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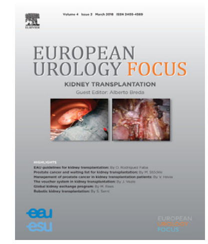

Andrology

Effects of a Lifestyle Change Intervention on Semen Quality in Healthy Young Men Living in Highly Polluted Areas in Italy: The FASt Randomized Controlled Trial

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Abstract

Background: Human semen quality is affected by lifestyle and environmental factors.
Objective: To evaluate the short-term effects of a diet and physical activity intervention on semen quality of healthy young men living in highly polluted areas of Italy.
Design, setting, and participants: A randomized controlled trial was conducted. Healthy young men were assigned to an intervention or a control group.
Intervention: A 4-mo Mediterranean diet and moderate physical activity program.
Outcome measurements and statistical analysis: The primary outcomes were sperm concentration, motility and morphology, concentration of round cells, and semen total antioxidant capacity. Secondary outcomes were adherence to Mediterranean diet and physical activity. All outcomes were measured twice, at the enrollment (t0) and at the end of the intervention (t4).
Results and limitations: A total of 263 individuals attended all visits, and underwent examinations and laboratory analyses: 137 in the intervention group and 126 in the control group. The adherence to Mediterranean diet and physical activity level increased more in the intervention group than in the control group from t0 to t4. Sperm concentration, total and progressive motility, and proportion of normal morphology cells increased in the intervention group but decreased in the control group, with statistically significant differences between the two groups at t4. The total antioxidant capacity increased in the intervention group but decreased in the control group, from t0 to t4.

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Conclusions: Study results showed that an intervention based on Mediterranean diet and regular physical activity can determine an improvement of semen quality in healthy young men.

Patient summary: Our study aimed to evaluate the effect of a lifestyle intervention on semen quality of healthy young men. We assigned the 263 enrolled individuals to an intervention or a control group. The intervention group followed a 4-mo Mediterranean diet and moderate physical activity program, at the end of which the participants showed an improvement of semen quality parameters.

1. Introduction

Couple infertility, the inability to conceive after 1 yr of unprotected sexual intercourse, affects 10–15% of couples of reproductive age worldwide, with males contributing to 50% of cases overall [1]. Testicular and post-testicular deficiencies are the causes of male reproductive dysfunction, and a dramatic reduction of semen parameters worldwide has been reported in last decades [2]. Several factors, including hormonal disorders, physical problems, chromosomal abnormalities, and environmental and lifestyle factors, have been found to be associated with semen quality parameters, especially sperm count, concentration, and motility [3–5].

Recent studies have found that low semen quality is associated with the risk of noncommunicable diseases, all-cause mortality, and life expectancy, suggesting that it may be an overall indicator of an individual's health status [6]. Furthermore, the growing interest in the transgenerational effects induced by environmental exposures and lifestyle through epigenetic modifications on gametes shifts the interest of prevention of chronic diseases toward the investigation of reproductive biomarkers [7].

Among lifestyle factors, dietary habits and physical activity have widely been investigated. Particularly, the traditional Mediterranean diet, characterized by high intake of vegetables, legumes, fruits, nuts, grains, fish, seafood, and extra-virgin olive oil, and moderate physical activity have been associated with better semen quality [3,8,9]. The positive effect of a Mediterranean diet has been demonstrated, particularly as regards the reduced risk of cardiovascular diseases, which is likely due to its antioxidant and anti-inflammatory properties [10]. However, the evidence of benefits of the Mediterranean diet and regular physical activity on semen quality is still uncertain, as it is based on observational epidemiological studies only, which are all at risk of bias [10].

Adolescents are a target population for lifestyle interventions, because of the highest efficacy of prevention activities for male infertility as well as chronic diseases at this age, due to high cell-tissue-organ plasticity, and the opportunity for acquiring positive lifestyle habits to be maintained in adulthood [11]. To the best of our knowledge, no randomized controlled trial (RCT) on the effects of dietary habits on semen quality in healthy young men has been carried out so far.

The primary objective of the "Fertilità, Ambiente, alimentazione, STile di vita" (FASt) study is to evaluate the effects of a lifestyle change intervention, based on better adherence to the Mediterranean diet model and regular moderate physical activity, on semen quality of healthy young men living in highly polluted areas of Italy through an RCT.

2. Patients and methods

2.1. Study design

FASt is an RCT (registered on ClinicalTrials.gov Protocol Registration and Results System; receipt release date: February 15, 2019; n. J59D1600132001), aimed to enroll healthy young men living in three polluted areas of Italy (Fig. 1): the Brescia-Caffaro area, Lombardy region (North Italy); the Sacco River Valley, Lazio region (Central Italy); and the "Land of Fires", the northern area of Naples, Campania region (South Italy). Details on the features of the areas are reported in the Supplementary material.

Fig. 1 – The three Italian areas from where study participants were recruited.

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Table 1 – Exclusion criteria for the selection of participants in the study

Exclusion conditions
Body mass index <18.5 or >25
Waist circumference >102 cm
Azoospermic or cryptospermic conditions
History of cancer
Chemo- or radiotherapy
Urogenital surgery for varicocele in the 6 mo before biological sample collection
Intake of dietary supplements or substances containing vegetal or animal extracts or trace elements
Regular use of steroids or anabolic hormones
Daily alcohol intake (>five drinks per week)[a]
Daily tobacco smoking (>five cigarettes, cigar, or pipe per week)[a]
Daily or weekly marijuana smoking (three times and over a month)[a]
any use of drugs (other than marijuana)
Postponing conditions[b]
Recent use of one of phosphodiesterase type 5 inhibitors (in the last 72 h)
Presence of fever or a recent antibiotic therapy (last 2 wk)

[a] "Low or very low consumption" of substances such as alcohol, tobacco, and marijuana was considered acceptable according to realistic exposure scenario in the 18–24-yr range in Italy.
[b] Temporary contraindications for collecting biological samples, because of possible alterations of semen quality. An individual with at least one of these conditions was invited to come back for the visit and biological sample collection after an appropriate period of time (some days or weeks).

After having provided informed consent, participants were randomly assigned to the lifestyle intervention (INT) or the control (CTRL) group. The INT group followed a Mediterranean diet pathway and a program of moderate physical activity for 16 wk under nutritionists' control. The CTRL group received only a booklet reporting the currently available national dietary guidelines issued by the Italian Council for Agricultural Research and Economics (CREA) [12].

Participants enrolled were high school and university students. Upon their recruitment (described in details in the Supplementary material), all participants underwent urological examination; measurement of weight, height, and abdominal circumference; and an interview on demographic data and lifestyle variables. Questionnaires for evaluating the adherence to the Mediterranean diet (PREvención con DIeta MEDiterránea questionnaire [PREDIMED]) [13] and physical activity (International Physical Activity Questionnaire [IPAQ]) [14,15] were administered. At the same time, a semen sample was collected and analyzed to determine semen quality (spermiogram) and its total antioxidant capacity (TAC). Questionnaire administration and semen analysis were carried out at the enrollment (t0) and at the end of the intervention (t4).

Study recruitment, with the first measurements (t0), was carried out between April 2018 and January 2019, and the end of the 4-mo intervention (t4) occurred between August 2018 and June 2019.

2.2. *Inclusion/exclusion criteria*

Following the recruitment, healthy young men aged 18–22 yr who have been living for at least 5 yr in the considered areas were eligible to participate. Selection was next carried out on the basis of demographic data and lifestyle variables, and the exclusion criteria are listed in Table 1.

2.3. *Sample size*

The computation of sample size was based on total sperm motility and semen TAC, two outcomes of a previous Italian study on 60 healthy young men living in a polluted area, in which a mean spermatozoa motility of 49% (standard deviation [SD] = 17%) and a semen TAC of 0.9 mM (SD = 0.3 mM) were found [5]. Based on the results of observational studies, we expected an improvement of at least 15% of both parameters in individuals following a lifestyle intervention compared with the CTRL group, according to 1:1 randomization plan. A sample size of 120 individuals for each group was considered enough for detecting the abovementioned differences between the two groups (effect sizes), using a two-tailed Student *t* test for unpaired data, with an α error of 0.05 and a power of 85%. Assuming a complete follow-up for at least 70% of participants in each arm, the study planned to enroll 340 individuals, 170 per group. However, since more loss of participants was expected in the INT group than in the CTRL group, due to the well-known difficulty for people to change and maintain a different dietary regimen for more than some days or weeks, we decided to oversize the INT group by about 20%.

2.4. *Randomization and blinding*

Eligible participants were randomly assigned to either the lifestyle INT group or the CTRL group in a 1:1 ratio, using a computer-generated randomization list. The allocation was performed by the trial statistician, who assigned a code to each recruited individual. Owing to the study design, participants and operators were aware of the intervention assignment. However, blinding was granted for the measurement of the biological variables and statistical analysis, as all semen and blood samples, as well as questionnaires and laboratory data were coded.

2.5. *Intervention*

Participants assigned to the INT group followed a nutritionist-counseled pathway based on a Mediterranean diet pattern for 4 mo. The nutritionists proposed each participant an individualized diet and physical activity plan, based on a step-by-step achievement of the objectives.

Face-to-face meetings and phone checks to verify and update nutritional and physical activity goals were carried out regularly, every week for the 1st month, every 2 wk for the 2nd and 3rd months, and once in the 4th month. Each meeting was conducted by nutritionists according to the Miller and Rollnick [16] management protocol aimed at increasing the intrinsic motivation to change.

Participants assigned to the CTRL group received only a booklet including the Italian national nutritional recommendations [12].

2.6. *Outcome measures*

Study outcomes were assessed twice during the study period: (1) at the enrollment (baseline, t0) and (2) after 4 mo, at the end of the intervention phase (t4). At each evaluation, individuals underwent measurement of weight, height, and abdominal circumference; gave a semen sample; and filled in the dietary and physical activity questionnaires (PREDIMED and IPAQ, respectively; details in the Supplementary material).

2.7. *Semen collection*

Each semen sample was collected in a sterile container through masturbation, after at least 3 d and at most 5 d of abstinence from sexual activity. The semen sample was delivered to the laboratory within 30–40 min after collection.

On delivery, a portion of semen (<50 μl) was processed immediately for the spermiogram, while an aliquot of 100 μl was frozen for the quantification of the TAC. Exhaustive methods are described in the Supplementary material.

2.8. *Data management and analysis*

All the data were collected in a database. First, a comparison of the variable distribution at baseline (t0) between the INT and CTRL groups and among individuals living in the three areas was performed using analysis of variance. Second, changes in the outcome variables between

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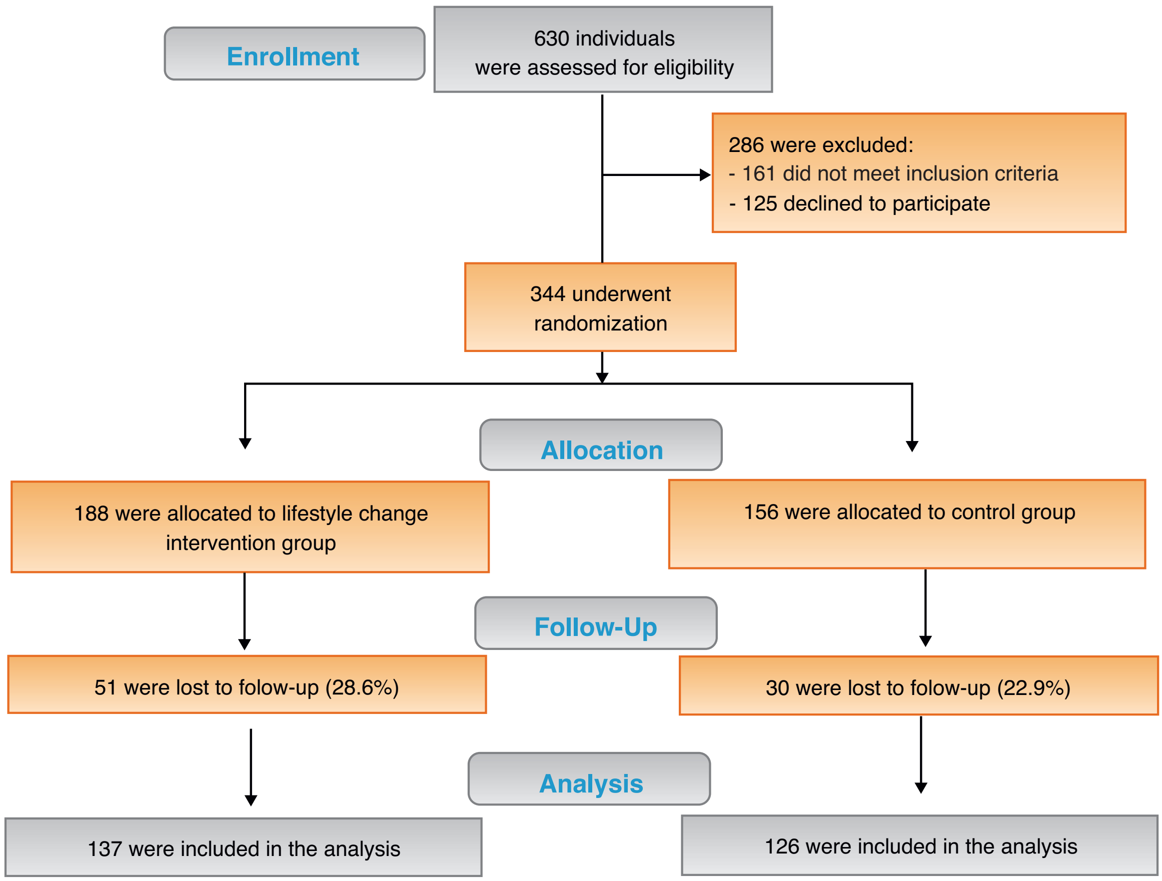

Fig. 2 – Flow diagram of the FASt study (according to the CONSORT statement). FASt = Fertilità, Ambiente, alimentazione, STile di vita.

before and after the intervention were assessed in the INT and CTRL groups, separately, using Student *t* test for paired data. Third, the differences between the INT and CTRL groups at baseline (t0) and t4 were assessed with Student *t* test for unpaired data. Additionally, t0-t4 changes in the two groups were compared with Student *t* test for unpaired data. Finally, the associations between lifestyle variables (IPAQ and PREDIMED) and semen parameters were assessed by computing the Spearman's correlation coefficient, considering of interest only the associations with a rho coefficient >0.4. Data analysis was carried out using the Stata 14.2 software (Stata Corp, College Station, TX, USA).

3. Results

Of the 630 students assessed for eligibility, 286 were excluded because they did not meet the inclusion criteria or declined to participate. Therefore, 344 students underwent randomization and were assigned to the INT (n = 188) or the CTRL (n = 156) group (Fig. 2). A total of 263 students (76%) completed the 4-mo follow-up by attending all visits, and undergoing examinations and laboratory analyses, and were included in the statistical data analysis: 137 in the INT group and 126 in the CTRL group.

3.1. *Baseline evaluation*

Baseline features of the study population are reported in Table 2. The mean age of the participants was 19.3 yr. No statistically significant difference was found between the participants in the INT and CTRL groups with regard to body measurements. Of the participants, 4.7% were affected by varicocele at recruitment and 6.7% were sporadic smokers (data not shown). Overall adherence to the Mediterranean diet was low: the majority of the participants (53.7% and 60.9% in the INT and CTRL groups, respectively) showed a "low adherence" level. In contrast, a high level of physical activity was reported by participants in both groups, indicating an active or highly active population, with only 18% of inactive students. The two groups showed no significant differences in semen parameters, except for the percentage of normal morphology cells, which was significantly higher in the INT than in the CTRL group (7.59% vs 5.76%). The mean value of the TAC was 1.06 mM, with no differences between groups.

Considering the reference values defined by the World Health Organization (WHO), a relevant proportion of participants had semen quality parameters lower than the WHO reference values (Supplementary Table 1): 20.1% and 16.3% had low volume and sperm concentrations, respectively; 44.2% and 55.2% had low total and progressive motility values, respectively; and 39% had <4% normal morphology cells.

Considering the three areas of recruitment, the participants were substantially similar with regard to general characteristics, adherence to the Mediterranean diet, and

Table 2 – Baseline characteristics of the study population

Variables	INT (mean ± SD)	CTRL (mean ± SD)	Whole cohort (mean ± SD)	*p* value[a]
No.	188	156	344	–
Age (yr)	19.3 ± 1.4	19.3 ± 1.4	19.3 ± 1.4	0.8318
Body measurements				
Weight (kg)	71.5 ± 9.1	71.7 ± 9.1	71.6 ± 9.1	0.7782
Height (cm)	176.7 ± 6.4	176.7 ± 6.1	176.7 ± 6.3	0.9912
BMI (kg/m^2)	22.8 ± 2.5	23.0 ± 2.6	22.9 ± 2.6	0.6847
Waist circumference (cm)	85.3 ± 7.4	85.6 ± 7.0	85.5 ± 7.2	0.7081
Lifestyle factors				
PREDIMED score	7.20 ± 2.28	7.08 ± 2.24	7.14 ± 2.26	0.6244
≤7	53.7%	60.9%	57.0%	0.181
>7	46.3%	39.1%	43.0%	
IPAQ score	2974.06 ± 2964.93	2524.52 ± 2428.98	2770.20 ± 2740.26	0.1300
<700	15.4%	21.2%	18.0%	0.172
700–2519	41.0%	44.2%	42.5%	
≥2520	43.6%	34.6%	39.5%	
Semen parameters				
Volume (ml)	2.74 ± 1.38	2.72 ± 1.26	2.73 ± 1.32	0.9137
Sperm concentration (10^6/ml)	55.32 ± 37.11	55.26 ± 44.41	55.29 ± 40.52	0.9886
Total motility (%)	42.88 ± 21.83	38.92 ± 23.27	41.09 ± 22.55	0.1047
Progressive motility (%)	29.27 ± 18.84	25.92 ± 18.40	27.75 ± 18.69	0.0987
Cell with normal morphology (%)	7.59 ± 6.48	5.76 ± 3.82	6.76 ± 5.51	0.0020
Round cells (10^6/ml)[b]	6.23 ± 4.56	5.98 ± 5.30	6.12 ± 4.89	0.6679
TAC (mM)[c]	1.05 ± 0.28	1.07 ± 0.24	1.06 ± 0.26	0.5295

BMI = body mass index; CTRL = control group; INT = intervention group; IPAQ = International Physical Activity Questionnaire; PREDIMED = PREvención con DIeta MEDiterránea questionnaire; SD = standard deviation; TAC = total antioxidant capacity.
Age, body measurements, lifestyle factors, and semen parameters, including spermiogram variables and TAC, are reported as mean ± SD for the intervention and control groups, and the whole cohort.
[a] *T* test for the comparison between the INT and CTRL groups for all variables except for PREDIMED and IPAQ classes, for which χ^2 was used.
[b] Participant number for round cells: INT, *N* = 158; CTRL, *N* = 123; and whole cohort, *N* = 281.
[c] Participant number for TAC analysis: INT, *N* = 154; CTRL, *N* = 139; and whole cohort, *N* = 293.

level of physical activity (Supplementary Table 2), while some differences were found in semen parameters.

3.2. *Effects of the intervention*

The intervention had substantial effects on the participants' lifestyle: adherence to the Mediterranean diet increased from t0 to t4, more in the INT group than in the CTRL group (Supplementary Table 3). Physical activity showed a similar trend, with a greater mean increase in the INT group than in the CTRL group. The comparison between the INT and CTRL groups showed no difference at baseline, but statistically significant differences at the end of the intervention between the two groups, for both the PREDIMED and the IPAQ mean score. Lastly, t0-t4 changes of the two groups were statistically significant only for the PREDIMED score (Supplementary Table 3).

The proportion of students with low adherence to the Mediterranean diet and that of inactive participants were lowered in both groups at t4, with statistically significant difference only for the PREDIMED level in the INT group (Supplementary Table 4).

The intervention induced a positive effect also on semen quality, especially on sperm concentration and motility (Table 3). Even if changes detected in the two groups separately were not statistically significant (except for progressive motility), the concentration of spermatozoa, their total and progressive motility, and the percentage of normal morphology cells increased in the INT group but decreased in the CTRL group, with statistically significant differences between the two groups at t4, whereas no differences were evident at baseline, except for the percentage of normal morphology cells. Sperm concentration of round cells, which is inversely proportional to semen quality, decreased in both groups, but the change was statistically significant only in the INT group. Accordingly, semen TAC increased in the INT group but decreased in the CTRL group, even if the difference between the two groups at t4 was not statistically significant. In addition, comparing t0-t4 changes between the INT and CTRL groups, statistically significant differences were found for total motility, progressive motility, percentage of round cells, and antioxidant capacity.

The percentage of individuals with semen parameters lower than the WHO reference values decreased in the INT group but not in the CTRL group, leading to significant differences of the proportions between the two groups at the end of the intervention (Supplementary Table 5). As a consequence, the proportion of individuals with an abnormal semen analysis according to WHO limits decreased in the INT group (from 46.0% to 41.6%) but increased in the CTRL group (from 59.5% to 69.0%; comparison at t4: $p < 0.0001$).

Finally, no association between lifestyle variables (IPAQ and PREDIMED) and semen parameters was found by applying the Spearman's correlation coefficient analysis.

Table 3 – Effect of the intervention on semen parameters, including spermiogram variables and total antioxidant capacity (TAC): comparison between intervention (n = 137) and control (n = 126) groups at baseline (t0) and at the end of the intervention (t4)

Semen parameters	Group	t0 (mean ± SD)	t4 (mean ± SD)	p value[a] INT vs CTRL (t4)	t4-t0 (95% CI)	p value[b] INT vs CTRL (t4-t0)
Volume (ml)	INT	2.84 ± 1.43	2.88 ± 1.50	0.3558	0.04 (−0.20; 0.28)	0.7122
	CTRL	2.74 ± 1.27	2.71 ± 1.52		−0.03 (−0.32; 0.25)	
Sperm concentration (10^6/ml)	INT	60.44 ± 38.61	62.72 ± 39.04	0.0278	2.28 (−3.30; 7.85)	0.2863
	CTRL	54.85 ± 44.71	52.55 ± 35.16		−2.31 (−8.75; 4.14)	
Total motility (%)	INT	43.25 ± 19.92	46.55 ± 19.82	<0.0001	3.30 (−0.36; 6.96)	0.0172
	CTRL	38.43 ± 23.45	34.98 ± 24.04		−3.45 (−7.68; 0.79)	
Progressive motility (%)	INT	29.55 ± 17.71	33.31 ± 18.19*	<0.0001	3.77 (0.40; 7.14)	0.0015
	CTRL	26.06 ± 18.61	22.34 ± 17.51*		−3.72 (−6.86; −0.58)	
Cells with normal morphology (%)	INT	7.26 ± 4.33	7.34 ± 3.88	0.0002	0.08 (−0.65; 0.81)	0.4506
	CTRL	5.89 ± 3.98	5.56 ± 3.86		−0.33 (−1.13; 0.47)	
Concentration of round cells (10^6/ml)	INT (N = 103)	6.68 ± 4.68	4.47 ± 3.86***	0.0632	−2.21 (−3.28; −1.14)	0.0441
	CTRL (N = 96)	6.33 ± 5.37	5.52 ± 4.11		−0.80 (−1.65; 0.04)	
TAC (mM)	INT (N = 103)	1.05 ± 0.26	1.07 ± 0.29	0.1043	0.03 (−0.04; 0.10)	0.0084
	CTRL (N = 94)	1.11 ± 0.21	1.00 ± 0.30**		−0.10 (−0.17; −0.04)	

CI = confidence interval; CTRL = control group; INT = intervention group; SD = standard deviation; TAC = total antioxidant capacity.
[a] p value for differences between the intervention and control groups at t4 (Student t test for unpaired data).
[b] p value for differences between t0-t4 changes in the intervention and control groups (Student t test for unpaired data).
* Statistically significant ($p < 0.05$) differences between t0 and t4 values of each group separately (Student t test for paired data).
** Statistically significant ($p < 0.01$) differences between t0 and t4 values of each group separately (Student t test for paired data).
*** Statistically significant ($p < 0.001$) differences between t0 and t4 values of each group separately (Student t test for paired data).

4. Discussion

The main findings of this randomized trial are that a lifestyle intervention based on diet and physical activity resulted in an increase of sperm concentration, total and progressive motility, and proportion of spermatozoa with normal morphology, and a decrease of round cell concentration in healthy young men. In contrast, all semen parameters worsened in the CTRL group. Overall, the proportion of individuals with abnormal semen analysis decreased in the INT group but increased in the CTRL group. Accordingly, TAC increased in the INT group and decreased in the CTRL group, but the difference between the two groups at the end of the intervention was not statistically significant. However, although TAC has widely been used as a diagnostic marker in male infertility [17] and a useful indicator of redox status alteration by environmental pollution [5], its sensitivity may not be sufficient for discriminating treatment efficacy in healthy individuals.

The comparison between the questionnaires filled in by participants at baseline and the end of the intervention confirmed significant changes in participants' dietary habits and physical activity in the INT group, and minor changes in the CTRL group.

Our intervention was based on the principles of the Mediterranean diet, which is rich in antioxidant and anti-inflammatory nutrients [18]. Our findings are in agreement with several observational studies showing a positive association between adherence to the Mediterranean diet and semen parameters [3,8,9,19].

A Spanish and a USA cross-sectional study among young university students confirmed positive associations between energy-adjusted nutrient intake of antioxidants and semen volume, concentration, motility, and morphology [20], and between a diet rich in fish, chicken, fruit, vegetables, legumes, and whole grains and sperm progressive motility [21]. The effects of diet may be explained through the antioxidant mechanisms of various nutrients, because high levels of oxidative stress are correlated with low semen quality, especially low sperm motility, and with high levels of DNA damage [22,23].

Observational epidemiological studies are prone to various types of biases; therefore, RCTs are usually considered to provide the best evidence of a putative association between a protective factor and health. Several intervention studies have been performed on the effect on semen quality parameters of administration of nutrients and dietary supplements, without changes in dietary habits: a recent systematic review of 28 RCTs concluded that some dietary supplements could positively influence semen parameters, although limited sample size and high heterogeneity among the studies did not allow drawing of firm conclusions [24]. A Cochrane systematic review of RCTs concluded that antioxidant supplementation in subfertile males may improve live birth rates for couples attending fertility clinics, although current evidence is inconclusive due to the low quality of trials performed so far [25].

To our knowledge, our study is the first RCT to evaluate the effects of a dietary and physical activity intervention on semen quality of healthy young men. The participants enrolled in the INT group modified their habits, on average, in a relatively short time (4 mo), compared with those in the CTRL group, who received only general recommendations on a correct lifestyle. The latter approach was considered equivalent to a "placebo" as it has not been found to modify individuals' habits according to the literature. Indeed, in our study, the control individuals showed only a modest improvement in the Mediterranean dietary score (PREDIMED) and physical activity index (IPAQ).

Apart from dietary habits and physical activity, various potentially avoidable risk factors for semen quality have been identified, including lifestyle factors, such as tobacco, alcohol and drug use, psychological stress, obesity, insufficient sleep, and others, and environmental factors, particularly chemical pollutants, such as air pollutants, endocrine disruptors, heavy metals, and others. The potential impact of environmental pollution on semen quality represents the reason why this study enrolled participants living in highly polluted areas: according to literature, residents in more polluted areas have worse health parameters, including semen quality, on average, than those living in less polluted areas [3,5] and therefore might be expected to achieve better results from a lifestyle intervention. Moreover, it has been suggested that the activity of various phytochemical compounds, which the Mediterranean diet is rich in may counteract the negative effects of environmental pollutants [26,27].

Some of the abovementioned factors could also explain differences at recruitment among individuals living in the three areas and the general worsening of semen parameters from t0 to t4 in the CTRL group.

This multicenter RCT has some strengths, including randomization of participants and the use of multiple biological measures along with international standardized questionnaires. However, it has some limitations, too. First of all, the number of participants, although relatively high with respect to other dietary RCTs, was not large enough to detect statistically significant differences between groups for some outcome variables due to difficulty in the recruitment of students, who were all volunteers and received no reward apart from some food samples. Second, the study design included only a short-term evaluation of the effects of the intervention; therefore, we cannot say whether students with improvement of their semen quality will maintain these results in the future. Third, a per-protocol analysis, including only the participants with semen parameter data at both t0 and t4, was performed. Even if an intention-to-treat analysis, including also individuals lost to follow-up, is usually performed in an RCT, such an analysis could not be performed because the participants who withdrew from the study did not undergo the final clinical visit.

This study investigated the impact of a healthy lifestyle (diet and physical activity) on semen quality by measuring sperm parameters and antioxidant capacity, which surely cannot define fertility potential of individuals. The observed changes in semen parameters were low and not clinically relevant. However, our results deserve consideration for various reasons. First, an improvement in semen parameters has been detected even if the lifestyle intervention lasted only 4 mo. We could speculate that longer and more stable lifestyle changes can also improve more semen quality. Second, the aim of our study was not the care of infertile people but the assessment of the effects of a lifestyle intervention on sperm quality of young, healthy, normal-weight men with a good level of physical activity. Therefore, the expected slight improvement in their sperm quality due to the active intervention may be of high relevance in a larger perspective, concerning the general population.

5. Conclusions

In conclusion, this study has shown positive effects of a moderate short-term lifestyle intervention, based on the application of the Mediterranean diet principles and regular physical activity, on semen quality in healthy young men. Although no long-term results are available, these findings suggest that lifestyle interventions can be applied successfully in healthy young men for protecting, and eventually ameliorating, their semen quality.

Author contributions: Luigi Montano had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Study concept and design: Montano, Donato, Lorenzetti, Notari, Ubaldi.
Acquisition of data: Ceretti, Bergamo, Viola, Pappalardo, D. Zani.
Analysis and interpretation of data: Ceretti, Donato, Bollati, Consales.
Drafting of the manuscript: Montano, Ceretti, Donato, Lorenzetti.
Critical revision of the manuscript for important intellectual content: Bergamo, C. Zani, Viola, Notari, Pappalardo, D. Zani, Ubaldi, Bollati, Consales, Leter, Trifuoggi, Amoresano.
Statistical analysis: Ceretti, C. Zani, Donato.
Obtaining funding: Montano.
Administrative, technical, or material support: Bollati, Consales, Leter, Trifuoggi, Amoresano.
Supervision: Notari, Ubaldi.
Other: None.

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chapter 3174, title of the project: "Un modello di intervento per la prevenzione dell' infertilità in adolescenti sani residenti in aree a forte impatto ambientale"). Moreover, the study was supported by some companies that supplied food products for the intervention group. Funders and supporters had no role in study design, interpretation of the data, or preparation of this manuscript. The EcoFoodFertility association covered publication cost for the article independent of its content.

Ethics statement: This study was conducted according to the guidelines laid down in the Declaration of Helsinki. The protocol was approved by the ethics committees of Southern Campania (29 November 2017) and by that of Brescia Province (13 March 2018), and accepted by the Italian National Institute of Health (20 December 2017).

Data sharing: All the data are available on request to the corresponding author (l.montano@aslsalerno.it). The results of the research will be disseminated to students and teachers of the participating institutions, communication to mass-media, and through peer-reviewed journals and scientific presentations.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.euf.2021.01.017.

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