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Impact of night shift work on telomere length and epigenetic age in older workers

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Abstract

Background Night shift work disrupts circadian rhythms and has been associated with various health disorders, particularly in older adults. Biological age indicators, such as telomere length (TL) and DNA methylation (DNAm) age, offer effective tools to assess early ageing-related changes linked to occupational exposures. This study aims to investigate the association between night shift work and biological ageing markers among workers aged over 50 years.

Methods Participants were classified as current, former, or never night shift workers. TL was measured via quantitative PCR, and DNAm age was estimated based on methylation at five CpG sites. Age acceleration (AA) was calculated as the residual from regressing DNAm age on chronological age. Associations between shift work and ageing markers were evaluated using univariate and multivariate analyses.

Results Out of 330 workers invited, a total of 262 (response rate 79.6%) were recruited, predominantly male (87%) with a mean age of 54.5 ± 3.1 years. Current night shift workers exhibited significantly shorter telomeres compared to non-current shift workers (adjusted $\beta = -0.07$, $p = 0.03$). Among former shift workers, longer cumulative exposure was associated with reduced TL ($\beta = -0.01$, $p = 0.004$). Additionally, TL increased and AA decreased with each year since night shift cessation ($\beta = 0.01$, $p = 0.001$ and $\beta = -0.08$, $p = 0.05$, respectively).

Conclusions Prolonged night shift work is associated with telomere shortening, suggesting increased cellular ageing, partially reversible after night-shift cessation. DNAm age appears less sensitive to recent or cumulative shift work exposure.

Keywords Ageing, Telomere length, Epigenetic age, Shift work, Occupational stress

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Background

In today's "24-hour society", flexible work schedules are becoming more widespread, with an increasing percentage of workers engaged in night shifts. This trend is driven by the need to maintain continuous production cycles on one hand, and ensure seamless connectivity across different regions of the world on the other. However, irregular sleep-wake patterns and chronic sleep deprivation can disrupt circadian rhythms—biological fluctuations that synchronize with the 24-hour light-dark cycle [1–3]. Such disruptions can have detrimental effects on overall well-being, as circadian rhythms govern a wide range of cellular processes, including metabolic homeostasis, cell cycle regulation, and immune and inflammatory responses [4–6]. These long-term effects are generally associated with an increased risk of inflammatory, degenerative, and fibrotic diseases, which are characteristic of ageing [7]. Considering current trends and the increasing average age of the global workforce, it is crucial to identify effect markers capable of detecting early alterations that may be linked to accelerated ageing or to age-related diseases.

In recent years, the concept of biological age has gained increasing importance, defined as the actual functionality of an organism, which can differ from its chronological age. In certain contexts, biological age may be greater (or lesser) depending on the risk factors to which an individual has been exposed throughout their life, such as chronic psychological stress, poor sleep quality, smoking, and low socioeconomic status [8].

Many models have been developed to estimate biological age through biological markers, and among them, estimating biological age via epigenetic markers is of particular interest due to the intrinsic role these markers play in determining the functional adaptive response to environmental stimuli. Specifically, telomere length (TL) and epigenetic age (DNAm age) are commonly used for this purpose [9]. Telomere length refers to the repetitive DNA sequences at the ends of chromosomes, which protect them from degradation. As cells divide, telomeres gradually shorten, which is associated with cellular senescence and ageing. While a gradual telomere shortening occurs physiologically over time, it is the rapid telomere attrition caused by exposure to specific risk factors that is particularly significant. This accelerated shortening can contribute to the early onset of age-related diseases and cellular dysfunction [10]. On the other hand, DNAm age refers to the age estimated from DNA methylation patterns that accumulate over time and can be influenced by environmental factors and lifestyle. DNAm age serves as a biological indicator of ageing, reflecting the cumulative effects of these influences on cellular function and overall health [11]. Unlike chronological age, DNAm age can

provide insights into an individual's biological age and potential risk for age-related diseases [12].

In the present study, using a cross-sectional approach, we investigated the effect of night shift work in a population of workers aged over 50 years and its potential impact on their health, through the assessment of TL and the estimation of DNAm age.

Methods

Participants

A subset of participants from the *ProAgeing* study [13, 14] was included in the present study. In particular, we recruited workers aged over 50 from two large companies (a packaging industry and a steel industry) during workers' periodic health surveillance visits. Participants were asked for socio-demographic information (age, sex, smoking status, BMI, etc.) and work-related information (job type, current or past exposure to night shifts, start and end years of night shift work). The presence of cardiovascular (hypertension, myocardial infarction), metabolic (hypercholesterolemia, dyslipidaemia), and musculoskeletal conditions diagnosed by a physician was collected through Sect. 3 of the Work Ability Index (WAI) [15], which systematically records medically certified diseases. Each Subject had a 7 ml blood sample withdrawn for laboratory analyses. The study protocol was published elsewhere [13]. Participants provided their written informed consent to participate in this study.

Night shift schedule and duration

Night shift workers were defined as workers with their standard work-schedule including at least one night per week. Usual work schedule in both companies was organized over a three shift models including two night shifts over a period of 10 days, and was stable over time.

The duration of night shift work was calculated by subtracting the questionnaire administration date or the year of the end of night shifts from the year they began night shifts, for current and former night shift workers respectively. For former night shift workers, we also calculated the duration since shift work cessation, defined as the number of years between shift work termination and questionnaire completion.

Telomere length analysis

Telomere length was analysed using real-time PCR, following previously described methods [13]. Briefly, relative telomere length was assessed by calculating the ratio of telomere repeat copy number (T) to a single-copy nuclear gene (S, human beta-globin gene, HBB) copy number (T/S ratio) in each sample, relative to a reference pooled DNA used to generate a standard curve included in every PCR run. The primer sequences have been reported in earlier studies [16]. The reference

pooled DNA was prepared from 10 individual DNA samples (1 µg DNA from each sample). A fresh standard curve, prepared from the pooled DNA with serial dilutions ranging from 24 ng/µL to 0.1875 ng/µL (1:2 dilutions), was included in every “T” and “S” PCR run. For each sample, 22.5 ng of DNA were used as a template, and each reaction was performed in triplicate. All PCR reactions were carried out on a 7900HT Fast Real-Time PCR System (Applied Biosystems, Waltham, MA, USA). At the end of each run, a melting curve analysis was performed to confirm the specificity of amplification and the absence of primer dimers. The average of the three T measurements was divided by the average of the three S measurements to compute the T/S ratio for each sample.

Determination of epigenetic age

DNA age was calculated measuring the methylation pattern of five CpG sites at five genes (ELOVL2, C1orf132/MIR29B2C, FHL2, KLF14, TRIM59) as previously described [17, 18]. The DNA samples (500 ng) were plated at a concentration of 25 ng/µL in 96-well plates. Sodium bisulfite conversion was performed by using the EZ-96 DNA Methylation-Gold™ Kit from Zymo Research (Irvine, CA, USA), according to the manufacturer's instructions. The DNA was eluted in a final volume of 200 µL. The PCR reaction was set up as follows in a final reaction volume of 50 µL: 10 µL of the bisulfite-treated template DNA was combined with 25 µL of GoTaq Hot Start Green Master Mix (Promega); 1 µL of the forward primer (10 µM); 1 µL of the 5'-end biotinylated reverse primer (10 µM) were added. The PCR reaction following cycling conditions were previously described [18], and primer sequences and sequencing regions are reported in the Supplementary Table S1. Biological (epigenetic) age (Y) was calculated as follows:

$$Y = 3.26847784751817 + 0.465445549010653 \text{ methC7} \\ - \text{ELOVL2} - 0.355450171437202 \text{ methC1} \\ - \text{C1orf132} + 0.306488541137007 \text{ methC7} \\ - \text{TRIM59} + 0.832684435238792 \text{ methC1} \\ - \text{KLF14} + 0.237081243617191 \text{ methC2} \\ - \text{FHL2}$$

In cases where one of the five CpG methylation markers used in the calculation formula was missing due to a laboratory error, we imputed the missing value using the median of that specific marker.

Statistical analysis

We represented data through frequencies and percentages or mean ± sd. Baseline characteristics were compared with chi-square test or Fisher's exact test as appropriate for categorical variables and with one-way ANOVA for continuous variables. To obtain an estimate

of biological ageing that is independent of chronological age, a measure known as age acceleration (AA) was used. It was calculated by applying a simple linear regression model with chronological age as the independent variable and biological age as the outcome. The residual of this statistical model, which is the difference between the observed biological age and the one predicted by the model, represents the acceleration of ageing due to epigenetic effects. Outliers (defined as values beyond 4 standard deviations from the mean) were excluded [19].

To explore the association between night shifts and telomere length and age acceleration, the differences among the three groups were investigated through one-way ANOVA. To measure the effect of night shift work on telomere length and biological age we implemented two linear regression models, where we included the categorical variable indicating night shift work (current/former/never) as independent variable and telomere length or AA as continuous dependent variable. In the latter case, the regression model was adjusted for age, sex, type of industry, BMI, and smoking habits.

All analyses were performed using R software, with statistical significance set at $p < 0.05$.

Results

A total of 262 workers out of 330 invited (response rate 79.6%) participated in the study. They were employed either in the steel industry ($N = 100$, 38%) or the packaging industry ($N = 162$, 62%). Characteristics of the study population are summarized in Table 1.

The average age was 54.5 ± 3.1 years (range 50–63), with a predominance of males ($N = 227$, 87%). At the time of recruitment, 117 participants (45%) were current shift workers, 65 (25%) were former shift workers, and 80 (30%) had never been exposed to shift work. Among current night shift workers, the mean number of years in night shift work was 26.6 ± 8.3 (range 2–38). For former night shift workers, the mean duration of exposure was 13.7 ± 9.7 years (range 0–34), and they had stopped night shift work an average of 16.8 ± 10.5 years prior to the study (range 1–36).

Sixty (23%) participants reported at least one cardiovascular disease diagnosed by a physician. Such as hypertension or myocardial infarction. Metabolic disorders, including hypercholesterolemia and dyslipidaemia, were present in 9% of participants ($N = 23$). Musculoskeletal disorders were reported by 36% of the sample ($N = 95$).

Age was similar among all Subgroups, with a mean around 54 years. BMI showed a statistically significant difference ($p < 0.05$), with current night shift workers having the highest mean BMI (26.7 ± 3.6), while never night shift workers had the lowest (25.1 ± 4.3).

Sex distribution differed between groups ($p < 0.001$): current and former night shift workers were

Table 1 Characteristics of the study population and summary statistics for telomere length (TL) and age acceleration (AA) of the total sample and stratified by night shift work status

	Total sample N=262	Current NSWs N=117 (45%)	Former NSWs N=65 (25%)	Never NSWs N=80 (30%)
Age, mean $\pm$ sd	54.5 $\pm$ 3.1	54.2 $\pm$ 2.8	54.4 $\pm$ 3.3	55.1 $\pm$ 3.3
BMI*, mean $\pm$ sd	26.1 $\pm$ 3.8	26.7 $\pm$ 3.6	26.1 $\pm$ 3.4	25.1 $\pm$ 4.3
Sex***, N (%)				
Male	227 (87)	112 (96)	64 (99)	51 (64)
Female	35 (13)	5 (4)	1 (1)	29 (36)
Smoking habits, N (%)				
Never	133 (51)	55 (47)	29 (45)	49 (61)
Former	78 (30)	37 (32)	26 (40)	15 (19)
Current	51 (19)	25 (21)	10 (15)	16 (20)
Workplace***				
Steel industry	100 (38)	59 (59)	30 (30)	11 (11)
Packaging industry	162 (62)	58 (36)	35 (22)	69 (42)
Physician's Diagnosed Diseases, N (%)				
Cardiovascular*	60 (23)	20 (17)	22 (34)	18 (22)
Metabolic	23 (9)	10 (9)	8 (12)	5 (6)
Musculoskeletal	95 (36)	44 (38)	24 (37)	27 (34)
Number of years in night shift, mean $\pm$ sd	-	26.6 $\pm$ 8.3	13.7 $\pm$ 9.7	-
Number of years since end of night shift, mean $\pm$ sd	-	-	16.8 $\pm$ 10.5	-
TL*, mean $\pm$ sd	1.02 $\pm$ 0.25	0.97 $\pm$ 0.24	1.05 $\pm$ 0.25	1.06 $\pm$ 0.25
AA, mean $\pm$ sd	-0.16 $\pm$ 4.53	0.23 $\pm$ 4.59	-0.13 $\pm$ 3.34	-0.65 $\pm$ 4.57

NSWs night shift workers

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ for Chi-square test (categorical variables) or one-way ANOVA (continuous variables)**Table 2** Association between night shift (current ($N = 117$) vs. non-night shift workers ($N = 145$) and telomere length (TL) and age acceleration (AA)

	Unadjusted		Adjusted*	
	β (se)	p -value	β (se)	p -value
TL	-0.12 (0.04)	0.002	-0.07 (0.03)	0.03
AA	0.64 (0.57)	0.26	0.47 (0.59)	0.42

*adjusted for age, sex, type of industry, BMI, and smoking habits

predominantly male (96% and 99%), while never shift workers showed a more balanced distribution (64% male, 36% female).

The proportion of participants who had never smoked was highest (61%) among workers never exposed to night shifts.

Cardiovascular diseases were significantly more prevalent among former night shift workers (34%) compared to current shift workers (17%) and never exposed individuals (22%) ($p < 0.05$). No significant differences were

observed for metabolic or musculoskeletal disorders across groups.

Significant differences in mean TL were observed among current, former, and never night shift workers (Table 1). As shown in Table 2, TL was significantly shorter in current night shift workers compared to those not currently engaged in night shifts (i.e., former or never night shift workers). After adjusting for age, sex, type of industry, BMI, and smoking habits, current night shift work remained significantly associated with reduced TL, with an adjusted regression coefficient of -0.07 ($p = 0.03$).

In contrast, no significant differences in mean AA were observed between current and former/never night shift workers (Table 1). Both unadjusted and adjusted models indicated a modest, non-significant trend toward increased AA in current night shift workers (Table 2).

Among former night shift workers, the number of years spent in night shift work was significantly and negatively associated with TL ($\beta = -0.01$, $p = 0.004$; Table 3; Fig. 1A),

Table 3 Association between night shift duration and years since end of night shift work and telomere length (TL) and age acceleration (AA)

	TL		AA	
	β^* (se)	p -value	β^* (se)	p -value
Years of night shift work (Current NSWs)	0.003 (0.002)	0.19	0.03 (0.05)	0.57
Years of night shift work (Former NSWs)	-0.01 (0.003)	0.004	0.08 (0.04)	0.07
Years since end of night shift work (Former NSWs)	0.01 (0.003)	0.001	-0.08 (0.04)	0.05

NSWs night shift workers. *adjusted for age, sex, type of industry, BMI, and smoking habits

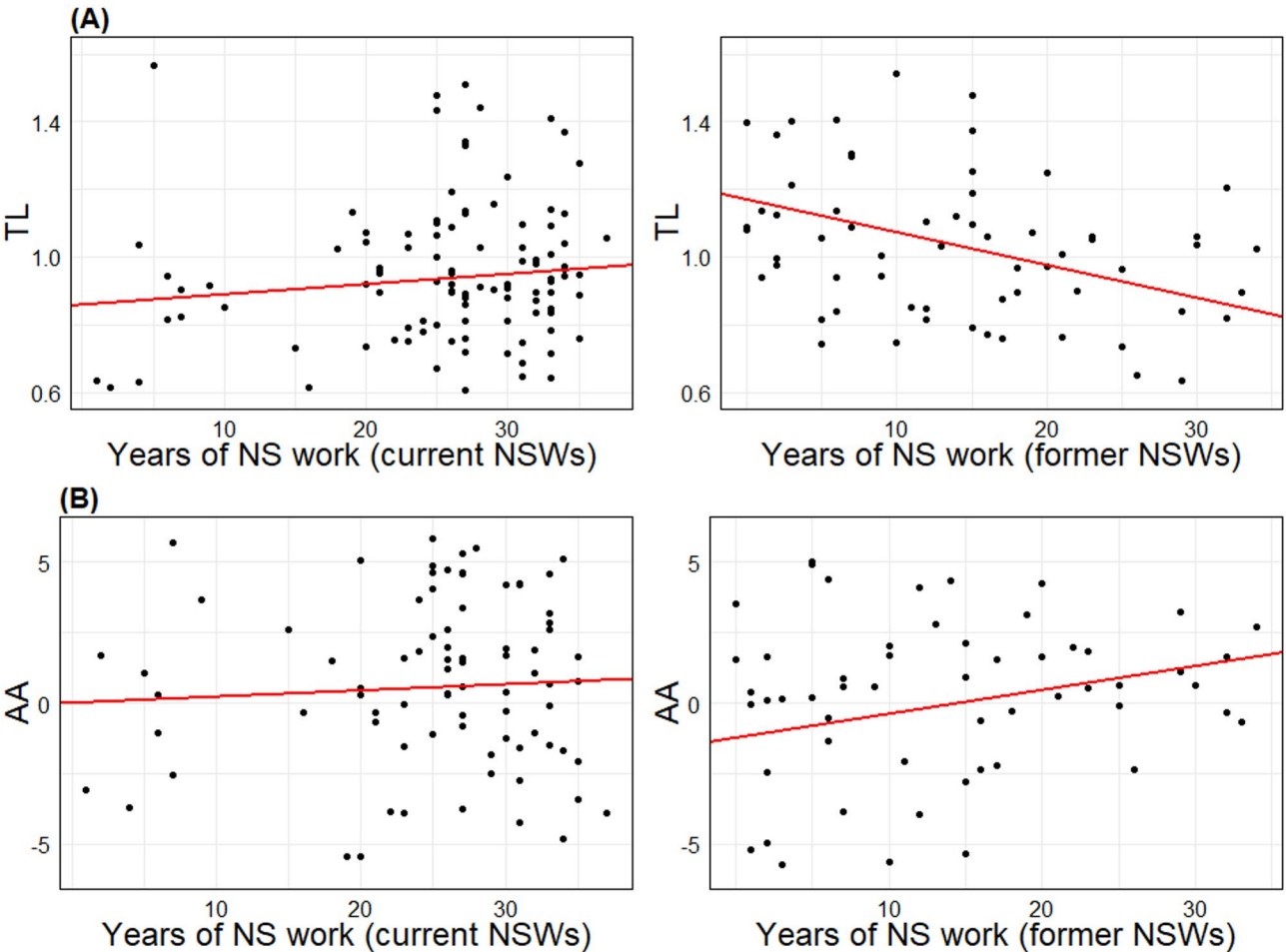


Fig. 1 Association between years spent in night shift work (NS) and (A) telomere length (TL) and (B) AA among current (left) and former (right) night shift workers (NSWs)

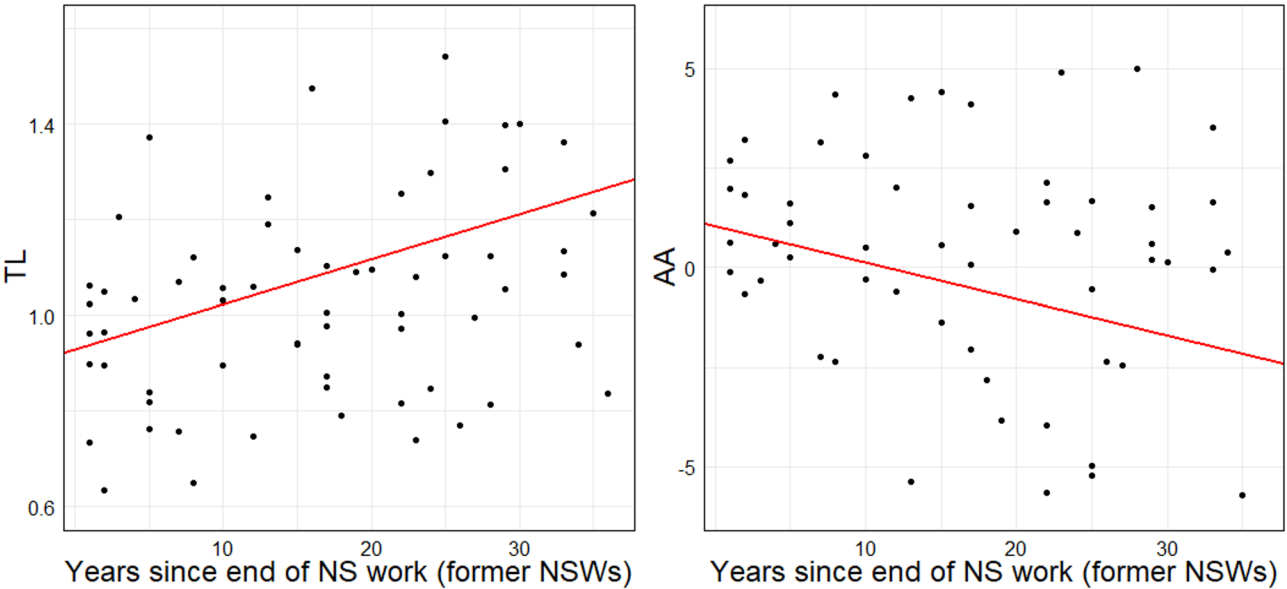


Fig. 2 Association between time since cessation of night shift (NS) work and telomere length (TL, left) and age acceleration (AA, right) among former night shift workers (NSWs)

whereas no such association was observed among current night shift workers. Additionally, in both current and former night shift workers, the duration of night shift exposure showed no significant association with AA (Table 3; Fig. 1B). Among former shift workers, each year since the cessation of night shift work was associated with a significant increase in TL ($\beta = 0.01$, $p = 0.001$) and a marginally significant decrease in AA ($\beta = -0.08$, $p = 0.05$) (Table 3; Fig. 2).

Discussion

This study investigated the impact of night shift work on TL and epigenetic age in workers aged over 50 years. Our findings highlight that prolonged night shift work is associated with significant telomere shortening, suggesting an accelerated cellular ageing process. However, its relationship with epigenetic AA is less evident.

Telomere shortening observed in current night shift workers is consistent with prior research showing that disrupted circadian rhythms and oxidative stress are associated with cellular senescence [20, 21]. The adjusted regression analysis revealed a significant reduction in TL among current shift workers compared to non-shift workers. Our results are consistent with the literature in the field, which, however, mainly focuses on female workers engaged in rotating shifts [22–24]. Additionally, the duration of night shift exposure showed a cumulative negative association with TL in former night shift workers. In a previous study conducted on a population of shift-working nurses, we observed a nonlinear association between night shift duration and TL [25]. In other studies conducted on populations of female shift workers, shift intensity has been associated with a reduction in TL [26]. This emphasizes the persistent biological impact of long-term exposure to night shift schedules, likely mediated through mechanisms such as oxidative stress and inflammation. Chronic circadian misalignment, a hallmark of shift work, disrupts the normal oscillatory expression of clock genes [27], leading to dysregulation of redox homeostasis and increased production of reactive oxygen species (ROS) [28]. The accumulation of oxidative damage, particularly at guanine-rich telomere regions, makes telomeres highly susceptible to attrition. Concurrently, shift work-induced sleep deprivation and metabolic alterations promote a pro-inflammatory state, with elevated levels of cytokines such as IL-6 and TNF- α , which have been implicated in telomere erosion [29]. These mechanisms collectively contribute to the accelerated cellular ageing observed in long-term shift workers.

Such biological alterations may also reflect clinical differences observed across exposure groups. Notably, cardio-metabolic diseases were more prevalent among former night shift workers. This pattern may suggest that

workers with pre-existing health conditions are more likely to be removed from night shift duties.

Interestingly, the cessation of night shift work appeared to offer some recovery, as evidenced by the increase in TL with each year since leaving night shift work. This suggests that the telomere attrition induced by chronic circadian disruption and associated stressors may be, at least partially, reversible when homeostatic balance is restored and oxidative stress decreases: during night shift work, mitochondrial dysfunction and an imbalance in the expression of superoxide dismutase (SOD) and glutathione peroxidase (GPx), lead to excessive production of ROS [30], which preferentially damage guanine-rich telomere regions. Upon returning to a stable circadian rhythm, the normalization of mitochondrial function and the upregulation of antioxidant defences may mitigate this oxidative burden, reducing further telomere damage. Another potential mechanism involves telomerase, the enzyme responsible for counteracting telomere shortening. Under conditions of sleep deprivation and metabolic dysregulation, telomerase activity is suppressed, limiting its ability to maintain telomere integrity [31, 32]. However, upon shift cessation, the restoration of sleep homeostasis and metabolic equilibrium may enhance telomerase function, promoting telomere elongation, particularly in highly regenerative cell types [33]. Indeed, removing circadian stressors allows for at least partial restoration of telomere integrity through multiple converging molecular pathways.

Although our results indicated a significant association between night shift work and telomere shortening, we recognize the potential for residual confounding and other biases inherent in observational studies. Similar to historical cases where initially attributed causes (e.g., extraterrestrial radiation among flight attendants [34]) were later replaced by night shift work exposure, careful consideration of all possible confounders is essential to avoid misinterpretation. Chronic inflammation, psychological stress, infections, and socioeconomic status are known to impact telomere length limiting its utility as a highly specific indicator of any single exposure [35]. Although we controlled for some lifestyle variables such as smoking and BMI, it is possible that unmeasured social or environmental confounders contributed to the observed inter-individual variation in telomere length.

In contrast to TL, no significant differences in AA were observed between current, former, and non-shift workers after adjusting for confounding variables. While there was a non-significant trend towards increased age acceleration among current shift workers, the lack of a robust association suggests that DNAm age may be less sensitive to the immediate effects of circadian disruption compared to TL. However, the slight reduction in age acceleration observed with increasing years since the

cessation of night shift work suggests a potential recovery process.

This discrepancy likely reflects the distinct biological processes captured by TL and epigenetic AA. TL shortening represents a direct measure of replicative ageing at the chromosomal level, with critically short telomeres triggering cellular senescence via activation of the DNA damage response (DDR) pathway [36]. Once telomeres reach a critical threshold, cells enter a state of growth arrest, which is associated with widespread epigenetic remodelling. Senescent cells exhibit characteristic DNA methylation changes, including both cis- and trans-acting effects [37]. In cis, local hypo methylation at sub-telomere regions may contribute to chromatin destabilization, reinforcing telomere dysfunction. In trans, broader alterations in DNA methylation patterns affect key regulatory genes involved in cell cycle progression, proliferation, and apoptosis, many of which are included in epigenetic age estimation models [38, 39]. These changes are thought to result from a combination of altered transcription factor activity, metabolic shifts, and inflammatory signalling, which accumulate over time rather than manifesting acutely in response to circadian misalignment.

Thus, it is possible that the effects of shift work on DNA methylation emerge more gradually and are influenced by additional factors beyond circadian disruption alone. The interplay between TL erosion and AA may become more pronounced with prolonged exposure to cellular stressors, such as chronic inflammation, metabolic dysregulation, and cumulative oxidative damage. This would explain why TL shortening is observed earlier in response to shift work, whereas alterations in epigenetic age markers may follow a more delayed trajectory.

We acknowledge some limitations of the present study. First, its cross-sectional design limits the ability to establish causality between night shift work and biological ageing markers. While significant associations were observed, longitudinal studies are needed to confirm these findings. Second, the sample was predominantly male (87%), which may limit the generalizability of the findings to female workers, who may experience different biological responses to night shift work due to hormonal and circadian differences. Moreover, the imbalance in sex distribution across exposure groups, despite the presence of female participants in all groups, limited the ability to explore potential sex-specific responses to shift work. Nonetheless, considering that previous research has largely focused on female cohorts, our study contributes novel and relevant data on older male workers. Third, although the duration of night shift work was calculated based on the number of years during which the worker was engaged in night shifts, no detailed records of cumulative night hours were available. This may limit

the precision of exposure quantification, particularly for workers with variable night workloads. Future studies should consider integrating other exposure metrics such as total hours worked at night or shift intensity. Fourth, telomere length was measured using a qPCR method, which, although widely applied due to its efficiency and relative simplicity, has some limitation. These include inter-individual variability influenced by both genetic and environmental factors, as well as potential technical variability [40]. Although the method used in the present study provides a relative TL value, it remains a valid approach to assess telomere length variations in population-based contexts when applied with standardized protocols, and it has consistently shown strong associations with chronological age across diverse populations [41]. A further limitation is the lack of information on individual chronotype, which may influence individual susceptibility to circadian disruption associated with shift work. Previous studies have shown that evening-type individuals may adapt differently to night shifts compared to morning-types.

However, despite the above-mentioned limitations, the observed results, highlights that TL could act as a sensitive marker for assessing the biological impact of occupational long-lasting (i.e. stable over time) potential wearing factors, including night shift work.

Conclusions

Our observational study conducted among workers aged 50 years or more, showed an association between NS exposure and TL, showing a less pronounced effect in former night shift workers the longer the time since their exposure ended. This finding is, to our knowledge, the first signal of a direct but reversible effect of night shift exposure and a biological marker of age in workers. Longitudinal studies are needed to investigate the relationship between TL, DNAm age, as suitable markers of biological aging across a more diverse range of industries in order to confirm our funding.

In a world where shift work is increasingly prevalent and the workforce is ageing, understanding the relationship between night shifts and biological ageing is crucial for accurately assessing the complex, multifactorial impact of work schedules across the workers' lifespan. Such insights are also essential for developing effective preventive strategies, including the promotion of healthy lifestyles and the reduction of other concurrent risk factors, to mitigate the impact of night shift work on exposed workers.

Abbreviations

AA	Age Acceleration
ANOVA	Analysis of Variance
BMI	Body Mass Index
CpG	Cytosine-phosphate-Guanine dinucleotide

DDR	DNA Damage Response
DNAm age	DNA Methylation Age
DNA	Deoxyribonucleic Acid
EZ	Easy (from kit name "EZ-96 DNA Methylation-Gold™ Kit")
GPx	Glutathione Peroxidase
HBB	Human Beta-Globin Gene
IL-6	Interleukin 6
IRCCS	Istituto di Ricovero e Cura a Carattere Scientifico
PCR	Polymerase Chain Reaction
ROS	Reactive Oxygen Species
sd	Standard Deviation
se	Standard Error
SOD	Superoxide Dismutase
T/S ratio	Telomere Repeat Copy Number / Single Copy Gene Ratio
TL	Telomere Length
TNF-α	Tumor Necrosis Factor alpha
WAI	Work Ability Index

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12995-025-00477-2>.

Supplementary Material 1

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Authors' contributions

LF and MB conceptualized the study. LF, AC, AF, TB wrote the original draft. AC performed the statistical analysis. ML, PB, SR, CC provided advice on study design and manuscript revision. BA and MH conducted laboratory analyses. MB was the principal investigator and supervised the manuscript writing. All the authors reviewed and approved the final manuscript.

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Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

The study was approved by the Ethical Committee of the Foundation IRCCS Ca' Granda Ospedale Maggiore Policlinico on June 22, 2021 (Milan Area 2 Ethical Committee, with decree number 616_2021bis). The participation was voluntary, each subject read and signed an extended informed consent to participate in the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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