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AGEING

Association between alcohol consumption in healthy midlife and telomere length in older men. The Helsinki Businessmen Study

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Abstract There are scarce data of alcohol consumption and telomere length, an indicator of biological age. In 1974, detailed alcohol consumption was available for a socioeconomically homogenous cohort of middle-aged men (The Helsinki Businessmen Study). Their alcohol use, divided into 5 groups (zero, 1–98, 99–196, 197–490, >490 g/week) has been repeatedly assessed until old age. In 2002/2003, leukocyte telomere length (LTL) and the proportion of short telomeres (less than 5 kilobases) were measured in a random subcohort of 499 men (mean age 76 years) using the Southern blot. Age-adjusted mean LTL in the 5 midlife alcohol consumption groups were 8.33, 8.24, 8.12, 8.13, and 7.87 kilobases, respectively ($P < 0.001$). The respective proportions (%) for short telomeres were 11.24, 11.52, 11.89, 12.08, and 13.47 ($P = 0.004$). The differences remained after further adjustments (ever smoking, body mass index, cholesterol, perceived fitness) for LTL ($P = 0.03$) and tended to remain for proportion of short telomeres ($P = 0.07$). Neither LTL, nor proportion of short telomeres, were associated with contemporary alcohol consumption groups in old age. Even minor alcohol consumption in midlife was significantly associated with shorter telomere length in old age. The differences represent an up to 10 year gap in biological age between zero and highest consumption.

Keywords Aged · Alcohol · Midlife · Telomere

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Introduction

Alcohol use has multiple influences on health and disease. Excess consumption involves well known medical ills, including risk for many cancers [1]. On the other hand, light to moderate drinking has been considered to exert beneficial influences, notably a reduced risk of cardiovascular diseases [2]. Moderate drinking has also been associated with better preservation of cognitive [3], and physical function [4, 5] in old age. Alcohol consumption thus exerts a dual effect on long-term health and wellbeing, and identification of the underlying mechanisms is important for aging societies.

Telomeres are specialized structures at the end of chromosomes, which preserve chromosome stability and integrity [6–8], and telomere length has been suggested to be a marker of biological age [9]. Shorter telomere length in peripheral blood leukocytes (LTL) has previously been associated with chronic diseases such as cardiovascular diseases [10–12], diabetes [13], cancer [14], as well as cardiovascular risk factors and lifestyle [15–17]. Very few studies have assessed the effect of alcohol on telomeres, although it may have been included as a covariate without an independent role [18]. In a case–control study comparing social drinking and alcohol abuse, the latter was

associated with shorter telomeres [19], possibly contributing to the association between excess alcohol and carcinogenesis. Conversely, it can be hypothesized that longer telomeres could be found among moderate drinkers. In the Helsinki Businessmen Study, detailed alcohol consumption of the participants has been repeatedly documented from midlife to late life. We sought to relate this long-term consumption to telomere length in old age.

Methods

Study population

A cohort of initially healthy Caucasian men from the highest social class, born 1919–1934, has been prospectively followed from the 1960s for cardiovascular risk factors, mortality, and quality of life. The study population and their alcohol consumption have been described in detail [16, 17, 20, 21]. The present analyses included 2360 men for whom we have detailed questionnaire data on alcohol consumption in 1974. At that time only 180 men (7.6 %) had some chronic condition or medication. Part of the cohort also participated in a multifactorial prevention study during the 1970s [20], but their exclusion did not affect the present results on telomere length.

After 1974, the cohort has been followed-up with mailed questionnaires on lifestyle, body weight, detailed alcohol consumption, diseases, and comorbidity. No specific data about diet nor environmental exposures were included, but in 1974 the men were asked how they rated their physical fitness on a 5-step scale ("very good", "good", "fair", "poor", "very poor"). Mortality has been followed-up using national registers. In 2002/2003, an unselected sample ($n = 622$) of all men who responded to the questionnaire survey ($n = 1070$, 69.9 % of survivors) were invited to the clinic whereupon venous blood samples were taken for genetic analyses, and extraction of DNA of peripheral blood leukocytes was performed with standard procedures. For 499 of them (80.2 % out of 622), we also had detailed data of alcohol consumption in 1974, and they formed the study group of the present analyses. Of them 98.4 % were clinically healthy and without regular medications in 1974.

The follow-up study with genetic testing has been approved by the ethics committee of the Helsinki University Hospital, Department of Medicine. All participants provided written informed consent.

Alcohol consumption

In 1974 and in 2002/2003, the participants were asked with identical questions to report their average weekly alcohol consumption, separately for beer, wine and spirits. Ex-drinkers were not identified among abstainers at baseline. Preference was based simply on which beverage was consumed the most during the week. One unit of alcohol (bottle of beer, glass of wine, or spirits) was calculated to contain 14 g pure alcohol [the average amount in a Finnish "restaurant unit" during the 1970 s, well known to participants]. In earlier analyses on mortality [21], we categorized alcohol consumption in 3 groups: zero, moderate (1–349 g/week, mean consumption of <3 drinks/d], and high consumption (>349 g/week, mean consumption of 5 drinks/d); or in 7 groups according to the Health Professionals Follow-up Study [22]. For the present analyses we also tested categorization in 5 groups according to the amount of daily drinks reported in 1974: I) zero, II) maximum one drink/day (1–98 g/week), III) maximum 2 drinks/day (99–196 g/week), IV) maximum 5 drinks/day (197–490 g/week), and V) over 5 drinks/day (>490 g/week). The conclusions were similar whatever categorization was used, and we present here the daily drinks-based categorization.

Telomere measurements

Telomere length of peripheral leukocytes of the 499 men was measured as previously described [23] using TeloTAGGG Telomere length assay kits (Roche Molecular Biochemicals, Basel Switzerland). Briefly, an aliquot (1 μL) of DNA was digested with HinfI and RsaI (20 U/μL DNA each) (Roche) at 37 °C for 2 h. Separation of digested DNA was done by 0.8 % agarose gel electrophoresis at 5 V/cm in 0.04 M Tris–acetate, 0.001 M EDTA, pH 8.0 buffer for 2–3 h. After electrophoresis, the DNA fragments were transferred by Southern blotting to a positively charged nylon membrane (Hybond N+, Amersham, Little Chalfont, UK) at room temperature using 3 M NaCl, 0.3 M sodium citrate, pH 7.0. The transferred DNA was then fixed on the blotting membrane by UV-cross-linking (UV Stratalinker 1800, Stratagene, La Jolla, CA). The blotted DNA fragments were hybridized to a digoxigenin-labeled probe specific for telomeric repeats in a hybridization oven (Techne, Burlington, NJ) at 42 °C for 3 h.

The membrane was incubated with a DIG-specific antibody covalently coupled to alkaline phosphatase and then visualized by virtue of alkaline phosphatase metabolizing CDP-*Star*, a highly sensitive chemiluminescence substrate. The membrane was then exposed to a hyperfilm ECL (Amersham, Little Chalfont, UK), and analyzed using Adobe PhotoShop and Science Lab 99 Image gauge software (Fuji Photo Film Co Ltd).

Mean size of the telomere restriction fragment (TRF) was estimated using the formula {sum}(ODi−

background)/{sum}(ODi−background/Li), where ODi is the chemiluminescent signal and Li is the length of the TRF fragment at position i. Interassay coefficient of variation (CV) was 3.70 % when calculated from an internal control DNA sample in 96 assays.

There is increasing evidence suggesting that regardless of mean telomere length, one critically short telomere may cause a cell to enter senescence [24–26]. Therefore, using the same films as for mean TRF analysis, we also calculated the percentage of short telomeres, shorter than 5 kilobases, in each telomeric sample. This value was chosen as it was the lowest cutoff limit providing reliable results.

The total chemiluminescence intensity of each sample was measured, and the signal intensity below molecular size marker 5 kilobases was quantitated. Percentage of short telomeres = (intensity of chemiluminescence signal below 5 kilobases−background) × 100/(total signal intensity−background).

Statistical analysis

Statistical analyses were performed with NCSS 2004 software (Number Crunching Statistical System, Kaysville, UT, USA). Continuous variables are shown as means with SDs, or medians with interquartile ranges. LTL and proportion of short telomeres showed a Gaussian distribution and were studied both as continuous variables and categorized in tertiles. Alcohol consumption was categorized as described above. We compared telomere characteristics in old age separately according to baseline (1974) and contemporary categories (see above) of alcohol consumption. Age, BMI, serum cholesterol concentration and perceived fitness were used as a continuous variables (also age-squared was tested but did not change the results). Smoking at baseline was dichotomized as never vs. ever (current plus ex-smokers). Spearman rank correlation, multiple regression, and ANCOVA (linear trend) were used to study the relationship between alcohol consumption and telomere data. P values <0.05 (2 sided) were considered to indicate statistical significance.

Results

General outline of the study is in Fig. 1. Clinical data at baseline in 1974 and at the time of telomere measurements in 2002/2003 for the initial cohort ($n = 2360$) and the random group with telomere measurements ($n = 499$) are compared in Table 1. Probably due to the homogeneity of the initial cohort, the group with telomere measurements well represented all 2360 men with alcohol data in 1974. Slightly lower risk factors at baseline probably reflect predictors of survival. In the initial cohort of 2360 men, alcohol consumptions reported in 1974 and 2002/2003 were significantly correlated ($r = 0.50$, $P < 0.001$), and the baseline alcohol consumption group seemed to track well during follow-up (Fig. 2). However, actual consumption remained more stable for lower than higher baseline consumption. While 88.0 % of those men reporting zero to maximum one drink/day consumption in 1974 reported similarly in 2002/2003, more than half (58.6 %) of those men reporting a consumption of >2 drinks/day in 1974 reported lower consumption in 2002/2003. Reasons (for example intervening illnesses) for lowered consumption could not be evaluated.

In the group of 499 men, telomere length was 8.17 (SD 0.45) kilobases, and the proportion of short telomeres (<5 kilobases] was 11.80 (SD 2.47) %. Mean LTL, but not the mean proportion of short telomeres, was weakly but significantly correlated with age ($r = -0.11$, $P = 0.02$, and $r = 0.06$, $P = 0.18$, respectively). No significant correlations were observed with alcohol consumption reported in 2002/2003 ($r = -0.08$, $P = 0.11$; $r = 0.04$, $P = 0.43$, for mean LTL and the mean proportion of short telomeres, respectively). There was a weak negative correlation (unadjusted) between mean LTL and alcohol use in 1974 ($r = -0.13$, $P = 0.004$), but the respective correlation with the mean proportion of short telomeres was not significant ($r = 0.08$, $P = 0.08$). Neither LTL nor the proportion of short telomeres were significantly associated with the preference of any alcohol beverage type in 1974 (data not shown).

Age-adjusted mean LTL and mean proportions of short telomeres show a significant and graded association with baseline alcohol consumption categories (Fig. 3, Panel A). Further adjusted for baseline ever smoking, BMI, cholesterol, and perceived fitness the associations remained significant for LTL ($P = 0.03$), and tended to be significant for the proportion of short telomeres ($P = 0.07$). Among the 499 men there were 72 men with a history of cancer in 2002/2003. Their exclusion did not abolish the association between 1974 alcohol consumption and LTL ($P = 0.002$), or the proportion of short telomeres ($P = 0.007$). Neither were the relationships with alcohol driven by the heaviest consumption, because the trend prevailed after the group with >5 drinks was excluded for LTL ($P = 0.009$), and tended to prevail for the proportion of short telomeres ($P = 0.06$). This was also observed in multiple regression analyses adjusted for age: With zero consumption (group I) as referent, consumption over one drink/day (groups III to V) was associated with shorter telomere length (P values 0.02, 0.03 and 0.001 for consumption groups III, IV and V, respectively). For the proportion of short telomeres, however, only group V significantly differed from zero consumption ($P = 0.002$). In contrast, there were no consistent associations between mean LTL nor the proportion of short telomeres with contemporary alcohol consumption categories in 2002/2003 (Fig. 3, Panel B).

General outline of the study

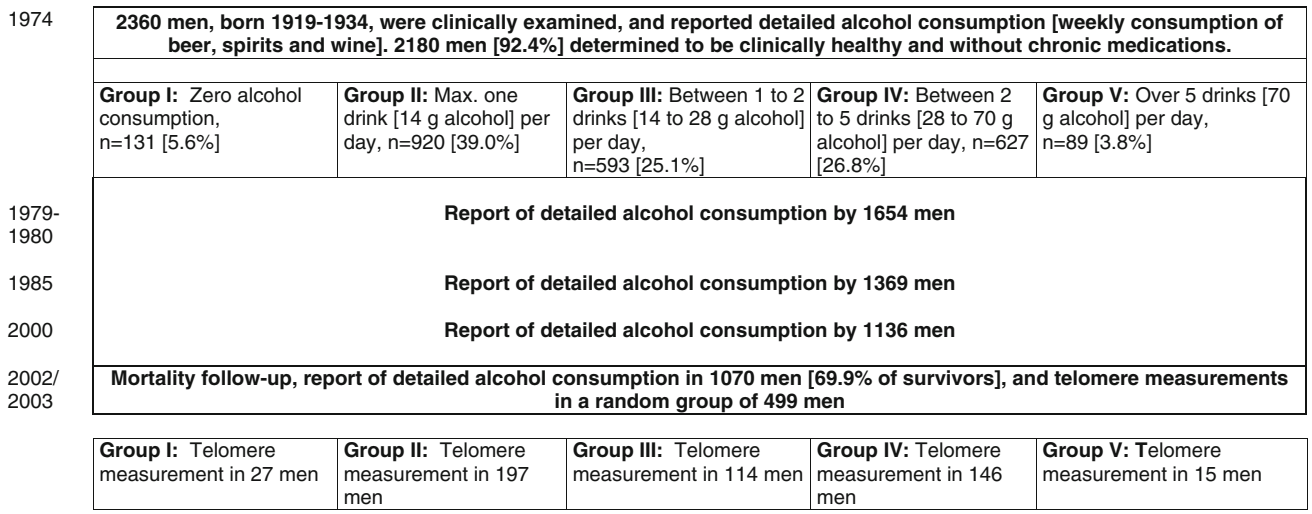

Fig. 1 General outline of the study

Table 1 Characteristics of the Helsinki Businessmen Study Cohort at baseline in 1974 ($n = 2360$), and the random group of 499 men selected for DNA analyses in 2002/2003[a]

Variable	Whole cohort, $n = 2{,}360$	Subcohort, $n = 499$
At start of follow up in 1974		
Age (year)	47.7 [4.1]	46.7 [3.9]
Body-mass index[b] − median [interquartile range]	25.7 [24.1–27.5]	25.1 [23.7–27.2]
Alcohol consumption (g/week)	163.4 [157.7]	161.9 [42.4]
Median [interquartile range] (g/week)	126 [56–238]	126 [56–238]
Blood pressure (mmHg)		
Systolic	142.9 [18.8]	140.3 [17.9]
Diastolic	91.8 [11.3]	90.7 [10.8]
Cholesterol (mmol/L)	6.3 [1.1]	6.2 [1.1]
Triglycerides, median [interquartile range] (mmol/L)	1.4 [1.1–1.9]	1.4 [1.0–1.8]
One-hour blood glucose, median [interquartile range] (mmol/L)	6.8 [5.5–8.3]	6.5 [5.3–8.1]
Smokers (n [%])	764 [32.4]	117 [23.4]
In 2002–2003		
	Whole cohort, n = 1070[c]	Subcohort, n = 499
Age (year)	76.7 [4.1]	75.7 [3.9]
Body-mass index + median [interquartile range]	25.3 [23.5–27.4]	25.3 [23.4–27.3]
Alcohol consumption, median [interquartile range] (g/week)	70 [28–154]	98 [28–168]
Current smokers (no. [%])	69 [6.4]	28 [5.6]
Telomere length, median [interquartile range] (kilobases)		8.14 [7.88–8.47]
Proportion of short telomeres, median [interquartile range] (%)		11.76 [10.04–13.42]

[a] Values are means [SD] unless otherwise stated

[b] The body-mass index is the weight in kilograms divided by the square of the height in meters

[c] Survivors of the initial alcohol cohort (69.9 %), who responded to the questionnaire survey in 2002–2003

Discussion

The results demonstrate a graded and highly significant inverse relationship between midlife alcohol consumption and later telomere length. Older men with long-term abstinence, or very light alcohol consumption had both the longest telomeres and the smallest proportion of short telomeres in their peripheral blood leukocytes. This relationship was independent of age and midlife smoking, overweight and high cholesterol concentration—factors

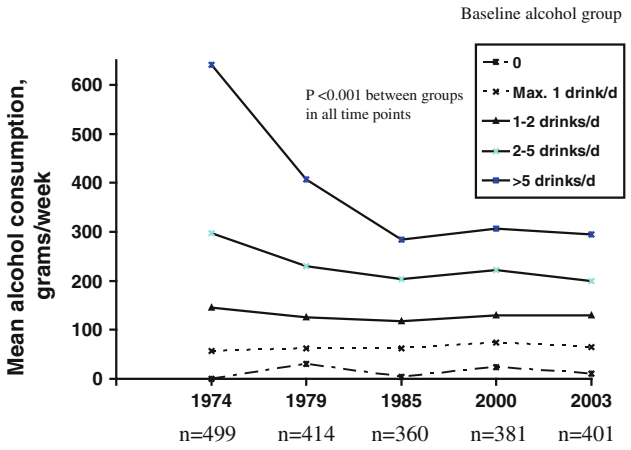

Fig. 2 Changes of alcohol use during follow-up in the baseline alcohol consumption groups of the 499 men with telomere measurements

shown to be associated with shorter telomeres in our cohort [16, 17]—as well as cancer in old age. Our results suggest that the effect on telomeres is seen already with modest, over one drink/day, alcohol consumption. It is of note that these relationships could only be revealed during long-term, because in old age there was no relationship between contemporary alcohol consumption and telomere length. The probable explanation is the decreasing trend of higher consumption levels (Fig. 2) which at the individual level may be due to intervening diseases also affecting telomere length.

Earlier studies on alcohol and telomeres are remarkably few. In some studies alcohol has been mentioned as a covariate, while three studies in cancer patients or older people reported inconsistent findings [27–29]. Our results are in accordance with an important study comparing heavy [abuse] and lighter [social drinking] alcohol consumption, where heavy use was associated with shorter telomeres. It is of note that in that cross-sectional study shortened telomeres were also associated with moderate (over one drink/day) alcohol consumption among the participants in their 40s [19].

These results may offer clues for the dual health effects of alcohol. Moderate alcohol consumption exerts various and well documented beneficial effects on vasculature, including correction of endothelial dysfunction, increase of HDL cholesterol, and antiaggregate effects [30]. These favourable effects may, however, be counteracted in the long-term through shortening of telomeres. Provided that in our cohort abstainers had lower levels of risk factors in midlife than alcohol consumers [21], and longer telomeres in old age, they should have had clearly lower mortality. As this was not the case [21], it can be speculated that harmful effects of alcohol on telomeres are offset by beneficial health effects.

However, the exact clinical significance and mechanism of telomere shortening by alcohol consumption are currently unknown. In general, telomere shortening may be related to disturbances of cell division, increased oxidative stress, impaired antioxidant function, or interference with telomerase activity [6–9]. Clinical factors include physical and psychological stress, socioeconomic status and diseases and their risk factors. Because telomeres were measured from immune cells (leukocytes), also periods of increased need for immune cells during the life course may contribute to telomere shortening, but it is unlikely that this would be connected to alcohol consumption at social drinking levels. Alcohol consumption was not related to telomerase activity in a small study of patients with oral cancer [31]. Although intensive lifestyle modification was related to increase of telomerase activity [32], the effect of reducing alcohol consumption is not known. The most plausible mechanisms are alcohol-induced increase of oxidative stress [33, 34], inflammation, as well as impaired defense systems against oxidative stress, which in long-term could be reflected in shorter telomeres. In this respect alcohol would be analogous to rigorous exercise, which exerts several positive effects on health, but has also been associated with oxidative stress and shorter telomeres [35].

Strengths of our study include the homogenous and at baseline largely healthy cohort, which reduces confounding by socioeconomical, psychological and nutritional factors, and may help to better elucidate the biological effects of alcohol. We also believe that the reporting of alcohol consumption by Finnish businessmen during the 1970s is likely to be reliable. The data on alcohol consumption probably reflect the peak of alcohol consumption during healthy lifetime on telomeres in old age. Telomere measurements were performed in a group of 499 men, but they represented alcohol consumption of the initial cohort of the 2360 men. For telomere analyses, we used the Southern blot method, which has been the termed as a "gold standard" in telomere research [36, 37].

Our study has also limitations. The cohort characteristics obviously limit generalizability to other ethnic or socioeconomic groups, or to women. Telomere length was measured in old age, and thus restricted to survivors, and we do not have data of changes in LTL with time. We related LTL measured in old age to consumption in midlife, and it can be argued that this does not take into account cumulative effects. However, because lower consumption remained relatively stable and higher midlife consumption clearly decreased, we assume that our results give a conservative estimate of the cumulative effect of alcohol on LTL. Decreasing consumption, possibly due to intervening diseases, is a putative reason for the non-existent relationship between LTL and alcohol consumption in old age. The sample size was not particularly large, but the age-adjusted

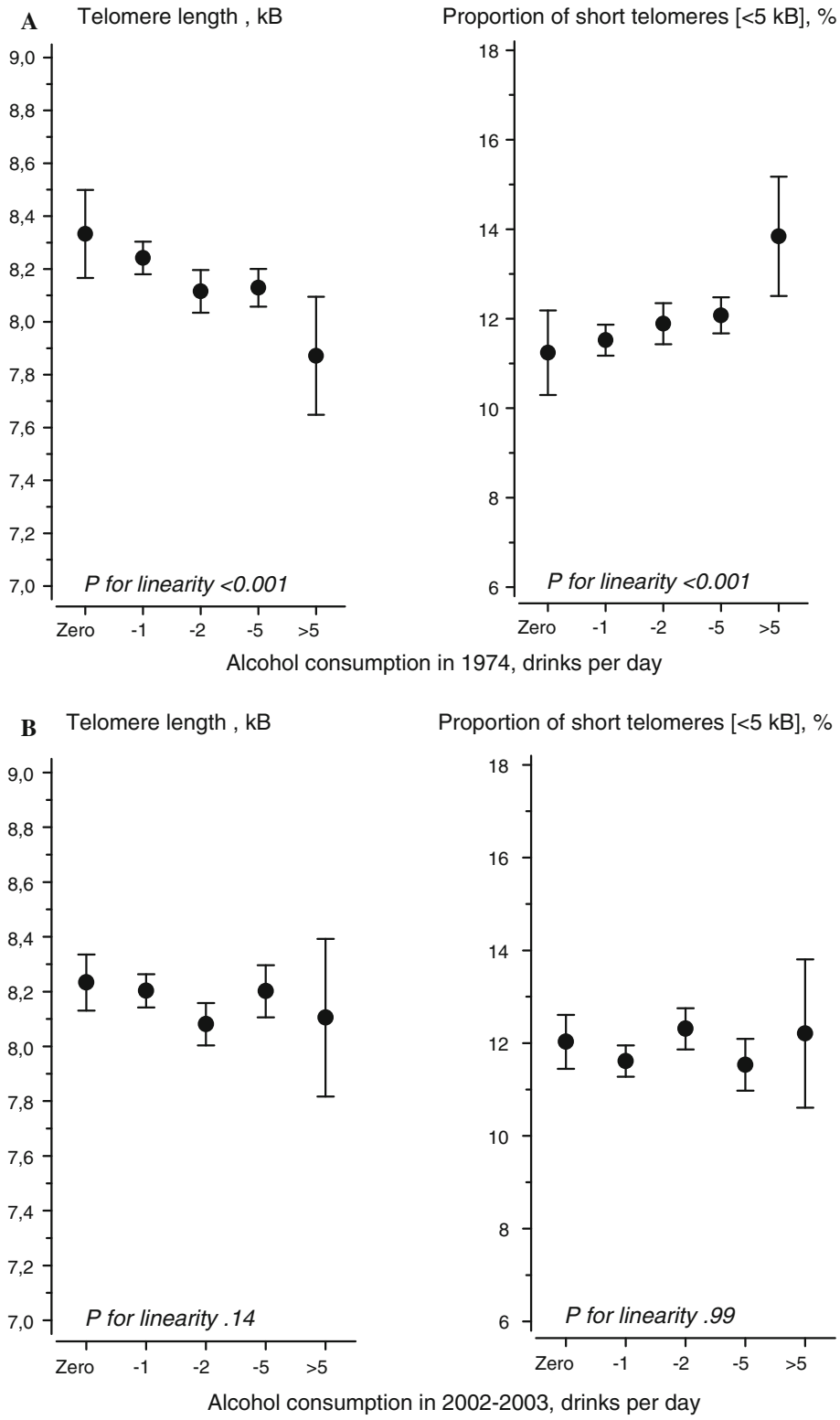

Fig. 3 Telomere characteristics in old age according to alcohol consumption groups in midlife (Panel A), and in 2002–2003 (Panel B). Data are adjusted for age. *kB* indicates kilobases of leukocyte telomere length

findings were nevertheless highly statistically significant. Although we adjusted for several factors related to telomere length, we cannot exclude residual confounding by, for example, genetic, parental (paternal age, [38]), and detailed lifestyle and environmental factors, such as use of omega-3 fatty acids [39], or pollutants possibly associated with alcohol use. We measured telomeres in leukocytes, but there is evidence that they reflect telomere length at least in the vascular cells [40]. Alcohol consumption was based on self-report, and problem drinkers were largely outside our

study. Furthermore, we do not have data about the pattern of drinking, and although we have information about preferences and noted no associations, it is not certain that all types of alcohol beverages have similar effects on telomeres. The clinical impact of the telomere differences is unknown. However, the difference in LTL at old age between alcohol abstainers and heavy drinkers in 1974, about 500 base pairs, corresponds to a difference in "biological age" of 10 years assuming an annual mean LTL shortening of 50 base pairs [38]. Our study is not powered enough to analyze the relations between alcohol, telomeres and diseases, such as cancer.

Conclusion

Our results suggest that even moderate alcohol consumption—which is often recommended for cardiovascular prevention—is in the long-term associated with shorter telomeres. This association was independent of other factors known to shorten telomeres in this male cohort. The findings may help to explain dual effects of alcohol consumption on health.

Acknowledgments TES, FF, OS and RST designed the study and drafted the manuscript. TES directed the implementation of the study, including quality assurance and control. AYS and KHP helped in analytic strategy and reviewed the manuscript. All authors approved the final version, and have participated sufficiently in the work to believe its overall validity and take public responsibility. Professor Tatu A Miettinen contributed to the design and initial preparation of this paper. He died accidentally in November 2011. The data were analyzed solely by the authors, but we wish to thank Hannu Kautiainen, M.Sc. for advice in statistical issues, and Emilie Kvist. M.Sc. for telomere measurements. This work was supported by the Jahnsson Foundation, the University Central Hospitals of Oulu and Helsinki [EVO funding], the Konung Gustaf V:s och Drottning Victorias Frimurarestiftelse, and the Wilhelm and Else Stockmann Foundation. The funding sources had no role in the design and conduct of the study; in the collection, analysis, and interpretation of the data; or in the preparation, review, or approval of the manuscript.

Conflict of interest The authors declare that they have no conflict of interest.

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