Cellular senescence in vitro and organismal ageing

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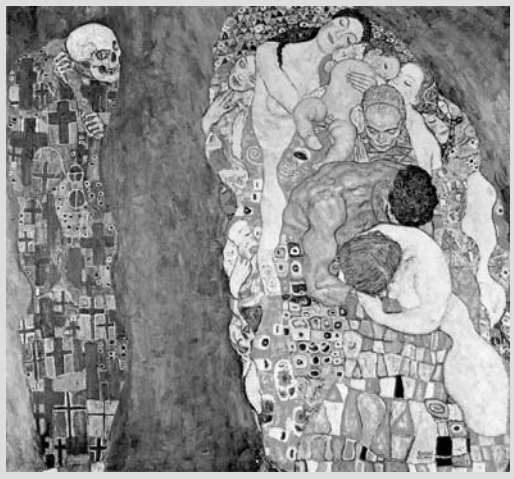

Chapter 6

Relation between body height and replicative capacity of human fibroblasts in nonagenarians

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Summary

Replicative capacity of fibroblasts has been used as a model of *in vitro* aging and is related to longevity of species. Recently, it has been shown across species that body size is the primary correlate of replicative capacity rather than longevity. To test the relationship between body size and replicative capacity within species, we cultured fibroblasts obtained from 57 nonagenarians until the onset of senescence. We found a highly significant inverse relation between height and replicative life span in humans of the same chronological age (r = -0.35, p = 0.007). Moreover, men were taller than women and had significantly lower replicative capacity (69.5 vs 75.2 population doublings, p = 0.03). No such correlation was found between replicative capacity and weight. The data suggest that tallness is associated with a lower replicative capacity of fibroblasts reflecting, when taken from nonagenarians, a diminished reserve capacity due to a higher number of past doublings during growth and regeneration over the life span.

Introduction

The maximal proliferative capacity of fibroblasts *in vitro* has been positively correlated with longevity across species (Hayflick, 1974; Rohme, 1981). Only a few researchers were not able to find this association (Stanley *et al*., 1975). As body size is positively related to longevity across mammalian species as well (Kirkwood, 1992; Promislow, 1993), it has also been studied whether replicative capacity of fibroblasts correlated with both longevity and body mass (Lorenzini *et al*., 2005). Interestingly, body mass is an even better correlate for the replicative capacity across species than average longevity (Lorenzini *et al*., 2005). A drawback of these studies is that they mainly compared differences between species from very different taxonomic groups and differed widely in life history characteristics and in the selective pressures to which these species have been exposed.

Within species, the remaining replicative capacity has been related to chronological age. Recently published studies in humans did not find a correlation between cellular characteristics and the chronological age of the participant (Cristofalo *et al*., 1998; Smith *et al*., 2002). In contrast to the positive relation of body mass and longevity across species, longevity declines with increasing body mass within species. This has been reported for various mammals including rat, mouse, dog, and human (Nohynek *et al*., 1993; Li *et al*., 1996; Rollo, 2002; Samaras *et al*., 2003; Speakman *et al*., 2003). No study has analyzed the relation between body size and cellular proliferative capacity in humans, and only one study did so in dogs, and, in line with the hypothesis, found this to be inversely related (Li *et al*., 1996).

Here, we tested the relation between body size and the replicative capacity of fibroblasts in 57 participants aged 90 years who were recruited from the second cohort of the Leiden 85-plus Study (der Wiel *et al*., 2002).

Material and Methods

All participants were in relatively good physical condition and undertook a comprehensive series of physiological tests, including the measurement of standing body height (cm), arm span (cm), and weight (kg). Three millimeter full-thickness skin biopsies were taken from the mid-upper medial arm. Fibroblast cultures were established and grown under standardized conditions to the end of their maximal replicative capacity (Maier *et al*., 2007). All participants gave informed consent, and the medical ethical committee of the Leiden University Medical Center approved the study. Anthropometric measurements and population doublings (PDs) are expressed as the mean ± standard deviation (SD); gender differences were analyzed with the *t* test. The maximal proliferative capacity was correlated with anthropometric measurements by using the Pearson correlation. *P* values < 0.05 were considered significant. All statistical analyses were performed using SPSS 12.00 software (SPSS, Inc., Chicago, IL).

Results

As expected, height, arm span, and weight were significantly higher in men compared to women (Table 1). The number of PDs at the stage of senescence

Table 1. Characteristics of test participants.

		Sex specific		
	All (n=57)	Women (n = 36)	Men (n = 21)	*p* value
Height, cm	160.6 (8.7)	155.7 (5.7)	169.1 (6.1)	< 0.001
Arm span, cm	168.7 (9.5)	163.3 (6.4)	178.0 (5.8)	< 0.001
Weight, kg	69.8 (11.2)	66.8 (11.5)	75.1 (8.6)	0.003
BMI	27.0 (3.9)	27.5 (4.3)	26.3 (3.3)	0.251
Max. proliferative capacity, PD	73.1(10.0)	75.2 (10.3)	69.5 (8.7)	0.030

BMI = body mass index.

differed widely, with an average number of 73.1 PDs (standard deviation [SD] 10, range 51–108). Fibroblasts from nonagenarian men had a significantly lower remaining replicative capacity when compared to their significantly smaller female counterparts ($p = 0.03$). As shown in Figure 1, shorter people had a significantly higher remaining replicative capacity compared to taller people (Pearson correlation $r = –0.35$, $p = 0.007$). Within men, the inverse correlation remained statistically significant but not so among women (Table 2). A significant inverse relation was also found between the maximal replicative capacity and arm span (Pearson correlation $r = –0.29$, $p = 0.031$), but not for the weight of the participants (Pearson correlation $r = 0.06$, $p = 0.65$).

Figure 1. Maximal proliferative capacity dependent on height and weight. Men are represented by open circles and women by closed circles.

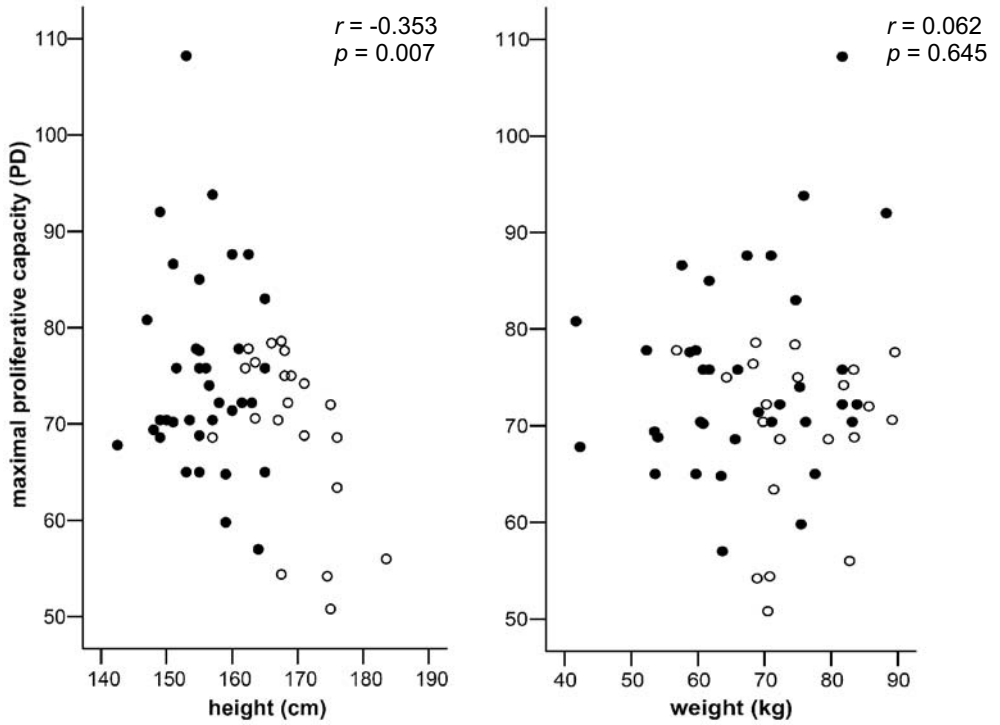

Table 2. Correlation between the maximal replicative capacity and body size in men and women.

	All (n=57)		**Women (n=36)**		**Men (n=21)**	
	r	*p* value	*r*	*p* value	*r*	*p* value
Height, cm	-0.353	0.007	-0.062	0.721	-0.554	0.009
Arm span, cm	-0.286	0.031	0.079	0.646	-0.576	0.006
Weight, kg	0.062	0.645	0.232	0.173	0.041	0.859
BMI	0.339	0.010	0.307	0.069	0.337	0.135

BMI = body mass index.

Discussion

The only corresponding study in dogs found a similar inverse correlation between the replicative capacity of fibroblasts and body size (Li *et al*., 1996). One explanation for the lower replicative capacity of fibroblasts within species is that a higher number of PDs are needed to achieve and maintain tallness, both during the growth phase and during the regeneration over the lifetime. All participants were aged 90 years and the relation is thus not distorted by difference in chronological age. As there could be factors other than height, which contribute to the replicative capacity of fibroblasts that were not accounted for, a causal relationship cannot be confidently asserted; however, in our earlier study we could not find an association between the replicative capacity and health characteristics (Maier *et al*., 2007).

Height is under strong genetic control (Silventoinen *et al*., 2000), whereas metabolic pathways have been shown to influence stature as well as longevity (van Heemst *et al*., 2005). In nonagenarians, a lower activity of the insulin/IGF-1 (insulin-like growth factor-1) signaling (IIS) pathway is significantly associated with lower body height and improved old age survival (van Heemst *et al*., 2005). A lower exposure to GH (growth hormone)/IGF-1 signaling triggers changes in cellular metabolism, such as higher levels of antioxidant defences and/or lower levels of reactive oxygen species (Brown-Borg and Rakoczy, 2003), which positively affects the remaining proliferative capacity of these cells *in vitro*

(Saretzki and von Zglinicki, 2002). Therefore, genetic differences in pathways regulating antioxidant defence mechanisms may significantly contribute to the identified correlation between PDs and height.

The fact that we detected this correlation in men, but not in women may have been due to the small sample size or reflect a sexual dimorphism. Gender differences in antioxidant defense have been found in rats, showing a lower production of mitochondrial reactive oxygen species in female compared to male rats (Borras *et al*., 2003; Jang *et al*., 2004). Interestingly, the mean life span of these female rats was significantly longer compared to male rats. In contrast to our results, gender differences have not been reported by others analyzing the replicative capacity of human fibroblasts (Cristofalo *et al*., 1998). But, in line with our findings, telomere length in adults have been reported to be shorter in men compared to women (Cawthon *et al*., 2003; Mayer *et al*., 2006), while these did not differ in the newborn (Okuda *et al*., 2002).

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