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ORIGINAL ARTICLE

Exercise Against Aging: Darwinian Natural Selection Among Fit and Unfit Cells Inside Human Body

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Abstract
Exercise inevitably induces damages and triggers a brief inflammation in challenged tissues of the human body. Nevertheless, regular exercise is associated with improved physical fitness and lower all-cause mortality among adults in a dose-dependent manner. The paradox between destructive nature of exercise and its anti-aging benefit can be best explained by decreasing aged cell population of the human body in a Darwinian natural selection fashion, resulting in tissue renewal. In this concept, the unfit-to-fit cell ratio of a multicellular system increases during growth (expansion of cell population and size) and decreases after exercise challenges. Inflammation serves as an innate mechanism to recognize cells in danger and triggers clearance mechanism to eliminate unhealthy cells followed by regeneration. A recent finding of decreased $p16^{INK4a+}$ senescent cells together with $CD68^{+}$ macrophage infiltration in human skeletal muscle after resistance exercise supports this concept. The senescent cells are mostly stem cells located in capillaries surrounding myofibers, functioning to replace short-lived endothelial cells. They can be found in young men aged 20–25 years. In this context, exercise controls weight gain (i.e. cell number and size) and decrease senescent cell proportion in capillaries of the human body, providing benefits in physical fitness and increasing life expectancy.

Keywords Anti-aging · Inflammation · $p16^{INK4a}$ · Macrophage · Fitness · Aged cell · Cell senescence · Exercise training · Intensity

Growth is the Fundamental Cause of Aging

There is a lack of consensus on definition of aging in scientific community. Lao Tzu, an ancient Chinese philosopher, described in Dao De Jing (a key literature of Taoism) that aging occurs at a time when accumulation of mass and energy of a growing life can no longer be persisted. This principle shares some similarity with the Second Law of Thermodynamics, implicating an inevitable collapse (decreases in mass followed by death) of any metabolic system that pursues endless growth (increases in mass). Unfortunately, growth is a major feature of life. Multicellular organisms like humans can be described as a society-like economic system, expanding from a single fertilized cell into trillions of cells until reaching peak weight followed by a weight loss (Fig. 1). It is quite comprehensive that accumulation of cells during growth brings physical instability regardless how sophisticated a metabolic system is designed (Fig. 2), witnessed by an increased baseline inflammation during weight gain [1]. Inflammation is a sign of unhealthy state in peripheral tissues. Slowing growth rate by restricting intake of building blocks for cell proliferation such as protein [2] and total calorie [3] delays death and improves metabolic health for small animals with similar genetic condition. Conversely, high protein intake is significantly associated with decreased longevity in humans [4]. Protein provides nitrogen and carbon sources for DNA synthesis during cell number increase. Lao Tzu's view suggests the transferability of this knowledge among all forms of life naturally evolved to maximize length of age.

Spontaneous physical activity, reflecting capability to recover from each voluntary movement within a short time, is a key measure for quality of life. Growth in size decreases spontaneous physical activity. In humans, the steepest decline in physical activity occurs during the ages of 13–18 years when body size increases rapidly [5]. Senescent cells in

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"Aging occurs at a physical state when flourished peak is achieved during growth"

(Chapter 30 物壯則老)

"Periodic loss could be beneficial for long-term survival"

(Chapter 48 為道日損)

Lao Tzu, ~6 century BC

Fig. 1 Definition of aging in Taoism

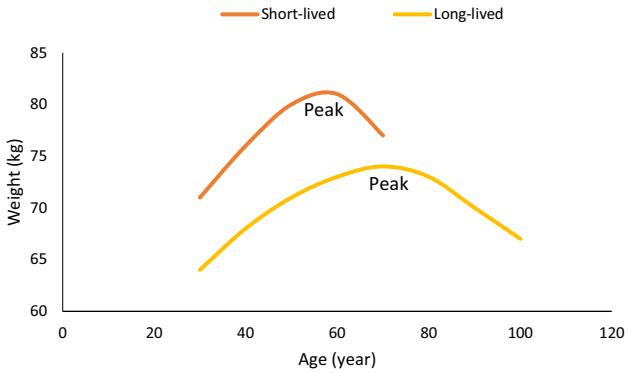

Fig. 2 Rise and fall in weight of human body. Human life is characterized by a long period of growth followed by a short drop in weight. Short-lived men are characterized by a faster growth to peak weight with a quicker weight loss at the end period in contrast with long-lived men. Early rise in weight is probably the cause of earlier and faster weight loss during the end stage. Weight trajectory data from short-lived and long-lived men were reported from Baltimore Longitudinal Study of Aging (1958–2005) observed beginning at age of 19 years until death with illustrated graph reproduced according to Alley et al. [23]

capillaries of human skeletal muscle can be detected by the age 20–25 years [6] (Fig. 3a). Further analysis reveals that those senescent cells in vastus lateralis muscle are endothelial stem cells ($CD34^+$) (Fig. 3b), which functions to replace short-lived endothelial cells [7, 8] (Fig. 3b). Endothelial cells in capillaries surrounding myofibers have a short lifespan for about 2 weeks [7, 8]. A positive correlation between senescent cell proportion ($p16^{INK4a+}$) and weight gain has been recently reported in small animals with the same birth age under manipulated condition to achieve different growth rate [9], supporting the view of that growth is the cause of aging.

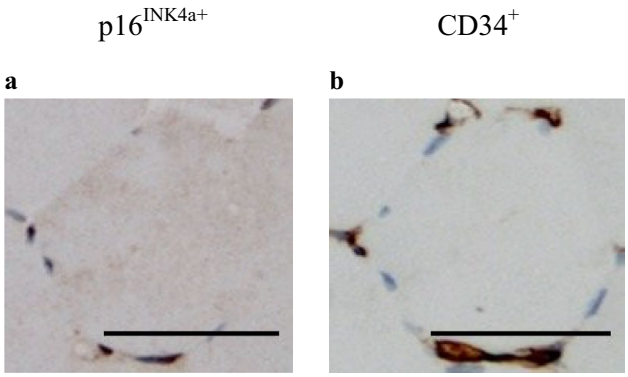

Fig. 3 Senescent stem cells in capillaries surrounding myofibers of human skeletal muscle from healthy young men aged ~20 years. Representative serial immunohistochemical staining Image **a** indicates that the detected senescent cells ($p16^{INK4a+}$) are mostly stem cells ($CD34^+$) Image **b** located in capillaries of human skeletal muscle

Increasing Physical Activity Level Extends Survival Time in Men

The gold standard to evaluate outcome of anti-aging interventions is longevity, or alternatively, all-cause mortality with a prolonged period of observation. All-cause mortality represents the fitness against miscellaneous challenges from our daily life. Despite exercise is an entropic challenge, an inverse relationship between all-cause mortality and physical activity level has been reported [10, 11]. Risk reduction in all-cause mortality is largest for vigorous exercise, whereas moderate-intensity activities of daily living are beneficial to a minor extent [10]. In a population based cohort study ($N = 2205$, aged > 50 years) with a follow-up over 35 years, increasing physical activity level from low or moderate to high significantly increases survival rate of participants, whereas those switches intensity from high to moderate or low level shows increased all-cause mortality [12]. Studies in animals adapting voluntary exercise model are not convincing due to its difficulty to encourage movement as animal ages.

Resistance Exercise Eliminates Senescent Cells in Human Body

Fitness of a human body relies on keeping normal cell population at relatively younger age. Recently, this idea has been supported by the evidence of extended lifespan through removing $p16^{INK4a+}$ senescent cells in a small multicellular system [13]. The protein $p16^{INK4a}$ is a widely used senescence marker for replicable cells, which increases as tissue ages [14]. We recently reported

decreased senescent cells in capillaries of human skeletal muscle immediately after and 48 h after resistance exercise [6] (Fig. 3), suggesting death of senescent cells in tissue after exercise (Fig. 4). Such type of exercise consists of eccentric muscle contraction leading to an increase in leukocyte infiltration. The senolytic effect of exercise appears to be associated with a damage-induced innate immune response. Unlike resistance exercise, aerobic exercise (i.e. cycling) that normally produces small muscle damages with leukocyte infiltration, does not show detectable senolytic effect [15]. However, elimination of senescence phenotype of most skeletal muscle from the same individuals conducting aerobic exercise occurs with increased iNOS expression when aerobic exercise is conducted after a pretreatment of ginseng compound Rg1. This compound has been shown to increase phagocytosis and high-intensity aerobic performance [15]. This suggests that leukocyte infiltration into skeletal muscle without activation of iNOS is not sufficient to remove senescent cells. Furthermore, different outcomes in senescent cell elimination during aerobic exercise might also explain distinctive metabolic benefits of endurance exercise and resistance exercise in late middle-aged men as previously reported [16].

Protein is Required for Regeneration and Resolution of Inflammation After Exercise

Amino acids contribute the majority of cell mass during proliferation [17]. After loss of senescent cells, both myogenesis and replenishment of endothelial cells immediately demand nitrogen and carbon sources from dietary amino acids or proteins for nucleotide synthesis and DNA replication [18]. The rate of endothelial cell proliferation in capillaries can occur at a very fast rate (doubling rate around 14 h) under optimal in vitro conditions [19]. Timing of protein supplementation appears to be important. With the same amount of protein, delayed

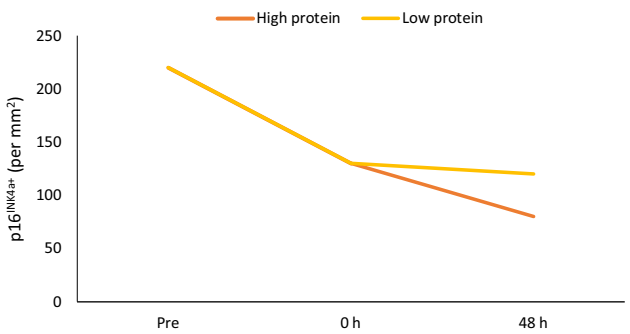

Fig. 4 Muscle damaging exercise decreases senescent cells ($p16^{INK4a+}$) in human skeletal muscle of young men (aged ~20 years). The illustration is reproduced according to Yang et al. [6]

supplementation following exercise significantly weakens muscle hypertrophy in men [20, 21], suggesting a far-reaching influence of protein supplemental timing around exercise challenge on long-term muscle remodeling. We have previously shown a faster resolution of muscle inflammation occurs when higher whey protein was supplemented in a carbohydrate diet immediately after resistance exercise [6].

It is generally known that cells in human body undergoes dynamical turnover [8], the current understanding on how senescent cells are recognized and cleared in human tissues is limited by a small amount of in vivo data. An early in vitro study indicates that the mechanism for senescent cell clearance may be associated with phagocytosis during inflammation [22]. In that study, aged red blood cells (RBC) were preferentially recognized and rapidly cleared by phagocytes in a fast rate. When freshly drawn RBC was separated into young and old RBC according to density and incubated with autologous phagocytes, less than 5% of the young RBC were cleared, whereas more than 30% of the old RBC were cleared. However, whether the phagocyte for senescent cell clearance is neutrophil, macrophage, or other types of phagocyte remains to be determined.

Can Life be Sustained if Number and Age of Cell Population are Well-Maintained?

Body weight of a multicellular organism during a lifespan is determined by the rate of cell death and regeneration [8]. During the early period of life, weight gain usually lasts for more than two-third of the entire life [23] (Fig. 2), followed by weight loss at the rest period of life. Loss of cell population causes collapse of the cooperative system bringing death of a human body. Weight gain at the beginning of human life is mainly associated with relatively higher rates of cell birth than death. In a multicellular system, cells are capable to survive for longer period due to advantage of cooperative partnership. Yet, this will gradually evolve an aging cell society and eventually lead to a relatively higher rate of cell death than birth during weight losing period at the end of life. Senescent cells ($p16^{INK4a+}$) in peripheral blood T-lymphocytes increase and saturate with age around 60 years when body weight is peaking during a normal human life (Fig. 5) [24]. In the cross-sectional study, the senescent cell proportion is relatively lower in individuals with exercise (> 240 min/month in a questionnaire) than their age-matched inactive peers [24].

Conclusion

Despite the evolvement as a highly specialized multicellular system by populating cells can increase fitness of the human body, persistent growth together with senescent cell

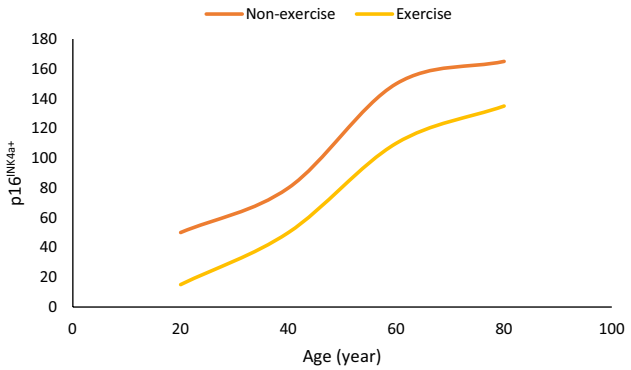

Fig. 5 Senescent cells ($p16^{INK4a+}$) of peripheral blood T-lymphocytes increases with age. Peripheral blood T-lymphocytes has a very short lifespan in humans (< 1 week). Saturation in $p16^{INK4a}$ signal after 60 years age (time around peak weight in humans) suggests that aging of peripheral blood cells is associated with body weight peaking. The illustration is reproduced according to Tsygankov et al. [24]

accumulation can be the fundamental cause of aging and eventual death. Exercise accelerates death of unfit senescent cells in the human body and slows down weight gain during early stage of a multicellular life. This in turn helps to maintain a relatively younger cell population of the human body at a thermodynamically stable size. Because cell death also triggers cell renewal and promote growth, inflammation and muscle damages in men after exercise should not always be regarded as a malign sign for long-term survival. The questions on optimal frequency and intensity of exercise challenge, together with nutritional supplementation aiming to maintain stable size and younger body, remains to be answered. The maximal intensity to eliminate greatest number of unfit cells without collapsing a multicellular system would be important knowledge to optimize physical fitness and survival at different age levels.

Compliance with ethical standards

Conflict of interest Part of the data reported in the paper is involved with a research funded by Nuliv Science, USA and Ministry of Science & Technology, Taiwan, China. US patent has been submitted in 2018.

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