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## Brief Communication

# The association between sperm telomere length, cardiorespiratory fitness and exercise training in humans

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## ABSTRACT

Telomeres protect genomic integrity and shorten in somatic cells due to the end replication problem. Sperm telomeres are, however, longer in older individuals and linked to semen quality. Exercise training may attenuate age-related telomere shortening in somatic cells, but the influence of exercise on sperm telomeres is unknown. Mature sperm from 34 healthy men were isolated by density gradient centrifugation and telomere length was assessed by qPCR. No significant correlations were observed between telomere length, fitness or exercise performance. Inter-individual variation in sperm telomere length responses to a 6-wk vigorous exercise training intervention ( $\Delta T/S$  ratio range:  $-0.49$  to  $0.87$ ) and a strong correlation between improvements in fitness and sperm telomere lengthening were revealed ( $r = 0.87$ ,  $p < 0.001$ ). These preliminary data suggest exercise training may regulate sperm telomere length and should encourage larger studies to explore the implications this may have on the health of the next generation.

Genomic integrity is conserved by a repetitive terminal stretch of DNA, called telomeres. Telomeres progressively shorten in somatic cells due to the end replication problem and this process is linked to biological ageing. Whilst the age-related telomere attrition in somatic cells is well accepted, average sperm telomere length is longer in older men relative to their younger counterparts [1]. This observation is due to an underrepresented number of sperm with short telomeres in the older men and provided an explanation as to why their offspring possess longer leukocyte telomeres compared to individuals from younger men [1]. These data suggest offspring from older men may inherit longer

telomeres, which could have implications to their health and lifespan.

The health benefits conferred by regular exercise training are well established. Interestingly, leukocyte telomere length is positively correlated to cardiorespiratory fitness, and findings indicate exercise training may maintain telomere length and attenuate the biological ageing of somatic cells [2]. Therefore, the purpose of this study was to: 1) determine the association between cardiorespiratory fitness and telomere length in human sperm isolated by density gradient centrifugation; and 2) elucidate the impact of a 6-wk vigorous exercise training intervention on sperm telomere length.

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## Material and methods

### Procedures

Thirty-four healthy Caucasian men were included in this study (mean  $\pm$  SD: age: 30.9  $\pm$  9.95 y; height: 180.9  $\pm$  7.68 cm; weight: 85.03  $\pm$  13.69 kg; body mass index: 25.87  $\pm$  2.98; maximum oxygen uptake [ $\dot{V}O_{2max}$ ]: 46.33  $\pm$  9.71 ml kg<sup>-1</sup> min<sup>-1</sup>; maximum power output, 313.23  $\pm$  56.34 W). All subjects donated a morning semen sample, completed a cardiopulmonary exercise test (CPET) to exhaustion on a cycle ergometer and were deemed apparently healthy by health questionnaires. A subset of men (n = 10) completed a supervised 6-wk vigorous exercise training intervention, comprised of thrice-weekly sprint interval training (4–6 maximal sprints with 4-min rest between efforts) performed on Wattbike ergometers (Wattbike Pro, UK). These men donated a second semen sample and completed another CPET approximately three days after their final exercise session. Participants gave written informed consent this study was approved by the University's Human Research Ethics Committee.

### Sperm telomere length quantitation

Semen donation and processing were completed according to the WHO and mature spermatozoa were isolated by density gradient centrifugation using the Puresperm 40/80 reagents (Nidacon). Average telomere length was quantified by quantitative PCR [3] and was expressed as the telomere to single copy gene (T/S) ratio, relative to the T/S ratio of leukocyte DNA from a healthy male donor (28 y). Specifically, DNA was extracted from sperm using the Purelink Genomic DNA Mini Kit (ThermoFisher Scientific). DNA purity was assessed by spectrophotometry (Nanodrop 8000, ThermoFisher Scientific) and was acceptable with average  $\pm$  SD 260/280 and 230/260 ratios, 1.80  $\pm$  0.11 and 1.80  $\pm$  0.50, respectively. Telomere assays were run twice, on separate days, to establish the reproducibility of the data. The intra-assay coefficient of variation (CV) for telomere and 36B4 primer sets was 1.25  $\pm$  0.89% and 0.59  $\pm$  0.32%, respectively. The inter-assay CV for telomere and 36B4 primer sets was 1.24  $\pm$  0.94% and 0.28  $\pm$  0.28%, respectively. As a quality control, only triplicate samples within 1 cycle threshold (Ct) were used in statistical analyses or the average of duplicate samples was used. The telomere, 36B4 primer sets and T/S ratios were highly reproducible ( $R^2 = 0.992$  and  $0.998$ , respectively) and a strong correlation was observed between T/S ratios from plate 1 and plate 2 (n = 33,  $r = 0.84$ ,  $p < 1.26 \times 10^{-9}$ , [Supplementary Figure 1](#)). Data are from two-tailed Pearson's correlation.

### Statistical analyses

Two-tailed Pearson's and partial correlations were used to identify linear relationships between telomere length, age and exercise parameters. Two-tailed independent samples t-tests were used to establish telomere length differences between young and older men. Two-tailed paired-samples t-tests and ANCOVA were used to determine significant changes before

and after the 6-wk training intervention. Statistical significance was set at  $p < .05$ .

## Results

### Older men have longer telomeres in mature sperm

Relative to younger men (n = 16, age = 22.31  $\pm$  1.99 y), older men (n = 18, age = 38.61  $\pm$  7.51 y) possessed longer sperm telomeres [[Fig. 1A](#)]. A positive correlation was observed between age and sperm telomere length ( $r = 0.40$ ,  $p = .02$ , [[Fig. 1B](#)]). The positive linear relationship between age and sperm telomere length remained statistically significant when adjusted for  $\dot{V}O_{2max}$  ( $r = 0.39$ ,  $p = .025$ ).

### Exercise, cardiorespiratory fitness and sperm telomere length

No statistically significant correlations were observed between sperm telomere length and maximal power output or  $\dot{V}O_{2max}$  ([Fig. 1C, D](#)), respectively, both  $p > .05$ . There were large inter-individual responses in sperm telomere length after 6 weeks of exercise training ( $\Delta$ T/S ratio range:  $-0.49$  to  $0.87$ ). No statistically significant changes were observed in sperm telomere length after the 6-wk exercise training intervention (mean T/S ratio  $\pm$  SD: 1.73  $\pm$  0.52 to 1.76  $\pm$  0.49,  $p = .80$ , [[Fig. 1E](#)]). A strong positive correlation was observed between the alteration in sperm telomere length and change in  $\dot{V}O_{2max}$  after exercise training ( $r = 0.87$ ,  $p < .001$ , [[Fig. 1F](#)]).

## Discussion

Exercise training may maintain leukocyte telomere length to attenuate premature biological ageing and prevent age-related disease [2], yet the impact of exercise on telomere biology in germ cells is unknown. This is the first study to explore the association between short-term vigorous exercise training, cardiorespiratory fitness and sperm telomere length in mature human sperm. Consistent with previous findings [1,4], older men, on average, possessed longer sperm telomere length compared to their younger peers. The correlation between age and sperm telomere length was relatively unchanged when controlled for  $\dot{V}O_{2max}$ , indicating the relationship is independent of cardiorespiratory fitness. Notably, the effects of age on telomere length are obvious even between individuals with a narrow age difference ( $\sim 16$  y) from young and older individuals from the present study. The translational health implications of these findings could be profound, given the link between leukocyte telomere shortening, mortality and age-related disease, and that paternal age is associated with longer leukocyte telomeres in offspring [1,5].

Observations have reported positive correlations between cardiorespiratory fitness and leukocyte telomere length [6–8]. Considering the linear association between leukocyte and sperm telomere lengths in young men [5], the lack of association between sperm telomere length and cardiorespiratory fitness was surprising. It will be important to confirm this

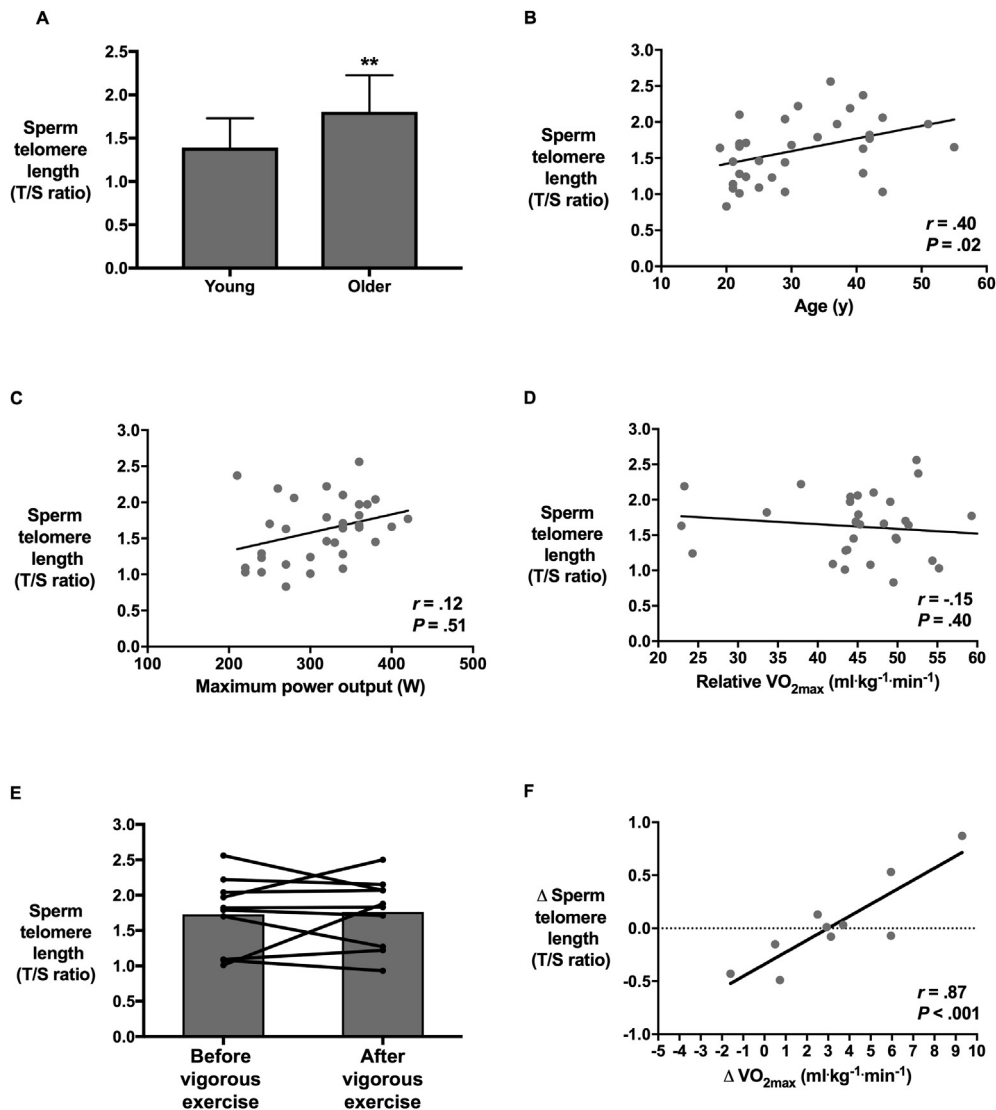


Fig. 1 Human sperm telomere length, age, cardiorespiratory fitness and exercise training. Sperm telomere length was longer in older ( $n = 18$ ; age =  $38.61 \pm 7.51$  y; T/S ratio:  $1.80 \pm 0.34$ ) versus the younger ( $n = 16$ ; age =  $22.31 \pm 1.99$  y; T/S ratio:  $1.39 \pm 0.42$ ) men ( $p = .004$ ) (A). Data are from independent samples t-test. A statistically significant positive correlation was observed between average telomere length in mature sperm and age (B). No statistically significant correlations were observed between sperm telomere length, maximum power output (C) and relative  $\dot{V}O_{2max}$  (D). Data are from Pearson's correlation. No statistically significant changes to sperm telomere length were observed after 6 weeks of three times per week vigorous exercise training (sprint interval training), comprised of 4–6 repetitions of 30-s maximal sprints on a Wattbike cycle ergometer, followed by 4-min rest periods (average T/S ratio  $\pm$  SD:  $1.73 \pm 0.52$  to  $1.76 \pm 0.49$ ,  $p = .80$ ) (E). Data are from Pearson's correlation. (F) A positive correlation between the change in sperm telomere length and improvement in  $\dot{V}O_{2max}$  after 6 weeks of vigorous exercise training. Abbreviations:  $\dot{V}O_{2max}$ : maximum oxygen uptake; CPET: cardiopulmonary exercise testing;  $\Delta$ : delta (change).

finding in a larger cohort, across a wider range cardiorespiratory fitness levels. No statistically significant changes in sperm telomere length were found after short-term vigorous exercise training, probably due to the modest sample size and inter-individual responses common amongst exercise adaptations. Interestingly, a strong correlation was observed between the exercise-induced improvement to  $\dot{V}O_{2max}$  and change in sperm telomere length, indicating high responders had telomere lengthening whereas low responders showed telomere shortening. This study was not designed to identify the potential mechanisms explaining this observation, but is

tempting to speculate that specific individuals may be vulnerable to the physiological stress caused by vigorous exercise, such as changes in semen quality or oxidative stress. Indeed, sperm telomere length is related to semen quality – sperm count, aneuploidy and DNA fragmentation [9]. Although mild oxidative stress may encourage sperm telomere maintenance, severe concentrations are associated with shortening [10]. Vigorous exercise increases reactive oxygen species [11,12] and the discordant sperm telomere length responses after training could be reflective of the different endogenous redox capacities amongst subjects. Nonetheless,

the inter-individual response in sperm telomere length after exercise training deserves future attention.

The modest sample size is a limitation of the study. This study, however, was an exploratory investigation to provide grounds for future work. Another limitation is that the observed changes in telomere length could be explained by day-to-day variations in semen quality. Although sperm samples were collected following the World Health Organisation's guidelines and motile sperm were isolated by an IVF-grade density gradient, future work should examine multiple sperm samples from the same individual before and after exercise interventions to control for such fluctuations in semen quality. A strength of the study is that the subjects were apparently healthy men and were not seeking sperm analyses or other prostate/ART services. Moreover, mature sperm were isolated by density gradient in this study, which prevented contaminating somatic cells, found in whole sperm samples, influencing telomere length.

## Conclusions

In summary, this is the first study to analyse the association between cardiorespiratory fitness and short-term vigorous exercise training in context with human sperm telomere length. This study provides additional evidence that suggests older men possess longer sperm telomeres compared to their younger peers. Additionally, a novel relationship between exercise-induced improvements to cardiorespiratory fitness and telomere dynamics was observed that could indicate exercise training influences telomere length in sperm. Although future work is required to verify these findings, they should encourage larger, detailed studies on the effect of different modes and doses – frequency, intensity and duration – of exercise training on human sperm telomere biology, and translational implications they may have on the subsequent generation.

## Conflicts of interest

The author does not have any conflicts of interest to declare.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.bj.2019.07.003>.

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