# Telomere Length, SIRT1, and Insulin in Master Athletes: The Path to Longevity?

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## ABSTRACT

Lower SIRT1 and insulin resistance are associated with accelerated telomere shortening. This study investigated whether the lifestyle of master athletes can attenuate these age-related changes and thereby slow aging. We compared insulin, SIRT1, and telomere length in highly trained male master athletes (n = 52; aged 49.9 ± 7.2 yrs) and age-matched non-athletes (n = 19; aged 47.3 ± 8.9 yrs). This is a cross-sectional study, in which all data were collected in one visit. Overnight fasted SIRT1 and insulin levels in whole blood were assessed using commercial kits. Relative telomere length was determined in leukocytes through qPCR analyses. Master athletes had higher SIRT1, lower insulin, and longer telomere length than age-matched non-athletes ($p < 0.05$ for all). Insulin was inversely associated with SIRT1 ($r = -0.38$; $p = 0.001$). Telomere length correlated positively with SIRT1 ($r = 0.65$; $p = 0.001$), whereas telomere length and insulin were not correlated ($r = 0.03$; $p = 0.87$). In conclusion, master athletes have higher SIRT1, lower insulin, and longer telomeres than age-matched non-athletes. Furthermore, SIRT1 was negatively associated with insulin and positively associated with telomere length. This suggests that in the studied middle-aged participants, there is a connection between reduced insulin, increased SIRT1 activity, and attenuation of biological aging.

## Introduction

Aging is caused by several biological processes, with the function of telomeres being a major regulator of cellular aging [1–3]. Telomeres are sequences of double-stranded DNA located at the end of chromosomes and are fundamental for DNA replication and chromosome integrity [4, 5]. Telomeres in somatic cells shorten in

each cell division and once shortened to a critical length, cells enter replicative senescence [4]. These senescent cells are more likely to undergo apoptosis if exposed to increased oxidative damage [4, 6]. The significance of excessive telomere shortening is reflected by its association with a higher risk of aging- and obesity-related chronic diseases, including insulin resistance and type 2 diabetes [7].

Recent studies have shown that sirtuin 1 (SIRT1) could play an important role in the maintenance of telomeres and insulin action during aging [8]. SIRT1 belongs to a class of NAD-dependent enzymes that can deacetylate a variety of genes, including FOX01, UCP2, PGC-1α, NF-κB, and p53, which results in their enhanced transcription that in turn reduces systemic inflammation and enhances glucose metabolism, insulin secretion, mitochondrial function, and cell survival [2, 9]. In this context, aerobic capacity and strength have shown promising associations with levels of SIRT1 [10–12] better glucose homeostasis [13], and longer telomeres [14, 15].

Master athletes are individuals over the age of 35 years who train and compete in organized events, designed specifically for middle-aged and older adults [16, 17]. As master athletes reach the upper limits of health and physical capacity, they are a unique model of healthy aging, not confounded by morbidities and reduced physical activity levels that are typically seen in the general aging population. There is some evidence that master athletes have higher levels of SIRT1 mRNA [18], better insulin sensitivity [19], and longer telomeres [14, 15] than age-matched controls. However, to our knowledge, the possible interactions between SIRT1 levels, insulin secretion, and telomere length in older individuals with a healthy lifestyle, such as master athletes, remains to be investigated.

Therefore, the aim of this study was (1) to compare insulin, SIRT1, and telomere length in high-level master athletes and age-matched non-athletes; and (2) to assess the relationships among these variables. We hypothesized that master athletes have lower insulin, higher SIRT1, and longer telomeres than their age-matched controls, where telomere length is positively associated with SIRT1 and inversely associated with insulin levels.

## Materials & Methods

### Ethical approval

This article is part of a larger study approved by the Human Research Ethics Committee of the Catholic University of Brasília (protocol number: 3.779.535). It was conducted according to the Declaration of Helsinki and in accordance with ethical standards of the journal [20]. Furthermore, all subjects provided written informed consent before participation.

### Participants

Master athletes were recruited from national and international athletic meetings and word of mouth from athletes to other athletes. The inclusion criteria for master athletes were: (1) continuously training for at least 15 years for various sprint or endurance events; and (2) previously and currently competing in national and international events at the time of data collection. The inclusion criterion for middle-aged non-athletes was to be sedentary. All participants were healthy.

### General procedures

All participants arrived at the laboratory in the morning (between 7:00–8:00 a.m.), in an 8-hour fasted state for venous blood sampling (8 mL) in tubes with EDTA (BD Vacutainer; Becton, Dickinson and Company, Franklin Lakes, NJ, USA). Participants also underwent an anamnesis, anthropometric measures, and completed a questionnaire regarding general training characteristics. Body composition was assessed as previously described with skin folds [14].

The SIRT1 (Abcam, São Paulo, Brazil) and insulin (Abcam) concentrations in serum were determined in duplicate using the specific human enzyme-linked immunosorbent assay (ELISA) kits, respectively. The detectable limit for insulin and SIRT-1 were 1.1 pmol/L and 132 pg/mL, respectively. Both assays had intra- and inter-assay CVs of less than 14 %. Telomere length was measured in DNA extracted from peripheral blood nuclear cells using real-time PCR as described previously [21, 22] and given as the T/S ratio (leukocyte telomere length adjusted by a young sample subject).

### Statistical analysis

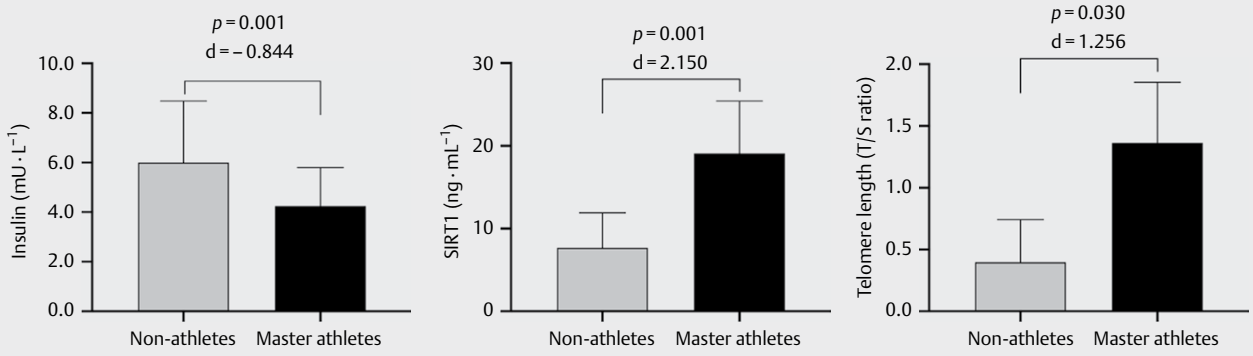
The normality of the data was assessed with the Shapiro-Wilk test. Data were expressed as mean ± standard deviation (SD). Independent samples t-tests were conducted to compare all variables between groups, expressed as t value (df). Pearson's correlation coefficients were calculated to determine the associations between SIRT1, insulin, and telomere length. Furthermore, Cohen's d was used to verify the effect size of the comparisons [23]. The following classification to measure the magnitude of effect size was used: small, d = 0.2–0.49; moderate, d = 0.5–0.79; and large, d > 0.8. The significance level was set at $p < 0.05$. For the main analyses, the total sample size conferred a statistical power of 87 % considering the significance level at $\alpha = 0.05$ and a moderate effect size of d = 0.6. The sample size (n = 26) of correlations involving the telomere length was lower than the total sample size owing the small amount of tissue available for analysis. All procedures were performed using Prism version 8.0 (GraphPad, San Diego, CA, USA), G * power version 3.1 (Heinrich Heine University, Düsseldorf, Germany), and SPSS version 26 (IBM Corporation, Armonk, NY, USA).

## Results

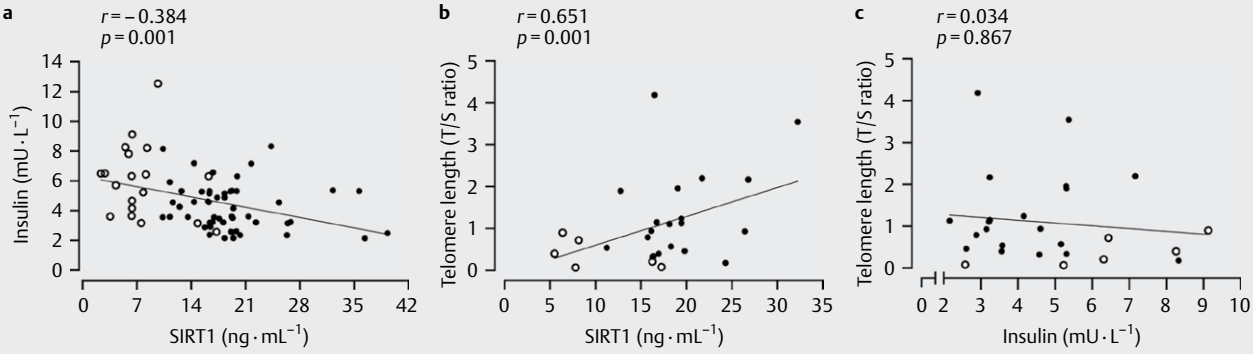
The sample of master athletes had 26.3 ± 9.2 years of training in endurance (10 km to marathon) or sprint/power events (60–800 m, 110 and 400 m hurdles, and the long and triple jump) and participated in 6.9 ± 3.8 competitions per year. The aerobic training volume per week was 6.3 ± 1.4 hours and the weekly strength/power training volume was 4.4 ± 2.1 hours. The maximal time of uninterrupted inactivity (e. g., due to injury, personal reasons, etc.) was 10.4 ± 16.7 months. All athletes in our sample reached the podium at least once in the national, South American, and/or world master championships. The final sample size (n = 71) was comprised of 52 male master athletes (21 endurance runners and 31 sprinters) and 19 untrained middle-aged men. The characteristics of the sample are displayed in ▶ **Table 1**, showing that age-matched non-athletes had an 18 % higher body mass (d = 1.386) and 41 % higher body fat (d = 2.252) content ($p = 0.001$) than the master athletes. Age (d = − 0.295) and height (d = 0.784) did not differ between the groups.

▶ **Table 1** General characteristics of middle-aged non-athletes and master athletes. Data expressed as mean and (±) standard deviation.

| | Non-athletes (n = 19) | Master athletes (n = 52) | t value (df) | *p* value | Cohen's d |
|---|---|---|---|---|---|
| Age (years) | 47.6 ± 8.9 | 50.0 ± 7.3 | − 1.13 (27.27) | 0.26 | − 0.295 |
| Body mass (kg) | 91.0 ± 14.8 | 74.7 ± 7.6 | 6.08 (21.59) | 0.001 | 1.386 |
| Height (m) | 1.79 ± 0.06 | 1.75 ± 0.04 | 1.05 (25.41) | 0.30 | 0.784 |
| Body fat (%) | 23.1 ± 4.6 | 13.6 ± 3.8 | 8.81 (27.70) | 0.001 | 2.252 |




▶ **Fig. 1** Insulin, SIRT1, and telomere length of middle-aged non-athletes and master athletes. * Statistical difference. Data expressed as mean ± SD. Insulin (non-athletes n = 19, master athletes n = 52); SIRT1 (non-athletes n = 19, master athletes n = 46); telomere length (non-athletes n = 12, master athletes n = 20).




▶ **Fig. 2** Correlation analysis among SIRT1, insulin, and telomere length of middle-aged non-athletes and master athletes. Figure **a** (n = 71); Figure **b** (n = 26); Figure **c** (n = 26). Master athletes are indicated by filled circles and non-athletes are indicated by empty circles.

Insulin of master athletes (4.24 ± 1.55 mU · L$^{-1}$) was lower ($p$ = 0.001) than the age-matched control group (5.99 ± 2.49 mU · L$^{-1}$). SIRT1 was higher ($p$ = 0.001) in master athletes (19.2 ± 6.3 ng · mL$^{-1}$) compared to age-matched non-athletes (7.69 ± 4.20 ng · mL$^{-1}$). Master athletes had longer ($p$ = 0.030) telomeres (1.38 ± 1.05 T/S ratio) than those of age-matched non-athletes (0.40 ± 0.34 T/S ratio). The effect size analysis indicated a large difference between the two groups for SIRT1 (d = 2.150), insulin (d = − 0.844), and telomere length (d = 1.256) (▶ **Fig. 1**). There were no significant differences between endurance runners and sprinters for SIRT1, insulin, and telomere length (p > 0.05).

SIRT1 correlated inversely with insulin ($r$ = − 0.38; $p$ = 0.001) and positively with telomere length ($r$ = 0.65; $p$ = 0.001). Telomere length and insulin were not correlated ($r$ = 0.03; $p$ = 0.872; see ▶ **Fig. 2**).

## Discussion

We found that master athletes have lower insulin, higher SIRT1, and thus longer telomeres than age-matched controls. Furthermore, our results indicate that lower insulin was associated with higher SIRT1, and higher SIRT1 with longer telomeres.

Mechanical and metabolic stimuli during physical exercise induce inflammatory and oxidative response and enhance DNA da-

mage repair, mitochondrial biogenesis, and insulin sensitivity and function [15, 19, 24, 25]. Improved insulin sensitivity reflects better glycemic control and lowered baseline insulin. Insulin levels and IGF-1 are directly associated with mTor and inversely correlated to AMPK pathways [26, 27]. AMPK and SIRT1 both regulate each other and share many common target molecules related to lifespan [28]. In our participants, the negative association of insulin to SIRT1 and positive correlation of SIRT1 to longer telomeres give clues of possible mechanisms by which exercise improves lifespan. Recent studies suggest that the increase in SIRT1 is a primary mediator of these changes [2, 8, 9]. Indeed, exercise-induced SIRT1 can decrease the expression of pro-inflammatory cytokines, such as TNF-α and IL-6, by deacetylation of NF-κB, and increased FOX03a activity, which leads to an increased catalase expression that help reduce reactive oxygen species level [10, 12, 13]. In addition, SIRT1 deacetylates PGC-1α, a transcription factor that plays a pivotal role in biogenesis and mitochondrial function [10, 12]. Recently, Koltai et al. [18] demonstrated that master athletes have an elevated level of SIRT1 mRNA compared to age-matched sedentary individuals. Taken together, our results and those by Koltai et al. [18] indicate that vigorous lifelong training elicits an enhanced antioxidant and anti-inflammatory defense system mediated by SIRT1.

The master athletes in our study had lower insulin than the age-matched non-athletes. Kusy et al. [19] reported that sprinter and endurance master athletes have greater insulin sensitivity and pancreatic β cell function compared to untrained individuals. In addition, master athletes (sprinter and long-distance runners) have a lower age-related decline in insulin sensitivity than untrained individuals [17].

In the current study, we found that SIRT1 correlated inversely with insulin. Several studies have shown that SIRT1 plays an important role in the regulation of insulin secretion from pancreatic β cells [2, 9]. SIRT1 deacetylates FOX01 in β cells, improving insulin secretion. In addition, SIRT1 reduction may lead to pancreatic β cell apoptosis [29, 30], and increasing SIRT1 might prevent a decrease in β-cell mass. In the liver, SIRT1-mediated deacetylation of FOX01 and PGC-1α is associated with improved hepatic glucose metabolism [2, 9]. Recently, Koltai et al. [18] demonstrated that master athletes have a higher level of FOX01 mRNA than age-matched controls. Therefore, altogether, these data suggest that glucose is maintained with less insulin, indicative of lower insulin resistance in master athletes.

Our observation of longer telomeres in master athletes than in non-athletes is consistent with previous studies [14, 15, 31, 32]. In the present study, telomere length was positively associated with SIRT1, something also seen by others [33]. Rather than a genetic predisposition of master athletes, our finding may represent an adaptation to increased activity level, as supported by a study in the elderly that 12 weeks of strength training resulted in both longer telomeres and a tendency to elevated SIRT1 expression [34]. This may well be a causal relationship, as it has also been reported that increased expression of SIRT1 results in longer telomeres and greater telomere integrity after DNA damage [8, 9, 35]. Therefore, it appears that SIRT1 plays a key role in reducing telomeric attrition with long-term training.

Some limitations of this study warrant consideration. We did not measure some proteins (e. g., FOX01, PGC-1α, and NF-κB) that could provide additional mechanistic understanding of the interactions among SIRT1, insulin, and telomere length. However, it is already well-established that SIRT1 deacetylates FOX01, PGC-1α, and NF-κB, and their deacetylation as a consequence of an exercise-induced rise in SIRT1 contributes to the positive effects of regular exercise on glucose metabolism, insulin secretion, mitochondrial function, inflammatory response, and telomere length [10–12]. A dietary assessment could be especially important for the insulin response. We acknowledge that insulin and glucose are considered crude proxy measures of insulin sensitivity at best. In addition, the sample size of the non-athletes would be higher to provide more significant differences and correlations, and lastly, due to the cross-sectional design, care must be taken in both causal and temporal interpretations.

## Conclusion

In conclusion, master athletes have higher SIRT1, lower insulin, and longer telomeres than age-matched non-athletes. Furthermore, SIRT1 was negatively associated with insulin and positively associated with telomere length. This suggests that in the studied middle-aged participants there is a connection between reduced insulin, increased SIRT1 activity, and attenuation of biological aging.

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### Conflict of Interest

The authors declare that they have no conflict of interest.

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