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Title:

Physical activity, sedentary time, and sperm DNA fragmentation index in healthy Japanese men

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Abstract

Research Question: Is physical activity (PA) or sedentary time (ST) associated with the DNA fragmentation index (DFI) and conventional semen parameters in healthy Japanese men?

Design: We utilized baseline data from the Preconception Research and Education Study for Men, an ongoing randomized controlled trial, to assess the long-term effects of preconception health education on male fertility. This cross-sectional analysis included 323 men aged 20–39 years recruited from September 2021 to December 2022 at workplace health checkup sites in Japan. Demographic, lifestyle, and health checkup data were collected, and PA and ST were measured using the International Physical Activity Questionnaire-Short Form. Semen quality was assessed with DFI and conventional semen analyses. Generalized linear models were used to evaluate the associations between PA, ST, and semen parameters, adjusting for confounders.

Results: Higher total PA was associated with a lower DFI (P for trend=0.002), with the third and fourth total PA quartiles showing a 21% ($P=0.013$, 95% CI: 5%–34%) and a 29% ($P=0.002$, 95% CI: 12%–43%) reduction in DFI from the first quartile reference, respectively. Shorter ST was associated with a lower DFI (P for trend=0.057), with the first ST quartile showing a 22% reduction in DFI from the fourth quartile reference ($P=0.024$, 95% CI: 3%–37%). There were no significant associations between total PA, ST, and conventional semen parameters.

Conclusions: Higher PA and shorter ST are associated with a lower DFI in Japanese men. However, these associations were not observed with conventional semen parameters.

Keywords: fertility, semen quality, physical activity, sedentary time, DNA fragmentation index.

Introduction

Globally, infertility affects many couples. Pooled estimates of lifetime and period prevalence of 12-month infertility from 1990 to 2021 are reported to be 17.5% and 12.6%, respectively (Cox et al., 2022). Male factors are the primary or contributing cause in approximately 50% of infertility cases (Agarwal et al., 2021). Recent meta-regression models indicate a global decline in total sperm count and sperm concentration, which has accelerated over the last two decades (Levine et al., 2023). Although the causal pathway of this decline in semen quality is not fully understood, previous studies have identified a variety of risk factors that possibly influence semen quality, such as adverse environmental factors (Kumar and Singh, 2022), poor self-rated health (Liu et al., 2024), unhealthy diet (Salas-Huetos et al., 2017), and various lifestyle risk factors, including reduced physical activity (PA) and prolonged sedentary time (ST) (Gaskins et al., 2014).

The impact of PA and ST on overall health has been frequently reported in epidemiological studies. PA offers benefits in preventing several chronic diseases (e.g., cardiovascular disease, diabetes, cancer, hypertension, obesity, depression, and osteoporosis) and premature death (Warburton and Bredin, 2017). In contrast, a long-time sedentary behaviour increases the risk for all-cause, cardiovascular, and cancer mortality (Dempsey et al., 2020). On the other hand, epidemiological findings on the associations between PA, ST, and semen quality are inconsistent. A recent systematic review suggested that recreational PA may have a positive effect on sperm concentration or progressive motility. However, the authors did not perform a meta-analysis due to limited data and high heterogeneity between studies (Ibañez-Perez et al., 2019). Some studies report no significant association between ST and sperm parameters, including sperm concentration, total sperm count, and motility (Støy et al., 2004; Sun et al., 2019) or morphology (Støy et al., 2004). In contrast, others indicate that high levels of ST are associated with lower sperm counts and concentrations (Magnusdottir et al., 2005; Priskorn et al., 2016).

Conventional semen analyses, which include sperm concentration, total sperm count, total motility, and progressive motility, are widely used and considered the gold standard for measuring semen quality. However, these have remarkable limitations. In addition to the considerable intra-individual variation (Ba, 2006), the conventional semen parameters do not reveal sperm functional

competence and reproductive potential (Okubo et al., 2023), as their association with pregnancy outcomes has shown mixed results (Keihani et al., 2021; Punjabi et al., 2021; Slama et al., 2002). Among the various sperm function tests developed to predict male fertility more efficiently (Wang and Swerdloff, 2014), the sperm DNA fragmentation index (DFI) test has become widely used as a complementary diagnostic tool in clinical settings (World Health Organization, 2021). The DFI test assesses the integrity and damage of sperm DNA, which can directly impact sperm's physiological functions and potentially transmit incorrect genetic information to offspring, making the DFI test useful for predicting fertilisation success and subsequent reproductive outcomes (Liu et al., 2023). Higher sperm DFI is correlated with impaired reproductive outcomes, including poor embryo quality, reduced implantation success, and increased rates of miscarriage (Borges et al., 2019). Recent research emphasizes the importance of including DFI in fertility assessments, as it is known to influence not only the ability to conceive but also miscarriage rates and birth weight (Li et al., 2024). However, its use in epidemiological research, particularly among the general population, remains limited (Szabó et al., 2023), and its association with PA and ST is still unclear.

Therefore, using baseline data from an ongoing, open-label, randomized controlled trial to assess the long-term effects of preconception health education on male fertility, we examined the association between PA, ST, and semen quality assessed by conventional semen analysis and DFI among healthy Japanese men.

Materials and Methods

Study participants

We used the Preconception Research and Education Study for Men (PRES-M) baseline data. The PRES-M is an ongoing, open-label, randomized controlled trial (UMIN Clinical Trials Registry UMIN000045389) that assesses the long-term effects of preconception health education on male fertility (Maeda et al., 2024). The recruitment period was from September 2021 to December 2022.

Participants were recruited at six types of institutions (i.e., universities, police offices, bank offices, hospitals, local governments, and clinical testing companies) in Akita Prefecture, Japan, during annual health checkups for regular employees required by the Occupational Safety and Health Act

in Japan. Inclusion criteria included male employees aged between 20 and 39, hoping to have children (more children) in the future. Those who understood the informed consent documents for this research in Japanese and voluntarily agreed to participate in the PRES-M provided their baseline semen sample, results of annual health checkups, and responses to a self-report questionnaire. Those who completed the baseline survey were given a gift card worth 4000 Japanese yen (JPY, approximately 36 USD in September 2021).

Physical and sedentary activity

We used the International Physical Activity Questionnaire-Short Form (IPAQ-SF) to evaluate physical and sedentary activity (Craig et al., 2003). The IPAQ-SF assesses PA during leisure time, domestic and gardening, work-related, and transport activities. Participants reported average minutes per day and number of days per week for vigorous-intensity activity, moderate-intensity activity, and walking, as well as average daily ST. The metabolic equivalent (MET)-minutes/week for each PA level was calculated by multiplying total minutes by the specific MET score for each activity, with 8 METs for vigorous PA, 4 METs for moderate PA, and 3.3 METs for walking (IPAQ Research Committee, 2005). The total PA (MET-minutes/week) for each participant was then calculated by summing up the MET-minutes/week for each category of PA. Participants were grouped into quartiles according to total PA and ST, respectively.

Semen collection and analysis

Participants were asked to provide semen samples after a recommended abstinence period of 2–5 days and a recommended delivery time to the laboratory of less than 2 hours. Samples were obtained by masturbation at home, collected in sterile containers, and allowed to liquefy at room temperature. The sterile containers used in this study (Transporter-S, Mitsuboshi Product Planning Co., Ltd., Tokyo, Japan) have less air exposure and greater liquid stability than conventional containers (Iwahata et al., 2019).

In accordance with the World Health Organization laboratory manual for semen analysis 6th ed (World Health Organization, 2021b), the ejaculate volume was estimated by sample weight. The

sperm motility analysing system (SMAS, DITECT Co., Ltd., Tokyo, Japan), a computer-assisted semen analyser (Komori et al., 2006), measured sperm concentration and sperm motility (progressive and non-progressive motility). Following the semen analyses using the SMAS, the samples were flash frozen in liquid nitrogen and preserved at -80°C . Frozen semen samples were thawed in a water bath at 35°C . The sperm DFI was measured using an in-house flow cytometric analysis (Osaka et al., 2022) based on the sperm chromatin structure assay (Evenson et al., 1999). A 10 μL semen sample, regardless of the sperm concentration, was diluted with Tris-NaCl-EDTA buffer (90 μL). The mixture was treated with 200 μL of pH 1.2 buffer for 30 seconds to denature the DNA at strand breaks. Then, 600 μL of acridine orange staining solution (0.0006% v/v in phosphate-citrate buffer) was added, and the sample was analysed using a CYTOFLEX flow cytometer equipped with CytExpert version 2.3 software (Beckman Coulter, Inc., Atlanta, GA, USA). Each sample was measured independently twice, with a minimum of 20,000 events analysed per measurement. The emission of red fluorescence from single-stranded DNA fragments bound to acridine orange compared to the green fluorescence emitted from intact double-stranded DNA bound to acridine orange, was used to calculate the proportion of sperm with red fluorescence within the total sperm population, thereby determining the sperm DFI. Flow cytometric data were processed using specialized software (Beckman Coulter, Inc.).

Sociodemographic and lifestyle variables

Participants completed self-report questionnaires, including age, university education, occupation, annual household income, marriage, having a child, body mass index (BMI), sleeping duration, smoking, drinking, and sauna habits. The occupations were divided into three categories: medical staff (i.e., those working for a hospital or clinical testing company), police officers, and office workers (i.e., those working for a university, bank, or local government).

We obtained the results of annual health checkups. We identified abnormalities based on the 2022 edition of the Judgment Criteria for Complete Medical Checkups (Japan Society of Ningen Dock and Preventive Medical Care, 2022), widely used for occupational health checkups in Japan. Blood pressure was flagged as abnormal when the systolic blood pressure was ≥ 140 mmHg or the diastolic

blood pressure was ≥ 90 mmHg. Glucose tolerance was flagged as abnormal when the Hemoglobin A1c was $\geq 6\%$, according to the National Glycohemoglobin Standardization Program. Lipid metabolism was flagged as abnormal when triglyceride levels were ≥ 300 mg/dL, high-density lipoprotein cholesterol was ≤ 34 mg/dL, or low-density lipoprotein cholesterol was ≥ 140 mg/dL. Liver function was flagged as abnormal when aspartate aminotransferase or alanine aminotransferase levels were ≥ 51 IU/L or gamma-glutamyl transferase levels were ≥ 101 IU/L. Participants were categorized as having abnormal test results if any of the above four items (blood pressure, blood sugar, blood lipids, and liver function) were abnormal.

Statistical Analysis

Continuous variables are presented as the mean and standard deviation (SD) or median (range or interquartile range [IQR]), and dichotomous and categorical variables are presented as counts and proportions. All missing values and outliers were treated according to the IPAQ-SF data processing and analysis guidelines (IPAQ Research Committee, 2005).

We described the distributions of sociodemographic, lifestyle, and semen sampling conditions by total PA or ST quartiles. We compared them using Chi-square tests, Fisher's exact test, or the Kruskal–Wallis test, where appropriate. We also described the distributions of semen parameters by total PA or ST quartiles.

Generalized linear models with Gaussian distribution and log-link function were employed to examine the crude and adjusted exponential of coefficients between the total PA or ST and semen parameters. Model assumptions, including the normality of residuals and homoscedasticity, were assessed, and robust standard errors were applied in the generalized linear models of the total and progressive motility due to the detection of heteroscedasticity. For multivariable models, the following covariates were selected as possible confounders or predictors of the outcome according to the previous findings (Szabó et al., 2023; Eisenberg et al., 2015; Donato et al., 2025; Le et al., 2020), age (20–24; 25–29; 30–34; 35–40 years), BMI (kg/m^2), annual household income (<2 million JPY; 2 to <6 million JPY; ≥ 6 million JPY; unknown), sleeping duration (minute/day), smoking (current smoker; former smoker; non-smoker), sauna habits (yes; no), abstinence days (days), sampling

season (spring; summer; autumn or winter), semen test time after sampling (minutes), and abnormal checkup results (yes; no). Annual household income was included as a possible confounder that can influence physical activity (Chastin et al., 2020) and semen quality (Janevic et al., 2014). Since the sample size of the underweight category (i.e. BMI < 18.5) was small, BMI was adjusted using a continuous variable.

Model 1 assessed the association between total PA or ST and semen parameters separately, while Model 2 included total PA and ST simultaneously. Using sensitivity analyses, we further evaluated the association between total PA, ST, and semen parameters in participants who strictly followed the guided abstinence period of 2–5 days.

Finally, a Spearman correlation analysis explored the relationship between conventional semen parameters and DFI. All the statistical analyses were conducted using SAS 9.4 (SAS Institute, Cary, NC). *P*-values less than 0.05 were considered significant.

Ethical approval

The study was conducted according to the Declaration of Helsinki for medical research involving human subjects. The ethics committees at Akita University Graduate School of Medicine and Hokkaido University approved the study protocol on July 30, 2021 (no. 2689) and January 22, 2024 (SEI 023-0136), respectively. All the participants provided written informed consent for all study procedures, including the use of annual health checkup data.

Results

A total of 382 men were enrolled in the PRES-M trial. After excluding those who withdrew consent (n=2), did not meet the inclusion criteria of age >39 years (n=1), did not respond to a self-report questionnaire (n=16), provided incomplete or unreasonable answers to the IPAQ-SF (n=23), had missing values on any covariates (smoking habits n=4, sauna habits n=1, total n=5), did not provide a semen sample after 2 to 7 days of abstinence (n=4), had missing data on semen parameters (n=5), or had semen tested more than 12 hours after collection (n=3), 323 participants were eligible for the present study (Fig. 1).

Table 1 displays the characteristics of the participant and semen sampling conditions by total PA quartiles. Of the 323 participants, the mean (SD) age was 30.4 (4.7) years, 47.1% were office workers, 65.0% had university education, 34.1% had an income of 2 to <6 million yen per year, 31.9% were overweight or obese (BMI ≥ 25.0 kg/m²), 60.1% were non-smokers, 23.2% had drinking habits with ≥ 20 g alcohol per day, and 15.8% had a habit of using a sauna or a long bath. The mean (SD) interval between semen collection and testing was 140.0 (72.3) minutes despite a recommended delivery time from collection to the laboratory of less than 2 hours. Participants with a higher total PA presented a significantly shorter ST ($P=0.003$). Participants in Q4 (most active) were younger ($P=0.017$) and less likely to be married ($P=0.017$) than those in other quartiles, whereas there were no significant differences among PA quartiles in other sociodemographic and lifestyle factors or semen sampling conditions. When assessed in ST quartiles, those with a longer ST were more likely to be office workers and had a longer interval between semen collection and testing (Supplementary Table S1).

The median (IQR) of the semen parameters for all participants were as follows: DFI, 7.0% (4.4%–10.0%); sperm concentration, $70.3 \times 10^6/\text{mL}$ ($41.7 \times 10^6/\text{mL}$ – $124.8 \times 10^6/\text{mL}$); total sperm count, 257.6×10^6 (129.3×10^6 – 418.5×10^6); total motility 71.1%, (58.2%–81.2%); and progressive motility 59.2% (46.9%–69.6%). Participants with a higher total PA or shorter ST had a lower median DFI (Table 2). Most participants had conventional semen parameters above the lower limit of the WHO reference range (World Health Organization, 2021b; Campbell et al., 2021) and a DFI below the cut-off value for infertility (Osaka et al., 2022) (Supplementary Table S2). The DFI cut-off value for differentiating fecundability among Japanese men is 24% (Osaka et al., 2022), and in our study, only 1.5% of participants exceeded this threshold.

Generalized linear models showed that higher total PA was associated with a lower DFI in all three models (all P for trend < 0.05 , Table 3). In Model 1, the third and fourth total PA quartiles had a 21% ($P=0.013$, 95% CI: 5%–34%) and 29% ($P=0.002$, 95% CI: 12%–43%) reduction in DFI from the first quartile reference, respectively. There were no significant associations between total PA and sperm concentration, total sperm count, total motility, or progressive motility.

A shorter ST was associated with a lower DFI in Model 1 (P for trend=0.057, Table 3). In Model 1, the first ST quartile had a 22% reduction in DFI from the fourth quartile reference ($P=0.024$, 95% CI: 3%–37%). Similar associations were observed in both the crude model and Model 2, but did not reach statistical significance. There were no significant associations between ST and sperm concentration, total sperm count, total motility, or progressive motility.

The sensitivity analysis among the participants with abstinence periods ranging from 2 to 5 days ($n=311$, Supplementary Table S3) showed similar results of decreasing trends in DFI across the total PA (Model 1: P for trend=0.027) and ST quartiles (Model 1: P for trend=0.016; Model 2: P for trend=0.043), respectively. No significant associations were found between other semen parameters, total PA, and ST.

Spearman correlation analysis indicated that DFI negatively correlated with total motility ($r_s=-0.376$, $P<0.0001$) and progressive motility ($r_s=-0.360$, $P<0.001$). However, the correlations between DFI and sperm concentration and between DFI and total sperm count were low ($r_s=-0.199$, $P=0.0005$) and null ($r_s=-0.038$, $P=0.491$), respectively (Supplementary Table S4).

Discussion

Our study found that higher levels of PA and shorter ST were associated with lower DFI, but not conventional semen parameters, among 323 healthy Japanese men. Participants in the fourth total PA quartile (most active) showed a 29% reduction in DFI compared to those in the first total PA quartile (least active), and those in the first ST quartile (shortest ST) showed a 22% reduction in DFI compared to those in the fourth ST quartile (longest ST). However, DFI values in our study were generally within the normal range, and the magnitude of these differences was relatively small. Further studies are needed to determine whether these small variations in semen parameters have meaningful implications for male fertility or reproductive outcomes.

The linear association between higher PA and lower DFI presented in this study adds new evidence to the existing literature. Previous findings regarding PA and semen quality are mixed and complex (Sadri et al., 2025). A systematic review showed that moderate- or high-intensity PA may have a positive effect on semen quality, except PA in elite athletes. However, the review

acknowledged considerable heterogeneity between studies (Ibañez-Perez et al., 2019). The conventional semen parameters significantly associated with PA varied between studies, such as sperm concentration and counts (Priskorn et al., 2016) and progressive motility and morphology (Vaamonde et al., 2012). Similarly, the most recent survey of 143 healthy young men demonstrated a positive association between total sperm motility and normal morphology with moderate PA levels (corresponding to Q3 in our sample), but no significant association with sperm concentration (Donato et al., 2025). Furthermore, consistent with this study's findings, no significant association between PA and conventional semen parameters was found in a cross-sectional study involving 215 university students (Mínguez-Alarcón et al., 2014) and a case-control study involving 207 sperm donors (Matorras et al., 2022). Inconsistent findings regarding conventional semen parameters may be due to biological intra-individual variability, sperm dysfunction not tested routinely, and poor standardization in semen analysis among laboratories (Esteves, 2014). In contrast, the DFI exhibits significantly lower biological variability (<9%) compared to the 28%–43% variability observed in conventional semen parameters across consecutive ejaculates (Esteves et al., 2021).

In this study, participants with the shortest ST (i.e., 270 minutes or less per day) exhibited a 22% reduction in DFI compared to those with the longest ST (i.e., 630 minutes or more per day). However, no association was observed with conventional semen parameters. Our findings align with a Polish study that reported no significant differences in the conventional semen parameters between groups spending <50% or ≥50% of their worktime in a sedentary position. However, that study did find a significant difference in DFI (Gill et al., 2019). Similarly, two other studies that did not assess DFI (Støy et al., 2004; Sun et al., 2019) concluded that ST was not associated with conventional semen parameters. Conversely, another study revealed that reduced television watching was significantly associated with higher total sperm count and sperm concentration (Gaskins et al., 2015), while additional studies have suggested a relationship between sedentary behaviour and declining semen quality (Magnusdottir et al., 2005; Priskorn et al., 2016). Given these inconsistencies, the complementary use of DFI testing in epidemiological studies, as demonstrated in our study, may provide further insights into the relationship between ST and semen quality.

Although the results of our study did not establish causal relationships in which higher PA levels or a shorter ST result in less sperm DNA damage, several studies have suggested the mechanisms of how PA and ST can affect semen quality. One mechanism is oxidative stress regulation. A systematic review of the effects of high-intensity exercise on oxidative stress found that physically active humans had better antioxidant systems than physically inactive humans (Lu et al., 2021). Oxidative stress refers to the production of free radicals in the body exceeding the clearance capacity of the antioxidant system, which not only damages spermatogenic cells and sperm but also impairs Sertoli cells and Leydig cells in the testes, affecting spermatogenesis (Simioni et al., 2018; Ye et al., 2021). Routine PA leads to the production of reactive oxygen species and reactive nitrogen species in the skeletal muscle while increasing the production of endogenous antioxidant enzymes (Meng and Su, 2024). A pilot study of infertile men showed that a three-month lifestyle intervention program, including diet and exercise, combined with oral antioxidant therapy intake reduced sperm DFI (Humaidan et al., 2021). Another mechanism is endocrine regulation, specifically testosterone. A study of men with idiopathic infertility demonstrated that follicle-stimulating hormone administration reduced sperm DFI by approximately 20%, with a significant inverse correlation between DFI and serum testosterone levels (Lispi et al., 2022), suggesting a positive effect of testosterone in reducing sperm DNA damage. A meta-analysis found that testosterone levels increased transiently after moderate- and high-intensity exercises (D'Andrea et al., 2020). Finally, testicular temperature is another potential mechanism linking shorter ST and DNA integrity. A study monitoring scrotal temperature over 24 hours revealed that the temperature decreases when moving and increases when sitting (Garolla et al., 2015). Sitting on a commonly used chair raises scrotal temperature by up to 3°C within 20 minutes (Koskelo et al., 2005). Since semen quality, including DNA integrity, deteriorates after exposure to heat, such as hot water baths (Rao et al., 2016; Sergerie et al., 2007), sedentary behaviour may negatively impact semen quality by increasing scrotal temperature. Heat stress induces the production of reactive oxygen species, activating the mitochondrial apoptotic pathway (Li et al., 2017).

The present study also revealed a significant correlation between DFI and sperm motility parameters, but not quantity parameters. Some studies have reported inverse correlations between

DFI and sperm motility, rather than semen volume or sperm counts (Zhang et al., 2021; Le et al., 2019; Evgeni et al., 2015). Still, others failed to find any association between DFI and conventional semen parameters (Khalili et al., 2006). Given that our study found a significant association between PA, ST, and DFI, but not between PA, ST, and conventional semen parameters, we suggest that conventional semen analysis and DFI should be used as complementary diagnostics to provide a more thorough evaluation of semen quality.

Our study would be strengthened by including semen data of a healthy Japanese population, not those of patients in fertility clinics. Due to a lack of large-scale sperm banks in Japan, semen data from fertile Japanese men is limited (Iwamoto et al., 2006). The median values for sperm concentration, total sperm count, total motility, and progressive motility of the present participants in any total PA or ST quartiles fell among the 50th to 75th percentile range of the global fertile population (Campbell et al., 2021). Although our participants were not randomly selected, it is essential to note that the sperm quantity and quality of Japanese men were comparable to those observed in other countries.

Although the magnitude of the effect on DFI is small, our results suggest that, even among a seemingly fertile population with good semen quality, sperm DNA integrity could be affected by PA and ST levels. The findings from our study hold significant public health implications, not only due to the rising global prevalence of infertility but also in response to the growing issue of physical inactivity. Physical inactivity is now recognized as the fourth leading risk factor for global mortality, and physical inactivity levels are rising in many countries with significant implications for the prevalence of noncommunicable diseases and the overall health of populations worldwide (World Health Organization, 2010). The 2020 World Health Organization guidelines (Bull et al., 2020; World Health Organization, 2020) recommend 150–300 minutes of moderate-intensity or 75–150 minutes of vigorous-intensity PA per week, or an equivalent combination. They recommend more than 300 minutes of moderate-intensity or 150 minutes of vigorous weekly activity for additional health benefits. Notably, our participants in the third and fourth total PA quartiles—who showed a significant reduction in DFI—fell within this additional recommendation level (>300 minutes of moderate-intensity activity or an equivalent combination). The association between higher PA,

lower ST, and improved sperm DNA integrity demonstrated in our study reinforces existing evidence that PA may serve as a preventive measure for reproductive health.

Several limitations in our study should be acknowledged. First, our participants consisted mainly of employees from large corporations, universities, banks, and government departments, and their PA levels were considerably lower than those reported in other studies (Mínguez-Alarcón et al., 2014; Sun et al., 2019), which may limit the generalizability of our findings to a broader population. Additionally, the semen parameters of our study participants indicated generally good semen quality (Supplementary table 2). This may limit the applicability of our findings to populations with poorer semen quality, where the results might differ. Further studies with participants from various occupations or with poorer semen quality are needed to confirm our findings. Second, each participant provided a single semen sample, which may not capture the variability in semen quality. Third, semen samples were collected at home. Although we requested participants to submit their samples within 2 hours of collection for immediate testing, the actual mean (SD) semen test time was 140.0 (72.3) minutes. Additionally, the semen test time after sampling was more extended among those with longer ST. Although we adjusted for this factor in our models, onsite semen collection could have minimised any potential variations caused by differences in collecting conditions. Fourth, sperm morphology was not assessed in this study. Although sperm morphology is a common test for male fertility, its analytical reliability, clinical validity, and utility remain controversial (Gatimel et al., 2017). Given the difficulty of standardization due to the subjective nature of this assessment (Agarwal et al., 2022), we prioritized the evaluation of sperm concentration, total sperm count, motility, and DFI to maximize the accuracy of semen quality measurement. However, semen quality is only a surrogate indicator of male fertility. The direct relationship between PA and ST with reproductive outcomes should be further explored. Finally, our study adopted a cross-sectional design and thus, could not establish causal relationships. Future longitudinal analyses that track changes over time would provide a more comprehensive understanding of the relationship between PA, ST, and semen quality.

Conclusion

This study demonstrated that Japanese men who engage in higher levels of PA and have a shorter ST are associated with less sperm DNA damage. However, these associations were not observed with conventional semen parameters, such as sperm concentration, total sperm count, total motility, or progressive motility. These findings underscore the potential benefits of increasing PA and reducing ST for improving semen quality, specifically DNA integrity. Further research is needed to investigate these associations in more diverse populations and to measure their impact for future reproductive outcomes.

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Conflict of Interest

None to declare.

During the preparation of this work the authors used ChatGPT [<https://chat.openai.com/>] and DeepL Write [DeepL.com] only to improve the language and readability of this paper, strictly following the Elsevier generative AI policies. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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Tables

Table 1. Participant characteristics and semen sampling conditions by quartiles of total physical activity (MET-minutes/week).

	Total	Q1 (least active)	Q2	Q3	Q4 (most active)	P value ^a
	N=323	n=80	n=81	n=82	n=80	
Total PA (MET-minutes/week), median (range)	1025 (0–11466)	198 (0–450)	662 (459–1020)	1501 (1025–2079)	3420 (2100–11466)	
ST (mins/day), mean±SD	465.6±244.5	493.7±258.6	522.2±230.6	461.0±255.9	384.9±212.2	0.003
Age (years), n (%)						0.017
mean±SD	30.4±4.7	31.3±4.4	30.5±4.5	31.1±4.7	28.8±4.8	
20–24	45 (13.9)	5 (6.3)	9 (11.1)	9 (11.0)	22 (27.5)	
25–29	95 (29.4)	22 (27.5)	25 (30.9)	25 (30.5)	23 (28.8)	
30–34	113 (35.0)	30 (37.5)	30 (37.0)	29 (35.4)	24 (30.0)	
35–39	70 (21.7)	23 (28.8)	17 (21.0)	19 (23.2)	11 (13.8)	
University education, n (%)	210 (65.0)	49 (61.3)	57 (70.4)	52 (63.4)	52 (65.0)	0.195
Occupation, n (%)						0.279
Medical staff	51 (15.8)	15 (18.8)	9 (11.1)	10 (12.2)	17 (21.3)	
Police officer	120 (37.2)	30 (37.5)	29 (35.8)	28 (34.2)	33 (41.3)	
Office worker	152 (47.1)	35 (43.8)	43 (53.1)	44 (53.7)	30 (37.5)	
Annual household income (millions JPY), n (%)						0.361
<2	74 (22.9)	15 (18.8)	22 (27.2)	14 (17.1)	23 (28.8)	
2 to <6	110 (34.1)	24 (30.0)	26 (32.1)	32 (39.0)	28 (35.0)	
≥6	78 (24.2)	24 (30.0)	22 (27.2)	17 (20.7)	15 (18.8)	
Unknown	61 (18.9)	17 (21.3)	11 (13.6)	19 (23.2)	14 (17.5)	
Marriage, n (%)						0.017
Married	192 (59.4)	56 (70.0)	45 (55.6)	55 (67.1)	36 (45.0)	
Unmarried (having a partner)	43 (13.3)	5 (6.3)	12 (14.8)	9 (11.0)	17 (21.3)	
Unmarried (no partner)	87 (26.9)	18 (22.5)	24 (29.6)	18 (22.0)	27 (33.8)	
Having a child, n (%)	136(42.1)	39(48.8)	32(39.5)	40(48.8)	25(31.3)	0.066
BMI (kg/m²), n (%)						0.670
mean±SD	24.1±3.9	24.4±4.2	23.2±4.0	24.5±3.9	24.2±3.2	
<18.5	11 (3.4)	3 (3.8)	5 (6.2)	2 (2.4)	1 (1.3)	
18.5 to <25.0	209 (64.7)	52 (65.0)	53 (65.4)	50 (61.0)	54 (67.5)	
25.0 to <30.0	80 (24.8)	18 (22.5)	18 (22.2)	22 (26.8)	22 (27.5)	
≥30.0	23 (7.1)	7 (8.8)	5 (6.2)	8 (9.8)	3 (3.8)	
Abnormal checkup results^b, n (%)	112(34.7)	28(35.0)	27(33.3)	28(34.2)	29(36.3)	0.983
Sleeping duration (minute/day), mean±SD	387.2±48.3	387.8±51.4	389.5±42.9	392.7±48.7	378.8±49.5	0.296
Smoking, n (%)						0.791
Current smoker	71 (22.0)	21 (26.3)	16 (19.8)	18 (22.0)	16 (20.0)	
Former smoker	58 (18.0)	15 (18.8)	11 (13.6)	16 (19.5)	16 (20.0)	

Non-smoker	194 (60.1)	44 (55.0)	54 (66.7)	48 (58.5)	48 (60.0)	
Drinking (g, alcohol/day), n (%)						0.092
None	62 (19.2)	9 (11.3)	15 (18.5)	20 (24.4)	0 (22.5)	
0.1 to <10.0	123 (38.1)	25 (31.3)	33 (40.7)	34 (41.5)	31 (38.8)	
10.0 to <20.0	45 (13.9)	12 (15.0)	16 (19.8)	7 (8.5)	10 (12.5)	
≥20.0	75 (23.2)	27 (33.8)	14 (17.3)	18 (22.0)	16 (20.0)	
Sauna habits, n (%)	51 (15.8)	10 (12.5)	12 (14.8)	15 (18.3)	14 (17.5)	0.736
Abstinence (days), mean±SD	3.2±1.3	3.2±1.2	3.2±1.3	3.4±1.4	3.2±1.3	0.843
Sampling season^c, n (%)						0.661
Spring	140 (43.3)	38 (47.5)	36 (44.4)	32 (39.0)	34 (42.5)	
Summer	160 (49.5)	37 (46.3)	40 (49.4)	46 (56.1)	37 (46.3)	
Autumn & Winter	23 (7.1)	5 (6.3)	5 (6.2)	4 (4.9)	9 (11.3)	
Semen test time (minutes), mean±SD	140.0±72.3	139.7±76.3	141.4±67.5	139.8±71.7	139.0±75.0	0.936

BMI, body mass index; MET, metabolic equivalent; SD, standard deviation; ST, sedentary time.

^a For continuous variables, Kruskal-Wallis test was used to test for associations across quartiles of total physical activity. For categorical variables, Chi-squared test or Fisher's exact test (when one or more cell counts were ≤5) was used to test the associations between quartiles of total physical activity.

^b Abnormal checkup results refer to any abnormal results in blood pressure, blood sugar, blood lipids, or liver function.

^c Spring (April, May, and June), summer (July, August, and September), and autumn & winter (October, November, December, January, February, and March).

Table 2. Semen quality parameters (median, interquartile range) by quartiles of total physical activity and sedentary time.

Total physical activity	Q1 (least active) n=80	Q2 n=81	Q3 n=82	Q4 (most active) n=80
DFI, %	7.6 (5.2–10.1)	7.5 (4.8–11.2)	6.5 (4.0–9.8)	6.1 (4.4–8.3)
Sperm concentration, 10 ⁶ /mL	66.6 (45.4–128.0)	65.7 (52.1–105.3)	79.8 (32.8–142.5)	68.0 (40.8–114.7)
Total sperm count, 10 ⁶	249.6 (118.8–417.6)	268.9 (158.4–416.3)	260.3 (108.2–408.7)	259.1 (109.8–435.5)
Total motility, %	70.0 (58.2–78.8)	70.5 (51.8–82.7)	73.5 (61.6–80.1)	69.8 (61.1–84.1)
Progressive motility, %	58.2 (44.5–67.7)	57.5 (43.7–68.7)	58.7 (51.0–68.1)	61.1 (50.2–72.7)
Sedentary time	Q1 (shortest) n=78	Q2 n=80	Q3 n=90	Q4 (longest) n=75
DFI, %	6.3 (4.4–9.6)	6.2 (4.0–9.3)	7.5 (4.6–10.0)	7.5 (5.1–11.4)
Sperm concentration, 10 ⁶ /mL	76.3 (43.1–140.9)	80.0 (52.1–121.0)	67.5 (44.0–126.5)	65.1 (38.7–107.2)
Total sperm count, 10 ⁶	283.2 (92.6–432.5)	308.0 (170.1–441.8)	255.3 (130.4–410.3)	207.5 (129.3–399.4)
Total motility, %	72.0 (61.6–81.2)	71.2 (60.0–83.4)	70.5 (58.7–81.2)	71.1 (56.3–84.1)
Progressive motility, %	60.5 (46.9–66.6)	58.8 (48.3–71.1)	57.7 (45.8–71.5)	60.7 (44.3–68.7)

DFI, DNA fragmentation index; MET, metabolic equivalent.

Table 3. Exponential of coefficients (95% confidence interval) in crude and adjusted generalized linear models: associations between semen quality parameters, total physical activity and sedentary time.

	Physical activity (MET-minutes/week)					Sedentary time				
	Q1 n=80	Q2 n=81	Q3 n=82	Q4 n=80	P for trend	Q1 n=78	Q2 n=80	Q3 n=90	Q4 n=75	P for trend
DFI										
Crude	1 (ref.)	0.93(0.76-1.14)	0.86(0.70-1.06)	0.76(0.60-0.96)	0.014	0.83(0.67-1.04)	0.85(0.68-1.06)	0.91(0.74-1.12)	1 (ref.)	0.086
Model 1	1 (ref.)	0.86(0.72-1.04)	0.79(0.66-0.95)	0.71(0.57-0.88)	0.002	0.78(0.63-0.97)	0.83(0.68-1.01)	0.91(0.75-1.10)	1 (ref.)	0.057
Model 2	1 (ref.)	0.86(0.72-1.04)	0.80(0.67-0.96)	0.74(0.60-0.93)	0.006	0.82(0.66-1.03)	0.87(0.71-1.07)	0.93(0.77-1.13)	1 (ref.)	0.185
Sperm concentration										
Crude	1 (ref.)	0.95(0.73-1.24)	1.06(0.82-1.36)	0.94(0.72-1.23)	0.861	1.15(0.88-1.51)	1.10(0.83-1.45)	1.04(0.79-1.37)	1 (ref.)	0.267
Model 1	1 (ref.)	0.92(0.73-1.17)	1.03(0.82-1.28)	0.91(0.70-1.17)	0.931	1.08(0.85-1.38)	0.89(0.69-1.14)	0.94(0.73-1.20)	1 (ref.)	0.535
Model 2	1 (ref.)	0.94(0.74-1.19)	1.03(0.83-1.29)	0.91(0.70-1.18)	0.797	1.09(0.84-1.40)	0.89(0.69-1.16)	0.95(0.74-1.22)	1 (ref.)	0.505
Total sperm count										
Crude	1 (ref.)	1.03(0.81-1.31)	1.02(0.81-1.30)	1.05(0.83-1.33)	0.723	1.06(0.80-1.40)	1.10(0.83-1.45)	0.95(0.71-1.27)	1 (ref.)	0.312
Model 1	1 (ref.)	0.94(0.76-1.17)	0.84(0.67-1.04)	0.83(0.65-1.06)	0.099	0.96(0.76-1.21)	0.94(0.75-1.18)	0.89(0.70-1.12)	1 (ref.)	0.791
Model 2	1 (ref.)	0.96(0.77-1.19)	0.83(0.67-1.03)	0.83(0.64-1.06)	0.100	1.02(0.80-1.31)	0.98(0.78-1.25)	0.90(0.71-1.14)	1 (ref.)	0.846
Total motility										
Crude	1 (ref.)	1.02(0.93-1.11)	1.05(0.97-1.14)	1.04(0.95-1.13)	0.307	1.03(0.95-1.12)	1.03(0.95-1.13)	1.01(0.93-1.10)	1 (ref.)	0.415
Model 1	1 (ref.)	1.01(0.93-1.09)	1.06(0.99-1.15)	1.04(0.96-1.13)	0.222	0.99(0.91-1.07)	1.00(0.92-1.08)	0.98(0.91-1.06)	1 (ref.)	0.898
Model 2	1 (ref.)	1.01(0.93-1.10)	1.07(0.99-1.15)	1.04(0.95-1.13)	0.211	0.98(0.90-1.06)	0.99(0.91-1.07)	0.97(0.90-1.05)	1 (ref.)	0.696
Progressive motility										
Crude	1 (ref.)	1.02(0.92-1.13)	1.05(0.95-1.16)	1.06(0.96-1.18)	0.180	1.02(0.92-1.12)	1.03(0.93-1.13)	1.02(0.92-1.12)	1 (ref.)	0.708
Model 1	1 (ref.)	1.01(0.92-1.12)	1.06(0.97-1.16)	1.07(0.97-1.18)	0.135	0.97(0.88-1.07)	0.98(0.90-1.07)	0.98(0.90-1.07)	1 (ref.)	0.487
Model 2	1 (ref.)	1.01(0.92-1.11)	1.07(0.97-1.16)	1.08(0.98-1.19)	0.098	0.95(0.87-1.05)	0.97(0.89-1.06)	0.98(0.90-1.06)	1 (ref.)	0.304

MET, metabolic equivalent; DFI, DNA fragmentation index

Model 1: Separate generalized linear models for physical activity or sedentary time, each adjusted for age, BMI, income, sleep duration, smoking, sauna habit, abstinence days, sampling season, semen test time after sampling, abnormal checkup results (lipids, blood pressure, blood sugar, or liver function).

Model 2: Generalized linear models with both physical activity and sedentary time, adjusted for age, BMI, income, sleep duration, smoking, sauna habit, abstinence days, sampling season, semen test time after sampling, abnormal checkup results (lipids, blood pressure, blood sugar, or liver function).

Bold font of numbers indicates a *P* value less than 0.05.

Figure legends

Figure 1 Flowchart of the study participants

