Greater organ involution in highly proliferative tissues associated with the early onset and acceleration of ageing in humans

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ABSTRACT

Domination of cell proliferation over cell death is a driving force for carcinogenesis, whereas reduced cell proliferation and increased cell death are characteristic of ageing. We employed published data to estimate representative mean values of cell turnover times for 31 different organs and tissues in adult humans and animals (when data in humans were lacking) as well as functional mass loss for 5 organs, accounting for actual mass loss and tissue conversion to fat, in humans over the adult period, age 25 to 70. We found that greater actual and functional mass loss was significantly associated (P = 0.001 and P < 0.0001, respectively) with the log of shorter cell turnover times. We propose that this is characteristic of stem cell exhaustion and replicative senescence. In addition, we provide quantitative evidence that, in many organs, involution is evident even in young adults. On the basis of published mass measurements of major organs, by analysis of covariance, we identified examples of significant (P ≤ 0.05), accelerated actual or functional mass loss and ageing from early to late adulthood. We hypothesise and quantitatively demonstrate that functional mass loss accelerates with ageing by incorporating the contribution of actual mass loss, tissue conversion to fatty or fibrous tissue, and the presence of apoptotic, necrotic and senescent cells. We propose that mass loss, linked to replicative senescence, is an evolutionary adaptation that effectively limits cancer in young adults, as mass loss is first apparent soon after the end of the growth period, accelerating in the more elderly as biological conditions deviate away from those prevailing in youth, when the selective pressure on pleiotropic genes is greatest.

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1. Introduction

Progressive atrophy or involution accompanies ageing and is characterised by common conditions including Alzheimer disease (Burns et al., 2010), sarcopenia and osteoporosis. During the age-related involution of organs and tissues (hereinafter “organs” for brevity), functional or fundamental tissue integrity is reduced, chiefly by one of four means: first, mass loss due to lack of replacement of cells after apoptosis, necrosis, mitotic catastrophe and perhaps autophagic cell death; second, irreversible cellular senescence; third, inflammatory cell infiltration; and fourth, conversion of a functional tissue to another form, such as fatty or fibrous tissue. Conversion to fat occurs in most organs, but particularly in the bone marrow, breast, muscle, pancreas and thymus. Both fibroblast material and fatty infiltrates are associated with chronic inflammation, a biomarker of ageing (Chung et al., 2009; Richardson, 2011b; Schaffler et al., 2006).

Multiple interwoven mechanisms promote age-related involution, including increased oxidative stress levels, loss of sex hormones, insulin resistance (e.g., fatty liver disease), lowered production of growth hormone, and tissue energy metabolism (Almeida et al., 2007; Krems et al., 2005). In the case of sex steroids (hormones), their loss due to gonadectomy or ageing is associated with involution of reproductive organs, osteoporosis and cognitive decline (Lin et al., 2011).

Shorter cell turnover times (i.e., average renewal time of a cell population) have been shown to be associated with an elevated tumour incidence in selected tissues in both humans and rodents (Baserga and Wiebel, 1969). The predominant cell populations in many organs are epithelial and stromal cells. In general, epithelial cells have a far higher turnover and cancer incidence than other types of cells, accounting for 80% of all human cancers. For example, for cells in the jejunum, the average age of all cells has been calculated at ~10.7 years. However, the cell replacement time varies from ~5 d for epithelial cells to 15.9 years for stromal cells (Spalding et al., 2005). The rapid-turnover epithelial cells give rise to adenocarcinoma, the most common cancer of the small intestine, while from the stromal cells rarer sarcomas originate.

While several qualitative reports have examined the relationship between cell proliferation and cell death in a specific tissue, a quantitative study of multiple organs is lacking. To advance our understanding of...
aging-related cellular processes that contribute to carcinogenesis, we investigated whether mass loss in a variety of organs was associated with cell turnover times for these organs. Furthermore, we determined the age at which organ mass peaks or plateaux, the age after which mass loss begins, and whether mass loss accelerates over adult life. Therefore, we systematically reviewed published studies of cell turnover time and organ mass loss, and analysed data from these studies to determine changes in the rate of mass loss between early and late adulthood. We then examined our results to discuss whether the relationships found support current theories of the effects of radiation and of normal ageing.

2. Materials and methods

2.1. Cell turnover time and organ mass loss

We searched the published literature for reported cell turnover times for various whole organs and tissues, or for their major and most rapidly replaced cell component. Data in humans were preferentially selected where available; in the absence of data in humans, we used data from rats or, if there were no data from rats, from mice. As organ turnover times vary with age, those tabulated were of younger, rather than older, adults. When turnover times were not available, they were estimated as the reciprocal of the fractional turnover rate (fraction per day) or from half-life (day) data by dividing by the natural logarithm of 2.

Reported turnover times for different parts of the respiratory tract, dominated by endothelial cells, are particularly divergent. The bronchioles, alveolar ducts and sacs, rather than the tracheobronchial tree, make up the major part (>90%) of the lung mass. No estimate was found of the turnover time for Type I epithelial cells, which constitute ~95% of the alveolar surface. The turnover time of 200 d for the bronchioles of rats by Bleykinsopp (1967) was selected as the representative value, although an even slower proliferation was measured by Rawlins and Hogan (2008) for bronchioles in mice (mean half-life 470 d).

As well, we searched for publications reporting the change in “actual” organ mass in adults from the age of 25 to 70 years, or as close to those years as possible. For example, organ mass for adults 25 years old were evaluated in some instances as the mean of two reported values for 20–24 and 25–29 years old. An allowance was made for small (within 5 years) deviations from the 45-year range (e.g., Dekaban and Sadowsky, 1978 reported results for ages 20–30 years to 70–80 years). The upper age range was limited to 70 years of age principally owing to a paucity of data beyond this age. For the bone marrow (Bain et al., 2010; Richardson and Dubéau, 2003), breast, muscle (Marcus et al., 2010), pancreas (Saisho et al., 2007) and thymus (Steinmann et al., 1985), we went some way to estimating functional mass loss by reducing the actual mass due to tissue conversion to fat. For the breast, the functional mass change from 25 to 70 years was evaluated as the decrease in non-fat tissue (collagen/fibrous and glandular) by 31% and 38% from the mammographic density studies by El-Bastawissi et al. (2000) and Li et al. (2005), respectively. Using the data of El-Bastawissi et al. (2000), the mid-range values for the non-fat tissue of four age groups was derived from four breast density ratings. Using the data of Li et al. (2005), the difference in the non-fat tissue of two age groups, mean ages 33 and 64 years, was linearly extrapolated to a 45-year range.

Linear correlation analysis was carried out to determine the strength of the relationship between organ turnover times and mass loss. Organs were excluded from the analysis if data regarding cell turnover times or mass changes with ageing were lacking, or if the representative cell type most rapidly replaced constituted ≤10% of the organ volume.

2.2. Mass loss onset and acceleration

The age at onset of mass loss was identified as occurring after peak mass, and specifically as the age or mid-range age that mass loss begins and continues with age. Organ mass loss onset and acceleration with ageing for most organs was analysed employing the published data in six different examinations, described below. For the remaining organs, the age at onset of mass loss was estimated on the basis of previously published studies (Table 1). Lastly, for all organs exhibiting continuous mass loss (defined as continuous loss except for one time point) with ageing, the age at onset for each organ was estimated by selecting the mid-point of an age range and averaging by laterality and sex, where appropriate. Further, the acceleration of mass loss during early and late age periods was calculated in the last three examinations. Slopes from regression analyses of mass change rates between the early and late age periods were statistically compared by analysis of covariance (ANCOVA).

First, we examined the constant mass loss rate of four organs during adulthood by linear regression analysis using autopsy data provided by He et al. (2009).

Second, age-dependent bone marrow mass was estimated, taking into account skeletal growth, bone marrow cellularity, and apoptotic cells (Bain et al., 2010; Richardson and Dubéau, 2003). Apoptotic cell data was interpolated and extrapolated from 6.5, 7.2 and 19.6% of human bone marrow cells exhibiting apoptosis at 10, 55 and 90 years of age, respectively (Ogawa et al., 2000).

Third, the age-dependent analysis of three female sex/reproductive organs were individually estimated from one published study if the age range of the data was adequate, or two separate studies if not. Representative parameter values of mass loss onset were estimated from data for breast tissue conversion to fat (Boyd et al., 2010), ovary volume (Cohen et al., 1990), and endometrial thickness (Amir et al., 2007; Gurbuz et al., 2004).

Fourth, for six organs we used autopsy data by Ogisu et al. (1997), for age points ti, (yearly until age 19 years, midranges of five-year periods for >19 years and a ten-year period, 85–95 years). We calculated the rate of actual mass change by a three-time-point (t1, t2, t3) moving-window, linear regression analysis. The onset age of mass loss was then conservatively identified as the age when the rate became consistently negative (one point exception allowed) from that age onwards. Linear regression analyses were carried out for an early period starting at onset age of mass loss (e.g., 22–52 years), followed by a late period with a generally similar number of age points (e.g., 57–90 years old). The same analysis was applied to the functional pancreatic mass changes (not tabulated). The adrenals, heart, thymus and thyroid were excluded from the analysis of data by Ogisu et al. (1997) due to lack of consistency in mass loss rate in adulthood.

Fifth, the functional mass loss (equivalent to functional volume loss assuming 1 g·cm−3) of the thymus was estimated from the data of Steinmann et al. (1985) for mid-range ages by evaluating the functional thymic volume as comprising the thymic epithelial space and lympho-lymphocytic perivascular space.

Sixth, changes in the actual body cell mass, and actual and functional skeletal muscle mass, were based on Kyle et al. (2001), which reported regression analyses for data divided into two age groups (17–59 years old and 60–94 years old). Comparison of early and late mass rates was carried out on data acquired by digitizing the relevant published figures. Onset of mass loss was evaluated from the mean mass of decadal age groups.

3. Results

3.1. Cell turnover time and organ mass loss

We examined reports of 31 organs or their major cell types (Table 1). Cell turnover times for the organs or their cell types had a very wide range, from 1.4 to 25 300 d (Table 1, Fig. 1A and B). The gastrointestinal tract, thymus and bone marrow were found to have high cellular turnover, whereas the heart, brain, bone, and muscle have low turnover and regenerative capacity. For consistency, we report cellular turnover times in young adults, where possible, as evidence shows that turnover ages...
Table 1

<table>
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<th>Turnover methods</th>
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<th>Human mass change, actual (mean) and [FM], %</th>
<th>Mass change references, Country of population</th>
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<tr>
<td>Adipose tissue</td>
<td>Adipose</td>
<td>H: 441, 444 (2448)</td>
<td>14C-DNA H2O</td>
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<td></td>
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<tr>
<td>Adrenal</td>
<td>Cortex, neuroendocrine</td>
<td>R: 455 [cortex]</td>
<td>3H-thy.</td>
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<td></td>
<td></td>
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<tr>
<td>Bone marrow, active</td>
<td>Osteopenia, osteoporosis</td>
<td>H: 7000</td>
<td>Ref. value</td>
<td>ICRP, 1975</td>
<td></td>
<td>−9, 3 (−3)</td>
<td></td>
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<tr>
<td>Brain</td>
<td>Dementia</td>
<td>H: 16,425 [grey matter]</td>
<td>14C-DNA</td>
<td>Spalding et al., 2005</td>
<td>−6, −3, −6, −9, −8, −7 (−6)</td>
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<tr>
<td>Breast</td>
<td>Lobular involution ET</td>
<td>H: 22, 88, 13, 143 (47) [ET]</td>
<td>3H-thy, 3H-thy, 2H2O</td>
<td>Meyer, 1977; Russo et al., 1987; Potten et al., 1988; Miselli et al., 2005</td>
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<td>Cervix</td>
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<td>H: 5.7 [ET]</td>
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<td>Cole and McKalen, 1961; Lipkin, 1965</td>
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<td>Colorectum</td>
<td>Mucosal and muscular atrophy (ET), smooth muscle cells</td>
<td>H: 3.5, 3.2 (3.4) [ET]</td>
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<tr>
<td>Duodenum</td>
<td>Mucosal and muscular atrophy (ET), smooth muscle cells</td>
<td>H: 7.0 [ET]</td>
<td>3H-thy</td>
<td>H: 25,300, 4290 (14,800)</td>
<td>14C-DNA, Ki-67</td>
<td>Bergmann et al., 2009; Kajstura et al., 2010</td>
<td></td>
</tr>
<tr>
<td>Heart</td>
<td>Enlarged heart Cardiomyocytes</td>
<td>R: 453, 201 (327)</td>
<td>3H-thy, 3H-thy</td>
<td>Macdonald, 1961; Zajicek et al., 1985</td>
<td>−13, −17, −16, −9 (−14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>(ET), nephrons, stroma</td>
<td>R: 270 [cortex]</td>
<td>3H-thy</td>
<td>Leblond, 1964</td>
<td>−7, −9, −7, −9 (−8)</td>
<td></td>
<td></td>
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<tr>
<td>Liver</td>
<td>Fibrotic &amp; fatty liver Hepatocytes</td>
<td>R: 453, 201 (327)</td>
<td>3H-thy, 3H-thy</td>
<td>Macdonald, 1961; Zajicek et al., 1985</td>
<td>−13, −17, −16, −9 (−14)</td>
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<tr>
<td>Lung</td>
<td>Pulmonary fibrosis</td>
<td>R: 200f</td>
<td>3H-thy</td>
<td>Blenkinsopp, 1967</td>
<td>+2, −6 (−2)</td>
<td></td>
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<tr>
<td>Muscle</td>
<td>Sarcoopia</td>
<td>H: 5510</td>
<td>14C</td>
<td>Spalding et al., 2005</td>
<td>−9, −8 (−9) [FM, −15]</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oesophagus</td>
<td>(ET), smooth muscle</td>
<td>H: 10</td>
<td>Colchicine</td>
<td>Bertalanffy, 1964</td>
<td>−46</td>
<td></td>
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<tr>
<td>Pancreas</td>
<td>Atresia (folicose loss)</td>
<td>M: 84, 190, 520 (265)</td>
<td>3H-thy, 3H-thy</td>
<td>Magami et al., 1990, Nakayama et al., 2003; Cameron, 1970</td>
<td>−3, −8, −4 (−5) [FM, −14]</td>
<td></td>
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</tr>
<tr>
<td>Parathyroid</td>
<td>Chief cells, (ET)</td>
<td>R: 1160</td>
<td>Ki-67</td>
<td>Wang et al., 1996</td>
<td>−5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pituitary</td>
<td>Nodular prostatic hyperplasia</td>
<td>ET</td>
<td>Somatotrophs</td>
<td></td>
<td>−2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prostate</td>
<td>Epidermal thinning</td>
<td>ET</td>
<td>3H-thy</td>
<td>Halprin, 1972</td>
<td>−29, −10, −15 (−18)</td>
<td></td>
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times decelerate with age, both in non-sexual organs, e.g., cardiac myocytes (Bergmann et al., 2009), and in sexual organs, e.g., breast epithelial cells (Kajstura et al., 2010; Meyer, 1977; Misell et al., 2005; Russo et al., 1987).

"Functional" mass loss was estimated for five organs in which the "actual" tissue mass is converted to fat with age. Actual and functional organ mass was lost from age 25 to 70 years in all organs studied, except the heart and prostate. Most measurements were based directly on autopsy mass, or on thickness in the case of cornea and skin (including use of optical coherence tomography, Gambichler et al., 2006); for ovary, ultrasound was initially used to obtain the volume.

Thirteen organs were excluded from the regression analysis of turnover and mass loss according to exclusion criteria (identified in Table 1): in particular, where data on mass loss for the gastrointestinal tract are unavailable: where no studies were found of epithelial cell turnover or organ mass loss in the prostate in the absence of hyperplasia, and where cells with the most rapid turnover constituted ≤10% of organ volume in cornea and bone. Table 2 provides the regression analysis results for a semi-log plot of overall organ turnover times in relation to the percentage change in actual or functional organ mass. We found that greater actual and functional mass loss was significantly associated with the log of shorter cell turnover times whether the turnover data were limited to organs with data obtained solely from humans (P = 0.008 for actual and P = 0.002 for functional mass loss) or extended to organs with data from humans and animals (P = 0.001 for actual and P < 0.0001 for functional mass loss). When breast and reproductive organs were excluded from the analysis, we found weaker but significant associations for organs with data solely from humans (P = 0.03), and with data from humans and animals (P = 0.001).

Our regression model indicates that more than two-thirds of the human variability of the actual (R² = 0.72) and functional mass loss (R² = 0.70) is explained by the log of the turnover times (Table 2).

3.2. Onset of mass loss

Organ mass loss with ageing was analysed employing published data in six different examinations. The first three considered a constant mass loss rate during adulthood, whereas the latter three examinations investigated the potential for accelerated mass loss (see Acceleration of mass loss section below). The onset of organ mass loss with ageing was identified from the data of all six examinations, three of which, Ogisu et al. (1997), Steinmann et al. (1985) and Kyle et al. (2001) are shown in Table 3. He et al. (2009), using US autopsy data for brain, kidneys, liver, and spleen, demonstrated highly significant negative relationships (P ≤ 0.01) between organ mass and age of adult males (aged 19–84 years) and females (aged 19–88 years). Investigation of mass loss onset age (and acceleration) was not possible using the data of He et al. (2009), as the study provided regression parameters rather than tabulating mass by age.

Skeletal bone marrow is gradually invaded by fat cells from birth onwards. Its functional mass, accounting for growth, cellularity (Bain et al., 2010; Richardson and Dubeau, 2003) and apoptotic cells (Ogawa et al., 1994)
et al., 2000) peaks at about 25 years of age and declines from 40 years onwards ($R^2 = 0.97, P = 0.01$; Fig. 2B).

An early onset (and continued decline) of actual or functional mass loss is also evident from analysis of published representative parameters for the mass of female sex/reproductive organs. Breast lobular involution and fat content is at a minimum in adolescents, increasing in young women of 19–30 years (Boyd et al., 2010; Milanese et al., 2006). Ovary volume peaks at 20–29 years and decreases at 30–39 years of age (Cohen et al., 1990), and endometrial thickness peaks at 17–25 years and thins at 26–35 years (Amir et al., 2007).

Ogiu et al. (1997) provided post-natal mass for the whole body and major non-reproductive organs in Japanese subjects. Mean body height peaked at 20–24 years of age in males and at 18 years of age in females, and then gradually decreased, whereas body weight for this low-obesity population was at its maximum at 35–39 years of age in males and 50–54 years of age in females. For this study, gradient trend analysis identified four of the 10 reported organs as exhibiting inconsistent mass loss during adulthood (adrenals also undergo involution in a different temporal pattern, beginning around birth). The adrenals, heart, thymus, and thyroid were therefore excluded from further analysis. By our analysis, the age at onset of continuous actual mass loss in adulthood, by organ from earliest to latest, was spleen at 22 years (for both sexes); brain at 27 and 37 years (males and females, respectively); liver at 42 and 47 years (males and females, respectively); kidneys at 47 years (for both sexes); pancreas at 47 years (for actual and functional mass, both sexes); and lung at 52 and 47 years (males and females, respectively). The average age of onset of continuous mass loss for the six organs combined was 42 and 47 years for males and females, respectively.

According to the data of Ogiu et al. (1997), the thymus exhibited erratic mass loss commencing a year after the peak actual mass at 12 and 10 years for males and females, respectively. This can be compared with post-natal measurements of the functional thymus (e.g., 24 cm$^3$ at 1/2 year, 22 cm$^3$ at 2.5 years, 17 cm$^3$ at 12 years, 13 cm$^3$ at 22 years, 5 cm$^3$ at 50 years, and 2 cm$^3$ at 88 years) obtained by Steinmann et al. (1985), who reported that its principal component, the thymic epithelial space, involuted rapidly from the ages of 1 to 10 years (Table 3).

Of the remaining organs for which mass change values are listed in Table 1, continuous mass loss was not evident in the parathyroid (Gilmore and Martin, 1937) or pituitary (Tanaka, 1992). However, after peaking in the previous period, continuous mass loss was reported for trabecular bone starting from 31–40 years (Popovic, 2006); peripheral, but not central, cornea thickness from 31 to 40 years (Martola and Baum, 1968); epidermis; but not superficial dermis, from 30 to 40 years (Branchet et al., 1990); and testis from 30 to 34 years (Tanaka, 1992).

In summary, age for the onset of actual mass loss (average of male and female) for the thymus is around 12 years old; spleen, 22 years; uterus, 31 years; brain, 32 years; testis, 32 years; ovary, 35 years; skin, 35 years; cornea, 36 years; trabecular bone, 36 years; muscle, 43 years; liver, 45 years; kidney, 47 years; pancreas, 47 years; and lung, 50 years. The onset of functional mass loss can be earlier than the actual mass, with the thymus around 1 year; breast, 25 years; bone marrow, 40 years; muscle, 40 years; and pancreas, 47 years.

3.3. Acceleration of mass loss

The potential for accelerated mass loss was investigated in three examinations by comparing linear regression mass change rates during early and late age periods and determining whether these rates (slopes) were significantly different ($P \leq 0.05$) by ANCOVA (Table 3).

Continuing study of autopsy data by Ogiu et al. (1997), we conducted linear correlation analysis for the organs with continuous mass loss to determine whether the mass loss rate changed between an early period commencing at the onset of mass loss and a late period, each period consisting of roughly equal number of age points (Table 3). In males, the kidney, liver, and lung exhibited a rate of mass loss greater in the late than in the early period, although this rate change was significantly different ($P = 0.008$) only for the liver. In females, the brain, kidney, pancreas, and spleen exhibited a rate of mass loss rate greater in the late than the early period, and this rate change was significantly different for the brain and spleen ($P = 0.05$ and 0.03, respectively). Surprisingly, the rate of mass loss accelerated strongly in the six major organs combined in males ($P = 0.0004$) but not in females ($P = 0.8$).

The functional mass loss rate of the thymus (Steinmann et al., 1985) significantly ($P = 0.05$), and surprisingly, slowed in adulthood compared to childhood (Table 3).

Also shown in Table 3 are the age-related changes in appendicular skeletal muscle mass and body cell mass reported by Kyle et al. (2001) for healthy European males and females in two groups, aged 18 to < 30 and >60 to 94 years old. Actual muscle mass values by Kyle et al. (2001) were modified in Table 3 and Fig. 2B by subtracting the age-dependent (linear) percentage fat infiltration, from the data of Marcus et al. (2010). Whereas the actual muscle mass loss accelerates in females only, the functional mass loss is greater and accelerates during adulthood for both sexes: the acceleration is significant for males only ($P = 0.03$).

Analysing the body cell mass data of Kyle et al. (2001), the rate of mass loss increased 2–4-fold from early to late periods of adulthood. These data, which are a good measure of functional tissue mass, exhibited a significant acceleration ($P = 0.0007$ in males; $P = 0.05$ in females) in mass loss when comparing periods before and after 60 years of age.

4. Discussion

4.1. Cell turnover, mass loss and implications for cancer and ageing

This work provides experimental evidence that the loss of functional mass in tissues with ageing may be related to the mitotic rate or rates of tissue turnover. This observation probably involves longevity regulator p53, the so-called “skinny gene”, down-regulation of which is associated with cellular senescence of endothelial cells and accelerated ageing (Bai et al., 2014). It is particularly relevant to our findings that the decline of p53 protein with ageing is greatest in mitotically active tissues (Sasaki et al., 2006). Similarly supportive is that highly proliferative tissues exhibit greater telomere erosion with ageing, hence a higher potential for replicative senescence (Ishii et al., 2006). The latter state is present at considerable levels (>15%) in mitotically active skin fibroblasts of aged baboons, whereas fewer (<3%) senescent cells are present in their mitotically inactive muscle (Jeyapalan et al., 2007).

In young adults, tissue maintenance involves the removal of old, damaged cells and their replacement by stem cells providing progenitors. In adults approaching old age, we have shown lack of homeostasis and mass loss to be more prevalent in the most proliferative tissues. Two means of losing functional mass involve the p53 protein, namely programmed cell death (without cell replacement) and cellular senescence. Inactivation of these processes can have severe consequences. On the one hand, one of us (Richardson, 2013) showed that p53 gene mutations are involved in approximately one-third of the ageing-related rise in the incidence of all cancers worldwide. On the other hand, mice with increased p53 activity had a cancer incidence only 1/8th that of normal mice, yet aged prematurely, with a 23% reduction in median lifespan (Tynor et al., 2002). By contrast, mice breed by Garcia-Cao et al. (2007) with extra copies of p53 genes were cancer-resistant but showed no accelerated ageing. These augmented p53 responses in mutant mice, with unambiguous benefits concerning p53 and cancer yet ambiguous effects on p53 and ageing, indicate that other factors also play a prominent role in ageing.

The breast is a well-documented example of an organ in which a slower turnover time and greater functional mass loss have been reported to influence the cancer rate (Milanese et al., 2006; Misell et al., 2005). The glandular epithelium in the breast is a tissue that atrophies commencing at age 19 to 30 years, with lobules replaced by fat and fibrous tissue (Boyd et al., 2010; Li et al., 2005). The post-menopausal hormonal decline contributes to a slower proliferation rate and increased cell death.
393 lower reactive oxygen species, less cancer, lower reproductive ageing 391 mitotic activity, as seen in calorie-restriction experiments with animals: 2005; Nolan et al., 1999). However, there are benefits that slow with ageing, attended by a reduced ability to repair 388 Similar to human breast epithelial cells, other organs and tissues, 385 acceleration of mass loss in some major organs and body cell mass than 382 age-dependent breast cancer rate (Misell et al., 2005; Pike et al., 1993). Non-obese populations are accompanied by a reduction in the (log 380 turnover for humans and animals (rats, mice), 16 organs, R² = 0.53, P = 0.001. 388 of ductal epithelial (stem) cells where breast cancer originates, which in non-obese populations are accompanied by a reduction in the (log–log) age-dependent breast cancer rate (Misell et al., 2005; Pike et al., 1993). Nonetheless, our tentative finding that males have, in general, a greater acceleration of mass loss in some major organs and body cell mass than females, yet accompanied by comparatively higher cancer rates, points to unknown complexities involving senile mass loss. Similar to human breast epithelial cells, other organs and tissues, 388 including non-sex-related organs (e.g., pituitary), have proliferative rates that slow with ageing, attended by a reduced ability to repair 390 double strand breaks by homologous recombination (Misell et al., 2005; Nolan et al., 1999). However, there are benefits to a slowing of mitotic activity, as seen in calorie-restriction experiments with animals: lower reactive oxygen species, less cancer, lower reproductive ageing and reduced general senescence (Lok et al., 1990; Marchal et al., 2013).

4.2. Cell turnover, mass loss and possible role of replicative senescence We found that, in normal ageing, organ mass loss is associated with high cell turnover. At the Hayflick limit, cells go into a senescent state of persistent cell cycle arrest, or undergo cell death, usually by p53-dependent apoptosis (Bree et al., 2002; Hayflick and Moorhead, 1961). This increase in apoptotic and senescent cells with ageing represents a loss of actual and functional tissue. We suggest that this mass loss may be characteristic of stem cell exhaustion as seen in muscle and marrow (Renaud et al., 2002; Richardson, 2011b) and that “replicative senescence” may play a role in this process. Stem cell pools can diminish with age. For example, the number of satellite cells in human skeletal muscle declines from young to old adults (Renaud et al., 2002). Furthermore, stem cell exhaustion may be due to cell dysfunction characterised by decreased self-renewal and quiescence, increased doubling time, degraded niches and impaired terminal differentiation (Zhou et al., 2008).

More rapid turnover of tissue stem cells and their progeny leads to faster rate tissue loss due to telomere erosion and replicative senescence (Alsopp et al., 1995), particularly if cells are telomerase-negative or if telomeres are less maintained due to the inevitable reduction of telomerase activity that occurs with ageing (Mariani et al., 2003). Although telomerase is generally more active in rodents than humans, even so, telomere erosion linked to rat development and ageing occurs in the kidney, pancreas, liver and lung but not brain, an organ with an exceptionally long turnover time (Cherif et al., 2003). Most human tissues and organs analysed consistently show telomere shortening with ageing (n = 9; Jiang et al., 2007), with the exceptions again having long turnover times, e.g., heart, brain, liver and muscle (Renaud et al., 2002; Verma et al., 2012). Employing data from Table 1, a highly statistically significant difference (t test, P = 0.004) was found, with turnover times (d) of 445 ± 1032 SD in organs with age-related telomere erosion and 9266 ± 7657 SD for those reported with more stable telomeres. In addition, accelerated telomere shortening occurs in various human diseases associated with ageing, e.g., cardiovascular disease, most cancers, type 2 diabetes and Alzheimer’s disease (Jiang et al., 2007; Panossian et al., 2003; Richardson, 2009).

Yet senescent cells themselves may have a limited lifespan of up to three years in vitro, followed by cell death by necrosis (Matsumura et al., 1979). We hypothesized that organs with shorter cell turnover would exhibit greater replicative senescence, and found that shorter cell turnover accounted for about two-thirds (Table 2) of subsequent age-related mass loss in humans due to apoptosis and necrosis, with the remaining mass loss probably due to other factors such as oxidative stress, mutation accumulation and stem cell dysfunction.

In fact, oxidative stress is an alternative mechanism for generating senescent cells (von Zglinicki et al., 2005). Both exogenous and endogenous forms of oxidative stress produce “stress-induced (premature) senescence”, indicated by the secretion of inflammatory chemokines and cytokines (Sabin and Anderson, 2011). For example, high levels of cell death and inflammatory markers are seen in atomic bomb survivors, in whom excess rates of cancer and non-cancer diseases, and shortened lifespan, are linked to premature ageing (Richardson, 2009). Although in radiation exposure replicative senescence also

Table 2 Regression parameters for percentage mass change, both actual and functional, in organs from age 25 to 70 years versus the log turnover time in days.

<table>
<thead>
<tr>
<th>Organ Type</th>
<th>Mass Change</th>
<th>Organs (n)</th>
<th>Slope</th>
<th>Intercept</th>
<th>R (95% CI)</th>
<th>R²</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human</td>
<td>Actual¹</td>
<td>8</td>
<td>12</td>
<td>-46</td>
<td>0.85 (0.36–0.97)</td>
<td>0.72</td>
<td>0.008</td>
</tr>
<tr>
<td>Human</td>
<td>Functional</td>
<td>10</td>
<td>11</td>
<td>-45</td>
<td>0.84 (0.44–0.96)</td>
<td>0.70</td>
<td>0.002</td>
</tr>
<tr>
<td>Human, excluding sex-related</td>
<td>Functional</td>
<td>6</td>
<td>9</td>
<td>-38</td>
<td>0.85 (0.13–0.98)</td>
<td>0.72</td>
<td>0.003</td>
</tr>
<tr>
<td>Human and animal</td>
<td>Actual¹</td>
<td>16</td>
<td>9</td>
<td>-33</td>
<td>0.73 (0.57–0.90)</td>
<td>0.53</td>
<td>0.001</td>
</tr>
<tr>
<td>Human and animal</td>
<td>Functional</td>
<td>18</td>
<td>14</td>
<td>-51</td>
<td>0.80 (0.52–0.92)</td>
<td>0.63</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Human and animal, excluding sex-related</td>
<td>Functional</td>
<td>14</td>
<td>13</td>
<td>-49</td>
<td>0.66 (0.20–0.88)</td>
<td>0.43</td>
<td>0.01</td>
</tr>
</tbody>
</table>

¹ Bone marrow and breast excluded owing to lack of mass change data.
The biological mechanism for the accelerated ageing phenomenon may involve inflammation stimulating the hypothalamus to produce increased cortisol (Dettenborn et al., 2012), causing glucocorticoid-induced skeletal and soft tissue atrophy as well as vulnerability to metabolic syndrome (Youm et al., 2013). Sex hormones could also play an important role in regulating both cell turnover and loss of tissue mass through apoptosis (Medth and Thompson, 2000). This acceleration trend is moderately strongly significant for body cell mass (Kyle et al., 2001), a good measure of functional tissue mass. We did account for tissue conversion to fat in five organs in Table 1 but not for reduction in functional tissue due to the presence of apoptotic, necrotic or senescent cells (Jayapalan et al., 2007; Ogawa et al., 2000). As few studies dedicated to these ageing changes indicate that these functional tissue losses may be considerable, this suggests that even measures of body cell mass underestimate the true accelerating loss of functional tissue with ageing. Indeed, accelerated functional mass loss could provide an increased elimination of precancerous cells in the very elderly, perhaps providing an explanation, among others, for the decrease in cancer rates observed after age 75 (Magdon-Maksymowicz and Maksymowicz, 2009).

Could this acceleration in ageing be linked to the non-linear increase in nuclear and mitochondrial mutation frequency, and associated apoptosis, replicative senescence, carcinogenesis and heritable defects (Pollack et al., 2002; Richardson, 2009; von Zglinicki et al., 2005)? A non-linear, age-related rise in nuclear mutations is seen in both somatic and germ cells accompanied by an exponential increase in the incidence of cancer, in somatic cells, and sporadic achondroplasia, in germ cells (Richardson, 2013; Tiemann-Boege et al., 2002; Vorobtsova et al., 2001). There is theoretical support for the view that children of older parents carry a larger "mutational burden", hence influencing the biological ageing and mortality rates of their progeny (Magdon-Maksymowicz and Maksymowicz, 2009). However, the experimental evidence is ambiguous regarding whether progeny of older parents are characterised by premature ageing. On the one hand, children of older parents have longer telomeres than progeny of younger parents, a process that may extend late-life function, as the average age at reproduction is delayed (Austad, 1993; Eisenberg et al., 2012). On the other hand, persons born of mothers younger than...
25 years are about two times more likely to survive to age 100 than their siblings born to the same mothers at older ages (Gavrilo and Gavrilo, 2012; Gavrilo and Gavrilo, 2007). As well, a variety of abnormalities in progeny are associated with greater age of the mother (trisomies) or father (autism, schizophrenia, sporadic achondroplasia). Regardless of the origin of ageing, these examples provide evidence that accelerated ageing, by increasing the likelihood of death, limits the risk that older parents will reproduce and create a mutational burden in their offspring.

### 4.4. Implications of results on theories of ageing

Reproductive value and contributions to fitness decline monotonically during the reproductive period as fecundity falls and mortality rises (Hamilton, 1966). At the same time, ageing is accompanied by an increased likelihood, and the participation of deleterious mutations. This has generally been viewed in terms of gene functional trade-offs and the evolution of pleiotropy (Guillaume and Otto, 2012): genome evolution being most influenced when selective pressure is greatest, before sexual maturity, and decreasing thereafter.

Medawar (1952) argued that ageing occurs and mutations accumulate because of its neutral effect on the selection of late-stage deleterious mutations, since mortality in the wild is mainly accidental and is sufficiently high to prevent organisms reaching old age. On this basis, Williams (1957) proposed the theory of antagonistic pleiotropy: genes such as TP53 or TGF-β, and biological processes that enhance reproductive success early in life, lead to an evolutionary trade-off, with later fitness decline and death.

In this view, replicative senescence would be one such biological process. In terms of cancer, replicative senescence is a double-edged mechanism: it can both hinder and help oncogenesis. It has been postulated to suppress cancer when cells become apoptotic or senescent upon their telomeres reaching a critical length, as long as the proliferation/apoptotic genes are functional (Campisi et al., 2001; Wright and Shay, 1992). A case could be made for replicative senescence protecting those with Alzheimer’s disease against cancer. Features of this form of dementia include telomere/telomerase dysfunction, shortened telomeres, reduced lean body mass, and brain atrophy. The risk of cancer for patients with this disease is halved compared with the risk in other people (Burns et al., 2010; Musico et al., 2013; Panossian et al., 2003).

Often, replicative senescence is a prerequisite for cancer cells, which they overcome. It is telling that most cancer cell lines exhibit very short telomeres but escape replicative senescence through mechanisms such as telomerase activation or telomeric recombination (Braut and Auteixier, 2011; Jiang et al., 2007). Extensive stem cell proliferation can foster a telomere erosion crisis, leading to oncogenic development, especially if accompanied by dysfunctional cell apoptosis or proliferation genes. A case in point: the unusually short telomeres observed in the bone marrow cells of myelodysplasia syndrome patients become shorter still upon the development of acute myeloid leukaemia with associated FLT3, RB1 or TP53 mutations (Sieglova et al., 2004). In this cancer, cells also exhibit exceptionally high levels of methylation ageing. At moderate or high doses, radiation is a telomere-eroding and ageing agent, which in A-bomb survivors has led to high relative risks of myelodysplasia syndrome and acute myeloid leukaemia (Horvath, 2013; Ilyen et al., 2011; Richardson, 2009).

We have shown that major organs exhibit a loss of actual and functional mass; attended by physiological or cognitive decline, surprisingly even in young adults, when reproductive ability and hence natural selective pressure is close to maximum. This early decline in fitness is contrary to Medawar’s (1952) postulate that ageing occurs after the reproductive period when selective pressure is lacking. This claim is therefore refuted by our study, and also by a study showing the onset of senescence in dogs at 2.2 ± 0.8 years old, when large dogs are not fully grown (Kraus et al., 2013). The first report for an early onset of ageing for animals living in the wild is for wolves, which found that the onset of ageing handicaps their fitness to escape predators (MacNulty et al., 2009), and presumably their reproductive success. The study found that the age of peak ability to attack, select and kill elk was at 1 to 3 years, around the time of first reproduction at 2 to 4 years old. One-fifth of wolves were still alive at 10 years, subjecting ageing to selective pressure. Similar conditions apply to premodern humans, including hunter-gathers, whose modal lifespan (or peak mortality) was estimated at about 72 years old, with a quarter of all adult deaths at or above mode (Curwen and Kaplan, 2007). Accordingly, evidence in young adult, wild animals and premodern humans compellingly suggest that ageing, and genes and mechanisms identified as pleiotropic and complicit in ageing (e.g., TP53, TGF-β and replicative senescence) are subject to strong evolutionary pressures.

Weismann (1889) suggested the controversial idea that ageing may have an evolutionary benefit: advancing death, thereby reducing the period of reproductive decline and post-reproductive senescence, hence making the best use of limited resources. In general, a species’ reproductive ability declines with adult age, with death commonly following shortly after reproductive senescence (Zhao et al., 2008). There are few exceptions to a short post-reproductive period, such as when the usually hermaphroditic Caenorhabditis elegans mates with rarer males (Mendenhall et al., 2011). Whales and humans constitute other exceptions, as long-lived, post-reproductive females (i.e., mothers and grandmothers) provide valuable aid to their progeny (Johnstone and Cant, 2010). Notwithstanding these exceptions, ageing may be beneficial in reducing the post-reproductive period and providing nutrients and resources for descendants either indirectly or directly and altruistically, as in yeast (Mendenhall et al., 2011).

Ageing in animals involves multifaceted mechanisms, including replicative senescence and epigenetic inheritance, which are generally considered to be genetically programmed. In most somatic tissues of
humans, telomere erosion begins post-natally, slows after growth finishes and sexual maturity is achieved, and continues into old age until a critical length is reached, when cell replication stops (Jiang et al., 2007). The general prevalence of the Hayflick limit in human somatic cells, including stem cells, means this aspect of human ageing is likely an evolutionary adaptation, as antioxidants against this shortening, such as telomerase, are not employed at sustaining levels in somatic tissues. However, telomere-maintenance mechanisms are fully operational in human germ cells, most neoplasms (clonal) and biologically immortal species such as Hydra vulgaris that reproduce asexually when food is plentiful (Boehm et al., 2012; Bridge et al., 2010). The immortality (and lack of reported mass loss) of Hydra is assigned to FoxO stem cell maintenance gene variants, which are also found in human stem cells, albeit at levels insufficient to maintain stem cells. Interestingly, a genetic variant in the FOXO3a gene region is more common in German centenarians compared with younger controls (Flachsbarth et al., 2009).

The fact that organ involution is first evident in young adults can be also interpreted to indicate that trade-offs and pleiotropic gene activities begin to be expressed well before actual mortality. Thymic atrophy, a form of immunosenescence, begins even earlier in human development: loss of thymic epithelial space commences at 1 year old in humans and proceeds at a faster rate before sexual maturity rather than after (Steinmann et al., 1985). This thymic involution is driven by the pre-pubertal release of glucocorticoids, or infectious or psychological stress. This demonstrates complex trade-offs, with an innate immune response predominating over an adaptive one, whereas impaired thymus function can, later in life, result in antagonistic pleiotropic features such as height-deficit, delayed puberty and increased vulnerability to acute lymphoblastic leukaemia (Kinouchi et al., 2012; Richardson, 2011a).

Another trade-off, in which human lifespan is extended at the cost of reproductive success, was reported by Westendorp and Kirkwood (1998) using historical data from the British aristocracy. Conversely, according to the disposal soma theory, finite food energy may be preferentially used for reproduction, compromising repair during ageing (Kirkwood, 1977). Indeed, the involution we documented in non-reproductive tissues could be redistributing energy away from mitotically active tissues to reproductive organs; however, this is doubtful, as we found a parallel mass loss in reproductive tissues.

The prevailing view is that antagonistic pleiotropy is the dominant influence on the genetic architecture of human ageing. According to this view, beneficial selective pressure on pleiotropic genes arises from early-stage survival and reproduction trade-offs, with ageing resulting from selective neutrality of late-stage deleterious mutations (Guillaume and Otto, 2012). Nevertheless, our research shows that mass loss in human bone and some soft tissue organs is first apparent soon after maturity, during the highly fertile period of young adulthood, contrary to Medawar’s (1952) assertion that ageing is not subject to evolutionary pressures. We therefore surmise that the mass loss processes involved are in place and operational even before maturity, yet are masked or effectively countered by growth. If this mass loss is linked to replicative senescence that suppresses cancer during a period of strong selective pressure, then it has proven a highly effective evolutionary adaptation, as evinced by the very low cancer incidence of late childhood and early adulthood.

We suggest, based on our findings, that the mass loss processes become observable post-maturity, when growth stops. As adulthood advances, this mass loss accelerates due to further temporal changes resulting from sex hormones and from an increase in mutation accumulation, chronic inflammation and chronic diseases. These temporal changes cause biological conditions to deviate from those existing before and soon after maturity, when the selective pressure is greatest on gene function, including pleiotropy. In light of this, epigenetic inheritance plays an important role in the ageing process by bringing about temporal changes in gene activity, such as those due to methylation ageing (Horvath, 2013).

4.5. Limitations of turnover/mass loss data

Our analysis has limitations and uncertainty arising from the use of published data from different species and human populations. Of necessity, we relied on data from different international sources utilizing various methods of analysis and interpretation. Each method has its advantages and limitations. For example, bromodeoxyuridine (BrDU) and atmospheric 14C in DNA were both employed by Bhardwaj et al. (2006) to estimate neocortical neuron turnover. The former method was preferable due to its high sensitivity to detect low-grade continuous cell generation, while BrDU-labelling labels newborn cells at a given point of time, with the disadvantage of inaccurate results. In order to aid the correlation analysis, a single representative mean value of both turnover and mass change was allocated for each organ. No uncertainty for this value was assigned, as it was generally estimated from multiple studies with data of different and often unknown errors. In addition, we acknowledge a confounding factor in our calculations based on data from different species, as organs from larger animals have lower metabolic and cell proliferation rates than the same organs from smaller animals (Porter and Brand, 1993).

There is a lack of continuous age-dependent mass loss data for reproductive organs and the gastrointestinal tract. Comprehensive measurements of alimentary tract wall mass or thickness are particularly scarce (Haber and Stern, 2000) and complicated by inflammatory bowel disease producing thickening or thinning. In addition to actual loss of tissue mass in organs, for five of them we also accounted for conversion of functional tissue to fat but did not account for either the percentage of tissue converted to fibrous material (e.g., as occurs in thyroid), or for the percentage of cells subject to apoptosis (e.g., bone marrow), macrophages, autophagic cell death, necrosis, mitotic cell death or senescence (e.g., skin). These omissions were primarily due to the lack of availability of published age-dependent data for humans, yet these biological mechanisms make important contributions to functional tissue mass loss.

5. Conclusion

Our review supports a strongly significant association between cell proliferation and functional mass loss, the latter being an important indicator of fitness and ageing. We found that two-thirds of the human variability of mass loss can be assigned to the log of tissue turnover times. We suggest that this is likely characteristic of replicative senescence of stem cells, which, as the immortal Hydra demonstrates, is not a biological imperative but an evolutionary adaptation, likely suppressing cancer in humans. The onset of functional mass loss first becomes apparent soon after growth terminates, during the early part of the reproductive period, when selective pressure is still considerable. We make the case that, although the deceleration of cell turnover helps mitigate the erosion of maintenance-deficient telomeres, there is an acceleration of functional mass loss in old age as biological conditions change from those existing in early development, when the selective pressure on genetic trade-offs is most influential.

Conflict of interest

The authors have no conflicts of interests.

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