2	.89
DFI (%) median [IQR]	5.9 [22.1]	16.3 [10.5]	.08
Reproductive hormone profile			
LH (IU/L)	3.2 ± 1.0	3.5 ± 1.7	.64
FSH (IU/L)	4.2 ± 2.6	4.4 ± 1.9	.83
Testosterone (nmol/L)	12.7 ± 4.2	12.3 ± 2.5	.80
SHBG (nmol/L)	22 ± 8	25 ± 9	.34
Study B	**LED (n = 20)**	**BDI (n = 23)**	***P* value**
Anthropometric parameters			
Age (years)	37.7 ± 6.6	39.5 ± 7.5	.40
Weight (kg)	116.8 ± 17.3	117.0 ± 21.9	.90
BMI (kg/m^2)	38 ± 4.0	38.0 ± 6.3	.90
Semen parameters			
Sperm concentration (million/mL) median [IQR]	5.6 [6.0]	7.8 [8.9]	.13
Sperm volume (mL) median [IQR]	3.4 [3.5]	2.7 [2.3]	.54
Total sperm count (million/ejaculate) median [IQR]	15.2 [14.6]	22.7 [28.8]	.20
Progressive motility (%)[b] median [IQR]	28.7 [23.0]	33.4 [25.9]	.35
Total motility (%)[b] median [IQR]	34.5 [26.5]	42.5 [28.0]	.32
Total motile count (million/ejaculate)[a,b] median [IQR]	5.0 [6.3]	9.6 [15.9]	.08
Morphology (%) [b] Median [IQR]	0.0 [0.8]	1.0 [1.4]	.03[c]
ROS (RLU) [b] Median [IQR]	8.5 [171.5]	3.6 [35.8]	.10
Reproductive hormone profile	14.1 [11]	10.9 [13.3]	.18
LH (IU/L)	3.7 ± 0.9	4.3 ± 1.5	.18
FSH (IU/L)	5.6 ± 2.9	5 ± 2.5	.51
Testosterone (nmol/L)	12.6 ± 5.1	14.6 ± 5.0	.21
SHBG (nmol/L)	21 ± 10	25 ± 10	.27

Data presented as mean ± SD unless otherwise stated.
Abbreviations: BDI, brief dietary intervention; BMI, body mass index; DFI, DNA fragmentation index; FSH, follicle-stimulating hormone; IQR, interquartile range; LED, low-energy diet; LH, luteinizing hormone; RLU, relative light units; ROS, reactive oxygen species; SHBG, sex hormone–binding globulin.
[a]Total motile count = sperm concentration × sperm volume × (total motility/100).
[b]Contains missing values.
[c]Denotes where differences between LED and BDI groups were statistically significant at $P < .05$.

TUNEL assay directly measures single- and double-strand DNA breaks using the enzyme terminal deoxynucleotidyl transferase to catalyze the attachment of florescent labels or deoxyribonucleotides to the 3′-hydroxyl "free ends" of single and double DNA breaks ("nicks") (19). The fluorescence, which is proportional to the number of strand breaks, is then quantified using flow cytometry (BD Accuri C6 Plus Flow Cytometer; Becton Dickinson, San Jose, CA, USA). The more DNA strand break sites are present, the more labels are incorporated within a cell.

ROS was measured using an established in-house validated chemiluminescence assay (20). In brief, 400 µL of neat

(native) semen was mixed with 100 µL working solution containing luminol. Each sample was vortexed to evenly disperse the samples before taking luminometer readings (GloMax; Promega Corporation; Madison, WI, USA). For each negative and positive controls, and specimen assay, 10 readings were taken every minute for ten minutes and the means were used. Chemiluminescence was expressed as mean relative light units per second (RLU/second). ROS value was calculated via the following formula:

$$\text{ROS} = \frac{\text{Mean seminal sample chemiluminescence} - \text{Negative control chemiluminescence}}{\text{Sperm concentration}}$$

The reference range for semen ROS was <3.8 RLU/second/million sperm (20). In-house validation was performed daily to ensure consistent positive and negative calibration.

Statistical Analysis and Sample Size

Håkonsen et al reported that weight loss increased total sperm count by 193 million (95% CI 45-341) (10). Based on these data, and assuming that NHS dietary advice would have no effect on semen parameters, we estimated that 10 subjects would be required to detect a significant increment in semen parameters during LED compared with a noneffective comparator, with 80% power ($\alpha = .05$, 2-sided). Accounting for dropouts, we aimed to recruit 12 per group in study A. The sample size for study B was increased due to the greater standard deviation in semen parameters in men with oligozoospermia compared with normal count and was based on previous unpublished pilot data. Quantitative data were assessed for normality using the Shapiro–Wilk normality test. Data are presented as mean ± SD if normally distributed and median [interquartile range] if not normally distributed. Comparisons between the groups were calculated using the independent samples t test for normally distributed data; not normally distributed data were compared using a Mann–Whitney U test. The paired samples t test and Wilcoxon signed rank test for parametric and nonparametric data, respectively, were carried out to assess the differences at baseline and end of intervention. Categorical data were compared using a chi squared or Fisher's exact test. Correlations were calculated using Pearson's correlation coefficient or Spearman's rank correlation coefficient for normally or not normally distributed data, respectively.

Outcome data following the end of intervention were analyzed using analysis of covariance, with the postintervention data as the response and the baseline measurements as an adjusting covariate. Fitting fixed effects in the regression model for the study group and the interaction between these 2 terms were performed to examine for group differences (LED vs BDI) between the 2 studies. Where an interaction was shown to be statistically significant, this was retained in the model and the effects of the intervention were quantified separately for each study. Outcomes meeting the assumptions of the linear regression model were analyzed on the original scale of measurement. Outcomes with a strongly positively skewed distribution were analyzed on the log scale. In all cases, $P < .05$ was considered to be statistically significant. Statistical analyses were performed using Prism v.9 (GraphPad Software Inc, La Jolla, CA, USA) and Stata v.15.1 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Results

Study A: Effects of Weight Loss on Semen Parameters in Men With Obesity and Normal-Count Sperm

The mean age did not differ between study groups (39.4 ± 6.4 years, BDI; 40.2 ± 9.6 years, LED; $P = .84$). Baseline BMI was not significantly different between the groups (36.2 ± 2.4 kg/m^2, BDI; 34.8 ± 3.8 kg/m^2, LED; $P = .29$). Baseline semen characteristics were also similar between the 2 groups, except for total sperm count and total motile count (Table 1). The remaining baseline demographic and anthropometric characteristics are shown elsewhere (Table S1 (14)).

Men in the LED group lost a mean of nearly 3-fold more weight than the BDI group during the 16-week study period (6.3 kg ± 6.4, BDI; 17.6 kg ± 7.7, LED; $P < .001$) (Fig. S2A (14)). Sperm concentration at the end of intervention did not differ significantly between the BDI and LED groups. Furthermore, sperm concentration did not increase significantly from baseline following either BDI or LED (Fig. 1A-1C).

TM at the end of the intervention did not differ significantly between the BDI and LED groups. However, TM increased significantly following both BDI (61 ± 6% vs 52 ± 8%; $P < .0001$) and LED (60 ± 10% vs 48 ± 17%; $P < .05$) compared with baseline (Fig. 1D-1F)

PM at the end of intervention did not differ between the BDI and LED groups. However, PM increased significantly following both BDI (54 ± 6% vs 45 ± 8%; $P < .001$) and LED (53 ± 10% vs 41 ± 16%; $P < .05$) compared with baseline (Fig. 1G-1I).

In addition to conventional semen parameters, we investigated the effect of weight loss on sperm DNA fragmentation in men with obesity and normal count. Sperm DNA fragmentation was significantly lower in the LED group compared with BDI group at the end of the intervention period (DFI: 18.3% [6.8] BDI; 4.1% [8] LED; $P < .001$) (Fig. 2A). Regression analysis also showed mean DFI in LED to be lower by 9.5 units than the DFI in the BDI group (95% CI −16.4, −2.5; $P = .009$) (Table 2). In men with obesity during LED intervention, the DFI decreased, but this was nonsignificant (Fig. 2B). There was a significant inverse correlation ($r = -0.56$, $P = .005$) between weight loss and DFI values at the end of the intervention (Fig. 2D), but the correlation between reduction in DFI with weight loss was nonsignificant (Fig. S3 (14)).

Three men randomized to LED intervention had asthenospermia. After excluding these men from the analysis, statistically significant increases in sperm motilities during LED were no longer observed in the remaining participants. The findings in sperm concentration and DFI remained unchanged (Fig. S4 (14)).

Study B: Effects of Weight Loss on Semen Parameters in Men With Obesity and Oligozoospermia

Baseline characteristics including demographics, anthropometric, metabolic, and hormonal parameters are shown in Table 1 and elsewhere (Table S2 (14)). Mean weight loss in the LED group was significantly higher than in the BDI group (14.4 kg ± 5.3, LED vs 1.8 kg ± 4.2, BDI; $P < .0001$) (Fig. S2B (14)). Baseline semen parameters did not differ between the 2 groups except for sperm morphology (Table 2).

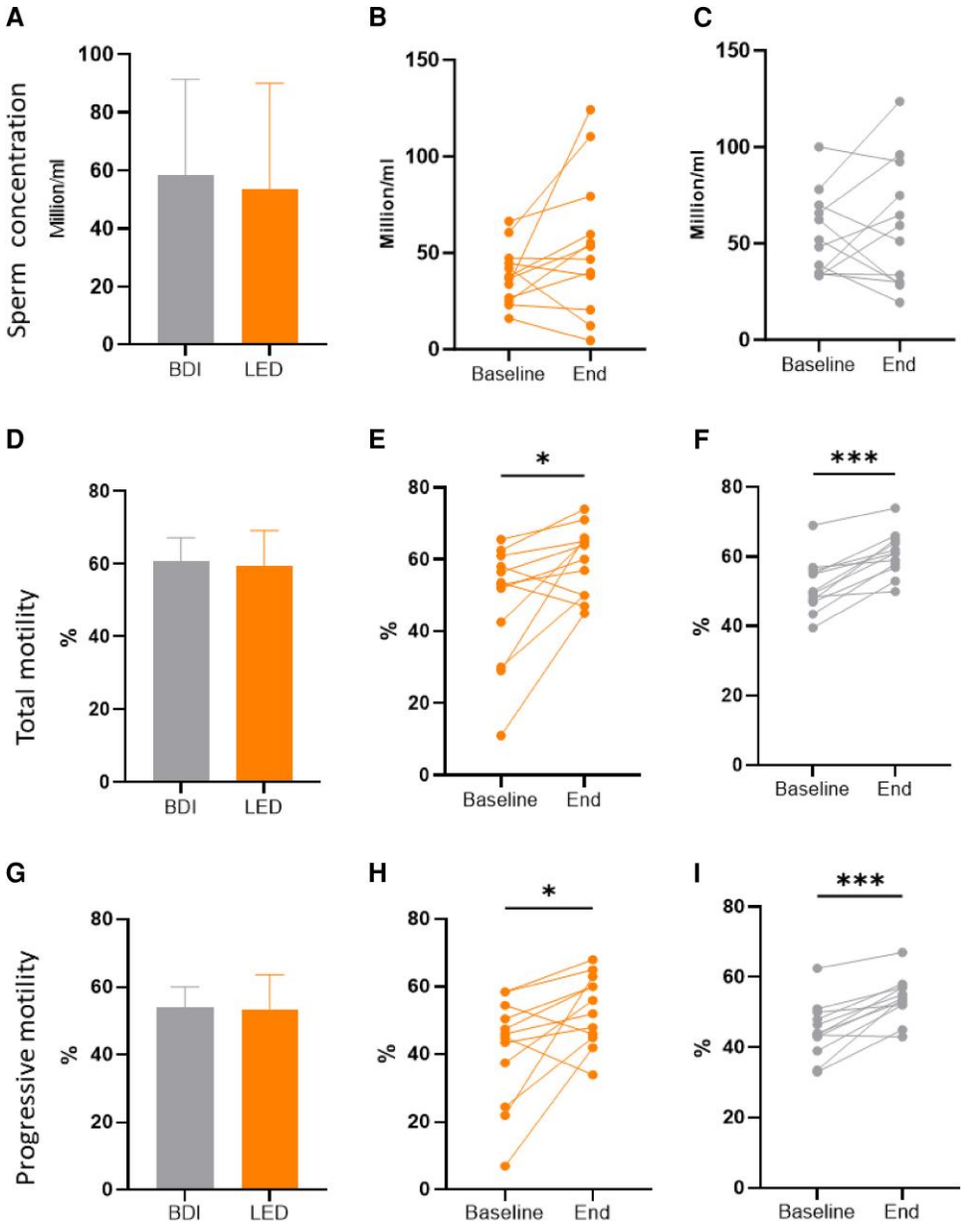

Figure 1. Effects of dietary interventions on semen parameters in men with normal count. Bar graphs showing (A) sperm concentration ($58.5 \times 10^6 \pm 32.8$, BDI; $53.6 \times 10^6 \pm 36.4$, LED; $P = .73$), (D) total motility (61% ± 6, BDI; 60% ± 10, LED; $P = .69$), and (G) progressive motility (54% ± 6, BDI; 53% ± 10, LED; $P = .85$) at the end of the dietary intervention in BDI groups and LED groups. Baseline and end of intervention for (B) sperm concentration ($P = .12$), (E) total motility ($P = .02$), and (H) progressive motility ($P = .02$) in the LED group. Baseline and end of intervention for (C) sperm concentration ($P = .59$), (F) total motility ($P < .0001$), and (I) progressive motility ($P < 0.001$) in the BDI group. (A, D, G) Data are presented as mean ± SD. $^*P < .05$; $^{***}P < .001$. BDI, brief dietary intervention; LED, low-energy diet.

Sperm concentration at the end of intervention did not differ significantly between the BDI and LED groups or change significantly from baseline following either BDI or LED (Fig. 3A-3C). The degree of weight loss did not correlate with semen quality (Table S3 (14)).

TM at the end of intervention did not differ significantly between the BDI and LED groups. TM increased significantly following the LED intervention (52% [16] vs 35% [26], $P < .05$), though TM following the BDI was non-significant (50% [23] vs 43% [28], $P = .0587$) (Fig. 3D-3F).

PM at the end of intervention did not differ significantly between the BDI and LED groups. However, PM increased significantly following both the BDI (44% [25] vs 33% [25]; $P < .05$) or LED (46% [18] vs 29% [23]; $P < .05$) groups compared with baseline (Fig. 3G-3I).

At baseline, approximately half of the participants in both interventions had reduced TM and PM as defined by the 2010 WHO reference range (16) (TM < 40% in 11/20 in LED group and 12/23 in BDI group; PM <32% in 12/20 in LED group and 11/23 in BDI group). In a subgroup analysis, in men with asthenospermia TM and PM at the end of intervention were significantly higher with LED than with BDI. There were no differences at the end of intervention in the men with normal sperm motilities at baseline (Fig. S5 (14)).

Sperm DFI at the end of intervention did not differ significantly between the BDI and LED groups or change

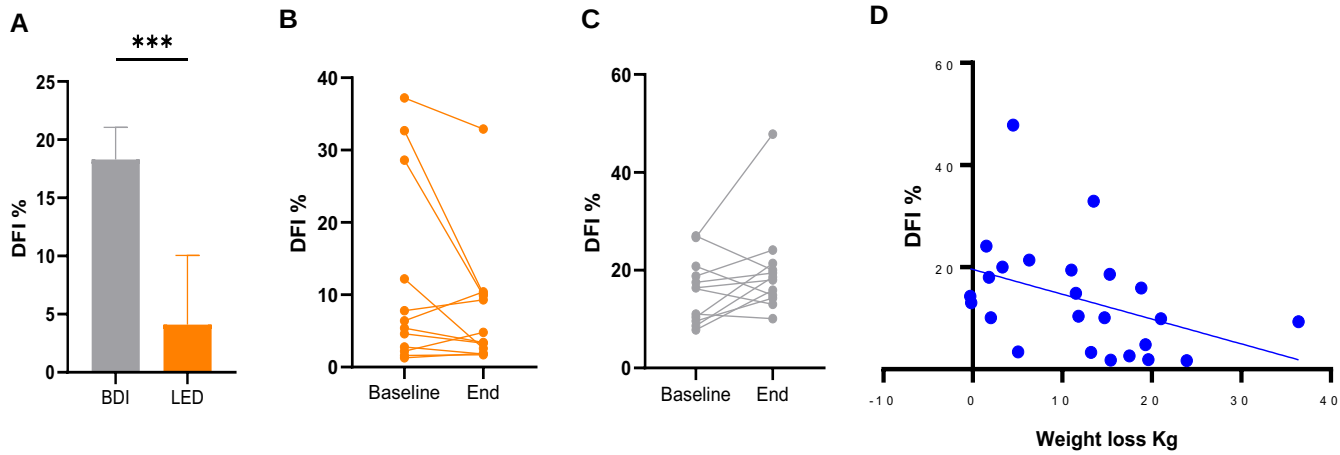

Figure 2. Effects of dietary interventions on DNA fragmentation index in men with normal count. (A) Bar graph of DFI at the end of the dietary intervention between the 2 groups (18.3% [6.8]; BDI, 4.1% [8], LED; $P < .001$). (B) Difference in DFI before and after the dietary intervention in LED group ($P = .23$). (C) Difference in DFI before and after the dietary intervention in BDI group ($P = .15$). (D) Scatter plot of weight loss and DFI as measured at the end of the study across both groups ($r = -0.56$, $P = .005$). (A) Data are given as median with interquartile range. ***$P < .001$. DFI: DNA fragmentation index; BDI, brief dietary intervention; LED, low-energy diet.

Table 2. Analysis for covariance and comparisons of outcomes from both studies

Outcome	Study by group interaction *P* value	Patients	Group difference Mean (95% CI)[a]	Group *P* value
Total motility	.42	All	2.4 (−3.6, 8.)	.43
Progressive motility	.62	All	2.8 (−3.3, 8.9)	.36
Semen volume	.81	All	0.44 (−0.12, 0.99)	.12
DFI	**.004**	Study A	−9.5 (−16.4, −2.5)	**.009**
		Study B	4.8 (−1.6, 11.2)	.14
Outcome	Study by group interaction *P* value	Patients	Group difference Ratio (95% CI)[b]	Group *P* value
Sperm concentration	.53	All	0.77 (0.40, 1.48)	.43
Sperm count	.79	All	0.87 (0.43, 1.74)	.69
Total motile count	.66	All	1.00 (0.52, 1.93)	.99
Morphology	.89	All	0.81 (0.60, 1.10)	.17
ROS	[c]	Study B	0.77 (0.18, 3.36)	.72

For outcomes analyzed on the original scale, the mean difference in outcome between groups is reported with a corresponding confidence interval (CI). For outcomes analyzed on the log scale, the ratio of values in the LED group relative to the BDI group is reported with a corresponding confidence interval. *P* values indicating the significance of the group differences are reported in the final column. *P* values reaching statistical significance are shown in bold.
Abbreviations: DFI, DNA fragmentation index; ROS, reactive oxygen species.
[a]Group difference expressed as outcome in LED group minus outcome in BDI group. Adjusted for outcome at baseline.
[b]Group difference expressed as outcome in LED group relative to outcome in BDI group. Adjusted for outcome at baseline.
[c]Interaction not relevant as outcome measured for Study B only.

significantly from baseline following either BDI or LED (Fig. S6 (14)). We observed no statistically significant difference in semen ROS between the 2 groups at the end of intervention (ROS in RLU/second/million sperm: 5.04 [36.5] BDI; 13.02 [36.14], LED; $P = .55$). In a subgroup of men with elevated ROS at baseline (validated threshold ≤ 3.8), 36% men normalized their ROS following the formula LED compared with 12.5% with the BDI ($P = .34$) (data not provided).

Combining the 2 Studies

The distinction between the 2 studies were based on the sperm concentration WHO 2010 cut off value (16). We combined the data from the 2 studies as participants received the same dietary interventions per group and were followed up for the same period. Data were adjusted for baseline measurements. We observed no difference in sperm concentration, TM, PM, semen volume, sperm count, total motile count, and morphology between the 2 intervention groups (Table 2). We additionally investigated for possible differences according to BMI severity. The results did not change after adjusting for baseline BMI (Table S4 (14)).

We analyzed levels of serum reproductive hormones from both studies. No differences between serum reproductive hormone levels were observed between BDI and LED groups at the end of the study. In the LED group, levels of serum testosterone and SHBG increased significantly from baseline to end of intervention. In the BDI group, levels of serum LH reduced

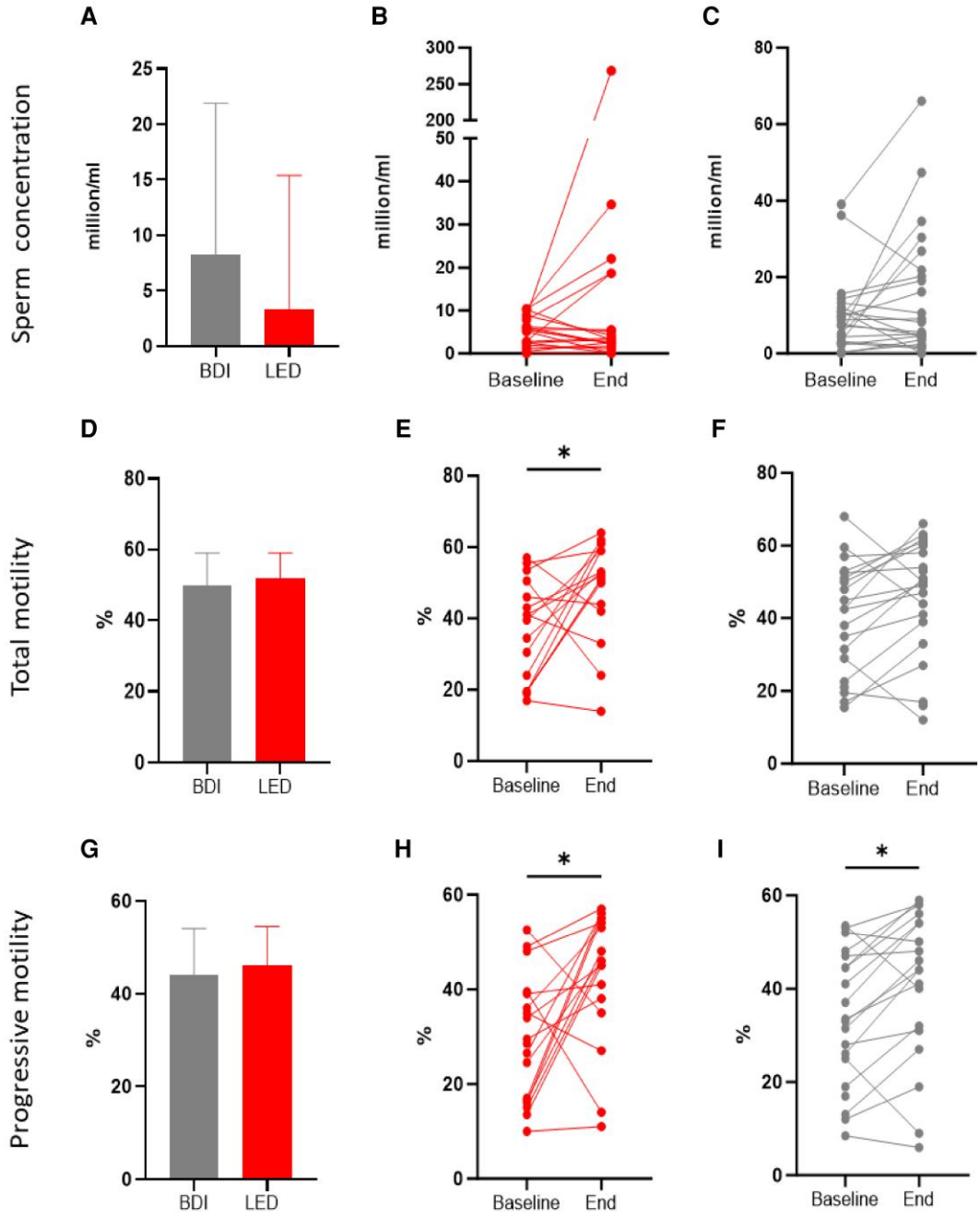

Figure 3. Effects of dietary interventions on semen parameters in men with oligozoospermia. Bar graphs of (A) sperm concentration (8.3×10^6 [18.3], BDI; 3.4×10^6 [13.1], LED; $P = 0.12$), (D) total motility (50% [23], BDI; 52% [16], LED; $P = .48$), and (G) progressive motility (44% [25], BDI; 46% [18], LED; $P = .67$) at the end of the dietary intervention in the 2 groups. Baseline and end of intervention for (B) sperm concentration ($P = .82$), (E) total motility ($P = .02$), and (H) progressive motility ($P = .01$) in LED group. Baseline and end of intervention for (C) sperm concentration ($P = .21$), (F) total motility ($P = .0587$), and (I) progressive motility ($P = .01$) in BDI group. (A, D, G) Data are given as median with interquartile range. *$P < .05$. BDI, brief dietary intervention; LED, low-energy diet.

significantly from baseline to end of study. No other before–after hormonal changes were observed (Fig. 4). Analysis of reproductive hormones for each study separately are shown elsewhere (Figs. S7 and 8 (14)). Baseline reproductive hormone profile is shown in Table 1.

Discussion

A recent meta-analysis concluded that reported mean sperm counts in both high and low/middle-income countries have halved during the last 50 years (21). In the absence of drug therapies, it is important to develop simple and affordable interventions to improve semen quality, and therefore male fertility. We expected that an intensive program of dietary weight loss would be superior to a control, brief dietary intervention for improving semen quality; surprisingly, similar improvements in sperm motility were observed following both dietary interventions. However, a high-intensity diet had a more favorable effect in improving sperm motilities in men with asthenospermia. Similarly, when men with asthenospermia

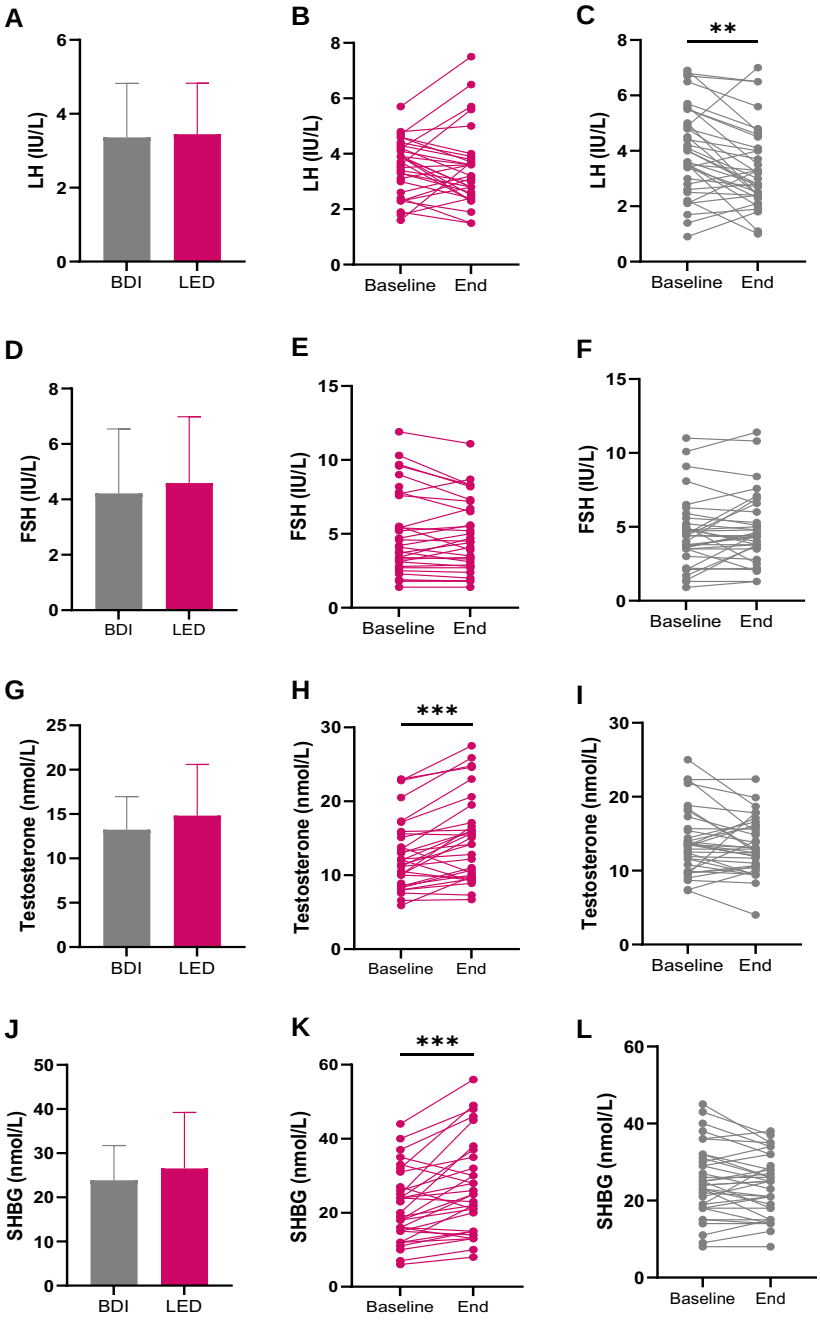

Figure 4. Effects of dietary interventions on hormonal parameters after combining the 2 studies. Bar graphs of (A) LH (3.4 ± 1.5, BDI; 3.5 ± 1.4, LED; $P = .81$), (D) FSH (4.2 ± 2.3, BDI; 4.6 ± 2.4, LED; $P = .52$), (G) testosterone (13.3 ± 3.7, BDI; 14.8 ± 5.8, LED; $P = .19$), and (J) SHBG (24 ± 8, BDI; 27 ± 13, LED; $P = .30$) at the end of the dietary intervention in BDI groups and LED groups. Baseline and end of intervention (B) LH ($P = .70$), (E) FSH ($P = .26$), (H) testosterone ($P < .0001$), and (K) SHBG ($P < .001$) in the LED group. Baseline and end of intervention for (C) LH ($P < .01$), (F) FSH ($P = .15$), (I) testosterone ($P = .28$), and (L) SHBG ($P = .15$) in the BDI group. (A, D, G, J) Data are presented as mean ± SD. $^{**}P < .01$; $^{***}P < .001$. BDI, brief dietary intervention; LED, low-energy diet.

were excluded, there were no significant increases in TM and PM during LED intervention. This suggests that improvements may be more pronounced in men with asthenospermia. Our data therefore support the growing view that dietary interventions may potentially be a method of improving fertility outcomes in couples in whom the male partner has obesity. However, we also suggest for the first time that even mild dietary interventions may be sufficient to improve semen quality, which may greatly increase the accessibility of fertility treatment for couples currently unable to access support.

Global rates of obesity have tripled since 1975 (22). Obesity may impair semen quality through several mechanisms, including suppressed hypothalamic gonadotrophin hormone-releasing hormone (GnRH) secretion, increased aromatization of androgens to estrogens, insulin resistance, oxidative and heat stress within the testes (23-25). However, the role of male obesity in semen parameters remains less clear (26, 27). The paucity of studies investigating the role of weight loss on sperm parameters and assisted reproductive technology outcomes creates an evidence gap of uncertainty. This

may reflect the technical challenge of studying semen parameters which have high degrees of biological variation. Furthermore, the relationship between weight loss and semen parameters may be complex, as demonstrated by lack of improvement of semen parameters with bariatric surgery (28). This may reflect that either significant energy restriction or inflammatory effects during substantial weight loss have negative effects on male reproductive function. We therefore hypothesized that a smaller degree of weight loss could ameliorate the detrimental effects of obesity on semen quality, without the impairments of semen quality observed during greater absolute weight loss. Our protocol for weight loss and sample size were based on pilot studies suggesting that a threshold of 12 kg of weight loss would significantly improve semen quality in men with obesity. Independent ethical review recommended that participants with obesity randomized to the control intervention should receive the level of dietary support available to the general population. Therefore, we provided a brief (10 minutes) intervention which is openly available online from the NHS, providing advice on healthy eating (15). Participants randomized to the BDI arm lost on average 3-fold less weight than the LED arm; this, however, did not result in greater improvements in sperm motility between the groups. Collectively, our data support the view that weight loss via dietary modification may improve semen quality in men. We also suggest for the first time that a universally available public health intervention could also provide clinical benefit for improving reproductive potential in men with obesity. Although our initial results are promising, larger studies with pregnancy outcomes are required. Our data would require replication in a larger study powered to live birth outcomes; if confirmed, dietary intervention might have substantial future potential for improving reproductive health in couples with infertility, including those in low to middle income countries without access to assisted reproductive technologies.

The current study broadly supports a limited number of prior studies suggesting that dietary intervention may improve semen quality in men with obesity. Håkonsen et al studied the effects of a 14-week residential diet and exercise program in 27 men with a mean BMI 44 kg/m^2; improvements in total sperm count and semen volume were observed in the 10 participants with greatest weight loss (mean 25.4%) (10). In the recently published S-Lite study (11), men with obesity underwent a mean 16.5 kg weight loss during 8 weeks of nonrandomized LED intervention; total sperm count increased by 40% vs baseline, and sperm concentration increased by 49% vs baseline. All participants were then randomized to 1 of 4 52-week intervention groups: placebo; exercise and placebo; GLP-1 agonist; and both exercise and GLP-1 agonist. S-Lite failed to demonstrate differences in semen parameters among the intervention groups. However, post hoc analysis suggested that improvements in semen parameters were sustained in men who maintained a median weight loss of more than 11.7 kg at 52 weeks regardless of the randomized group. Mir et al studied the effects of a "healthy diet and exercise" program in 105 men (mean BMI 33.2 kg/m^2) and reported significant improvements in sperm morphology and PM with approximately 10% of BMI reduction (29). In summary, there is concordance among our study and other studies that intensive forms of dietary intervention are associated with improved semen quality in men with obesity. However, our study is the first to suggest that a single, publicly available intervention may also be sufficient to improve semen quality in men with obesity. Larger studies would be needed to resolve whether LED has differential effects on semen quality compared with BDI in men with obesity.

Sperm DNA fragmentation is an important mechanism of sperm damage (30). Men with elevated DFI are known to have adverse fertility outcomes (30) and an increased risk of recurrent pregnancy loss (31-33). We observed a 4-fold improvement in sperm DFI for normal-count men with obesity during LED compared with BDI. This finding was replicated during regression analysis after adjusting for baseline value. This is of particular importance as seminal oxidative stress and sperm DNA fragmentation have widely been accepted as major causes of male infertility (34). In our study, participants losing more weight were more likely to have lower DFI sperm values by the end of the study. However, the correlation of weight loss with DFI reduction was not significant. Our results were not replicated in men with oligozoospermia; this may reflect that DFI could not be measured in some participants owing to an insufficient sperm concentration required for the TUNEL assay. We noticed similar results with analysis of covariance. Our data are in concordance with 2 recent studies. Mir et al observed improvements in sperm chromatin dispersion, an indirect marker of DFI, in men undergoing a 12-week diet and exercise program (29). Faure et al observed reductions in TUNEL DFI in 6 men after 3 to 8 months of a dietary weight loss program (35). However, Håkonsen et al failed to observe any significant improvements in sperm chromatin structure assay in men with morbid obesity undergoing weight loss and exercise (10). Hence, our study data suggest that in men with obesity, the degree of dietary weight loss may be correlated with degree of sperm DNA damage. Further studies would be needed to determine if different methods of weight loss would provide clinically significant differences in sperm DNA fragmentation which were sufficient to affect live birth outcomes in couples with male infertility. Seminal oxidative stress is a potential mediator of sperm damage and DNA fragmentation in men (36). Seminal ROS are correlated with reduced fertility outcomes (37, 38) and recurrent pregnancy loss (39). One study of 6 men observed significant increase in seminal superoxide dismutase protein 2 levels, which is a ROS scavenger, with abdominal fat loss (35). We observed reductions in ROS during weight loss, but not significant differences between the study groups. Further studies are needed to investigate whether reductions in semen ROS represent a mechanism for improving semen quality in men during weight loss.

We additionally measured serum LH, FSH, testosterone and SHBG in men with normal count and oligozoospermia. On combined analysis, serum testosterone and SHBG increased significantly following LED but not BDI. This is expected as it is widely known that weight loss either through dietary intervention or bariatric surgery results in rising of testosterone and SHBG (40-43). However, post-treatment levels were not different between the groups. Caloric restriction is known to suppress pulsatile GnRH/LH secretion (44); we are unable to explain why LH reduced significantly following BDI, but not following LED which provides more extensive weight loss.

This study utilized a randomized design and measured WHO and advanced semen parameters using validated assay techniques. A further strength of our study is its investigation of an effectively cost-free intervention available to the general public. Furthermore, unlike some published studies in this field, our study period was long enough to investigate effects

of interventions on a complete cycle of spermatogenesis in men (range 42-76 days) (45). The semen analyses were performed by biomedical scientists in the andrology laboratory who were not part of the study team and were blinded to the intervention given. However, all interventional studies of semen quality are limited by large, biological variations observed in semen parameters of men (46). This probably prohibited any associations of weight loss with semen quality. The study failed to detect any significant differences in its primary outcome (sperm concentration). Positive findings were shown regarding sperm motilities (secondary outcomes), but one needs to consider that these would have been underpowered. We cannot exclude that undetected differences between semen parameters may exist between men in the intervention groups. Additionally, the observed improvements in sperm motilities (but not in concentration) raise the possibility of regression to the mean. We attempted to mitigate this phenomenon at the design stage by including a randomly allocated placebo and intervention group and taking 2 baseline measurements for semen parameters (47). We cannot exclude though that the observed changes in sperm motility were by random chance. Many men were screened to find sufficient participants for the study. We speculate that the embarrassment of producing semen samples together with the social stigma of male infertility (48) may have contributed to the relative low number of patients who agreed to participate. We also cannot exclude the possibility that participants may have voluntarily changed their exercise behavior during the study, and the potential effects this may have had on our results. Furthermore, it should be recognized that regular visits to see a medical team within a specialist hospital may have encouraged weight loss in the BDI group which might not be achieved within a community setting. Lastly, compliance with diet was not measured. Eight to sixteen weeks' duration of LED or very–low-energy diet results in 10% to 15% of weight loss (49, 50). In our cohort, 25 out of 32 men (78%) receiving LED achieved >10% weight loss and only 1 man had <8% weight loss, indicating a moderate compliance with LED diet. Prior studies have reported 53% to 94% of participants achieving the target of >10% weight loss with LED or very–low-energy diet (51-53). Various factors have been deemed barriers to adherence to LED/very–low-energy diet meal replacement such as product unpalatability, unrealistic weight loss expectations, poor program accessibility, unforeseeable circumstances, and externalized weight-related stigma (54).

In conclusion, our data suggest that dietary interventions resulting in modest degrees of weight loss may be sufficient to improve sperm motilities in men living with obesity, thus having the potential to improve fertility in couples with male infertility. Current clinical guidelines for the management of infertility do not identify weight loss as a potential method of improving semen quality for male partners (55-57). Further studies are required to determine the feasibility, clinical and cost-effectiveness of publicly available dietary intervention program to improve fertility outcomes for couples with infertility. Such approaches may be particularly suited to geographical regions with both a high prevalence of obesity and limited fertility healthcare provisions.

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Data Availability

Some or all datasets generated during and/or analyzed during the current study are not publicly available, but are available from the corresponding author on reasonable request.

Role of Funder

NIHR funded the study, but was not involved in its design, conduct or analysis. Cambridge Weight Plan Ltd, Corby, Northants, UK, and LighterLife, Harlow, Essex, UK, provided food products free of charge, without condition, and without any other involvement in the study. LogixX Pharma sponsored the flow cytometer but was not involved in its design or conduct.

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