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A mathematical model for the interpretation of nuclear bomb test derived ^{14}C incorporation in biological systems

Samuel Bernard[1], Jonas Frisén[2] and Kirsty L. Spalding[2]

[1]Université de Lyon; Université Lyon 1; INSA de Lyon, F-69621; Ecole Centrale de Lyon; CNRS, UMR5208, Institut Camille Jordan, 43 blvd du 11 novembre 1918, F-69622 Villeurbanne-Cedex, France.

[2]Department of Cell and Molecular Biology, Karolinska Institute, SE-171 77 Stockholm, Sweden.

Abstract

Human tissues continually replace dying cells with newborn cells. However, the rate of renewal varies by orders of magnitudes between blood cells, which are renewed every day and neurons, for which renewal is non-existent or limited to specific regions of the brain. Between those extreme are many tissues that turn over on a time scale of years, although no direct measurements have been done. We present here a mathematical method to estimate cell turnover in slowly renewing biological systems. Age distribution of DNA can be estimated from the integration of radiocarbon derived from nuclear bomb testing during the cold war (1955-1963). For slowly renewing tissues, this method provides a better estimate of the average age of the tissue than direct estimates from the bomb curve. Moreover, death, birth and turnover rates can be estimated. We highlight this method with data from human fat cells.

Introduction

Biomedical research has progressed enormously the last decades, and we now understand many biological processes in minute detail at the molecular level. Nevertheless, there are a few fundamental processes that have remained very difficult to study and where our knowledge is scant. One of those is cell turnover in the human body. It is quite remarkable how little is known about the age of cells in many regions of the adult human body. The stability or turnover of cells in different tissues is a fundamental feature that may influence the response of different organs to insults and aging.

Traditional methods used for dating cells are limited in the information they provide, or are not appropriate for human use (all available techniques for studying cell turnover are considered unsafe for use in humans). As such, much of our view on cell turnover in the adult human body has been inferred from studies in rodents, which in most cases are only a few months old at the time of analysis. This may

not be an ideal model for man, who can live for a century, and can potentially have a greater need to replace cells over a lifespan.

Making use of drastically altered atmospheric ^{14}C levels due to nuclear bomb testing during the cold war, it is possible to retrospectively determine the birth date of a population of cells based on its DNA ^{14}C content. ^{14}C in the atmosphere reacts with oxygen and forms CO_2, which enters the biotope through photosynthesis. Our consumption of plants, and of animals that live off plants, results in ^{14}C levels in the human body paralleling those in the atmosphere. Most molecules in a cell are in constant flux, with the unique exception of genomic DNA, which is not exchanged after a cell has gone through its last division. Nucleotide exchange in postmitotic cells is minimal (1). The level of ^{14}C integrated into genomic DNA thus reflects the level in the atmosphere at any given time. In this way, ^{14}C levels in genomic DNA can be used to retrospectively establish the birth date of cells in the human body (2-4). One example is fat tissue in man. Recent work using radiocarbon dating of fat cells demonstrated that the fat mass in humans is constantly turning over. Neither adipocyte death nor generation was found to be altered in early-onset obesity, suggesting a tight regulation of fat cell number in adulthood (3). Elucidating the high turnover of adipocytes by modelling ^{14}C adipocyte data therefore identified a new therapeutic target for pharmacological intervention in obesity.

Mathematical model of cell turnover

In tissues with significant turnover, lost cells are continuously replaced by new cells. Atmospheric ^{14}C is integrated into the DNA of newborn cells and provide a time stamp of when they were born. Depending on the turnover rate, a wide distribution of birth dates can be expected in a tissue sample. The measured ^{14}C level of the sample is an average of all ^{14}C DNA content weighted with the distribution of cell birth dates. During development, the number of cells in many tissues is increasing while in others it is constant

after birth. For instance, the number of adipocytes increases during childhood until early adulthood. During adulthood, most tissues replace lost cells with new one to preserve homeostasis, but the balance in renewal may change with aging. For instance, it is possible that a reduced capacity for regeneration is compensated by cell hypertrophy. Thus, a model for DNA ^{14}C integration in new born cells must be specific to the development, maintenance and aging of the cell population. Here, we present a method for estimating turnover rates in biological systems that can have a wide range of renewal potential, during development and aging. We illustrate the method with ^{14}C data from fat cells in lean and obese adults.

Because little is known about the mechanisms that regulate the development and maintenance of renewing tissues, it is necessary to make some assumptions on the dynamics of birth and death. We assume that cells die with a probability $dt\,\gamma(t,a)$ during a small time interval dt. The resulting death rate, $\gamma(t,a)$, depends on the age of the individual t (in years, $t \geq 0$) and the age of the cells a (in years, $0 \leq a \leq t$). The number of new cells born each year, $\beta(t)$, is composed of cells replacing a fraction (or all) of dead cells plus cells that are added independent of cell death. Cell number is allowed to increase, stay constant or decrease at different life stages. Newborn cells are assumed to come from a stem cell pool and have undergone enough divisions to dilute the original stem cell DNA. Furthermore, we assume that the DNA content is stable over the cell lifespan. Thus, cells born in an individual aged t have a ^{14}C level corresponding to the year $t + t_b$, where t_b is the birth date of the individual.

These assumptions may be formalized into a linear partial differential equation with an age-structure:

$$\frac{\partial n(t,a)}{\partial t}+\frac{\partial n(t,a)}{\partial a}=-\gamma(t,a)\,n(t,a)\ . \qquad (1)$$

The function $n(t,a)$ is the density of cells of age a for a subject of age t (unit of n: cells/year, units of a and t: year, unit of γ: 1/year). The initial condition at $t = t_0 \geq 0$ is $n(t_0,a) = N_0\,\delta_0(a)$, where δ is the Dirac delta function (i.e. all cells at t_0 have age $a = 0$) and N_0 is the initial adipocyte number. A boundary condition describes the birth of new cells from progenitor cells, $n(t,0) = \beta(t)$ (unit: cells/year). Equation 1, with the

initial and boundary conditions, is related to the more general McKendrick-von Foerster equation used in population dynamics (5).

When γ and β depend on the age of the subject and γ also depends on the age of the cells, $\gamma=\gamma(t,a)$ and $\beta=\beta(t)$, Eq. 1 has the closed form solution, for $a \leq t - t_0$,

$$n(t,a) = N_0 \delta(t-a-t_0) e^{-\int_0^a \gamma(t-a+s,\, s) ds} + \beta(t-a) e^{-\int_0^a \gamma(t-a+s,\, s) ds}. \tag{2}$$

The total cell number for an individual of age $t \geq t_0$ is,

$$N(t) = \int_0^{t-t_0} n(t,a) da = N_0 e^{-\int_0^{t-t_0} \gamma(t_0+s,\, s) ds} + \int_0^{t-t_0} \beta(t-a) e^{-\int_0^a \gamma(t-a+s,\, s) ds} da. \tag{3}$$

For simple functional forms of γ and β, the integrals can be evaluated explicitly. This can be an advantage because numerical simulations can become heavy. The average age of cells in an individual aged t is:

$$\langle a \rangle = \frac{\int_0^{t-t_0} a\, n(t,a)\, da}{N(t)}. \tag{4}$$

It is also possible for some mature cells to re-enter the cell cycle and divide. In this case, cell age distribution would be altered; however cell turnover would be unchanged. When each dying cell is replaced, $N(t)=N_0$ and the production term must match the number of cells dying each year. Because the actual cell number is not important to calculate ^{14}C levels, it is convenient to define the relative production $b=\beta/N_0$. For cell age-independent death rate, death and relative production rates can be equated: $b(t) = \beta(t)/N_0=\gamma(t)$. When the death rate is cell-age-dependent ($\gamma(a)$), the production rate depends on the history of cell birth and death and must satisfy a Volterra equation of type II (also called renewal equation (6)):

$$b(t) = \gamma(t) \exp\left(-\int_0^t \gamma(s) ds\right) + \int_0^t b(t-a) \gamma(a) \exp\left(-\int_0^a \gamma(s) ds\right) da. \tag{5}$$

This equation is to be solved separately. Analytical solutions exist for a handful of functions $\gamma(a)$; hence numerical integration must be used in general (6).The cell turnover rate of a tissue is only well defined when the cell number is constant or slowly changing, so that the number of newborn cells is equal to the number of dead cells in a given time period. This is a realistic assumption in healthy tissues that preserve homeostasis. In that case, the turnover rate is equal to the death rate, γ. Therefore, the death rate is the essential parameter to estimate, along with any change in cell number during lifetime.

Estimating cell turnover from ^{14}C data

The cell density in a tissue of an individual aged t (in years) is given by the solution of Eq. 1, $n(t,a)$. The average ^{14}C level C of a DNA sample collected at calendar year t_d (which may correspond to age at death or age at sampling) of an individual aged t ($t = t_d - t_b$) is

$$C = \frac{\int_0^{t=t_d-t_b} K(t_d - a)\, n(t,a)\, da}{N(t)} \quad (6)$$

The bomb-curve function K is the atmospheric ^{14}C level, and is expressed in relation to a universal standard and corrected for radioactive decay, giving Δ^{14}C values (in ‰, Fig 1 and ref (7)). Once cell production is expressed as a relative production $b(t)$, Equation 2 depends on three unknown parameters: γ, b, and t_0. In contrast to forensic applications, it is supposed that birth date and date of collection (or death) are known. These three parameters must be inferred from the ^{14}C profiles of the samples. Depending on the nature and number of samples, it is usually not possible to find robust estimates for all of these parameters because different dynamics of cell birth and death can correspond to the same ^{14}C profile. This is especially true when samples come from adults and yield little or no information on the dynamics of cell turnover during growth and development. Thus, different scenarios must be envisaged that fix one or more of the parameters, leading to a robust estimate of the remaining parameters.

This leads to the problem of selecting the scenario that best describes the data. The goodness-of-fit is taken as the SSE between the model prediction and the data $\sum_{i=1}^{n}\left(x_i - c_i\right)^2$, where x_i is the prediction and c_i is the Δ^{14}C level for subject i. Models with more parameters are expected to fit the data better, so a criterion that penalizes models with many parameters, such as the Akaike Information Criterion may be used for selecting the most parsimonious model (8). Akaike Information Criterion is $AIC = \log SSE + n_{\mathrm{par}}$ (SSE = sum of squares of the errors). It states that each additional parameter must reduce the SSE by a log. In log base 2, SSE must be halved for every additional parameter.

The characteristic peak shape of the bomb curve is valuable as a way to univocally identify DNA synthesis in pre-bomb subjects (born before 1955). If synthesis is found to occur in pre-bomb subjects, it is not possible to say when it occurred. ^{14}C levels can then correspond to two dates, one on the upward slope of the bomb-curve, and one on the downward part of the curve. By analyzing many individuals dispersed along the bomb-peak axis, it is possible to get an estimate that is consistent between the pre- and post-bomb subjects (born after 1955).

Application to fat cell turnover

Analysis of ^{14}C DNA provides an extremely sensitive method to detect cells born after a subject's birth date. As little as 1% of cells born at the peak of bomb-testing can be detected in a population of pre-bomb cells (2). We have recently analyzed fat samples obtained during liposuction or abdominal wall reconstruction from 35 adult lean and obese adults (3). Ages ranged from 21 to 72 years. In both pre-bomb and post-bomb subjects, ^{14}C levels were similar and corresponded to dates in the middle of the 1990's, suggesting birth of new fat cells in adults of all ages (Fig 1). However, fat cells couldn't be all born during that small time window, in adults born at different years. Fat cells are more likely to be produced continuously, with sampled ^{14}C levels representing the average of ^{14}C content of all combined

individual cells. This approach allows one not only to estimate the average age of fat cells, but also their turnover rates. Because cells of all ages could possibly be sampled, the measured ^{14}C depends on the bomb curve at all years between the birth of the individual and the collection date.

We had little information about the fat cell dynamics in childhood and adolescence; therefore we used two separate data sets (3; 9) to define the course of fat cell numbers from birth to adult. The cell number increased during childhood and adolescence and stabilized in early adulthood, indicating that in adults, fat cell number is constant. Three possible scenarios were consistent with cell number data. (A) At birth few fat cells are present; cells are produced at a constant rate and die at a constant rate. (B) At birth few fat cells are present; fat cell number rapidly grows during childhood and cell number is set afterwards, cells are replaced at a constant rate. (C) Like B, but number is allowed to change after the initial expansion. Fitting these three scenarios lead to similar results concerning fat cells death rate estimates, indicating that ^{14}C samples can be used to find robust estimates of cell turnover rates. However, other parameters t_0 (age at which cell number expands) and b (the relative fat cell production rate) were not consistent among the three scenarios. This is because few cells, if any, survive many decades, thus no ^{14}C signature would be left from childhood in most of the subjects.

It is also of interest to find turnover estimates for individual samples. However, it is not possible to estimate more than one parameter in Eq. (2) for individual samples, due to over-parameterization. Thus more complex (or realistic) models can only be fitted on a population. Scenario A has only one parameter to fit, the death rate. The cell density (Eq. (2) with t_0=0, N_0=0 and γ constant) simplifies to $n(t,a) = \beta \exp(-\gamma a)$, $a \leq t$, and 0 otherwise. Because the total cell number is proportional to β, it cancels out in Eq. (6) and only the death rate remains to be fitted. The production rate β can then be calculated based on the actual cell number at the collection date: $\beta = \gamma N_{measured}/(1 - \exp(-\gamma t))$.

Because fat cell number is constant in adults, the fat cell turnover rate is well defined and can be equated to the death rate. Results of fitting the death rate for two subjects, one pre-bomb and one post-bomb, are

shown in Fig 2. The post-bomb subject was aged 27 at fat cell collection and had a fitted fat cell death rate of 0.104 per year, indicating that 10.4% percent of the cells are replaced each year. The pre-bomb subject was 70 at fat cell collection, and had a fitted fat cell death rate of 0.084 per year, indicating a turnover rate of 8.4% per year. The individually fitted turnover rates were not correlated with the age of the subjects, indicating that a constant turnover rate throughout life was a realistic assumption.

Discussion

The method presented here is suitable for dating biological sample that turn over in years or decades. In addition to adipose tissue, the heart and the brain are tissues with limited capacity for regeneration. Quantifying turnover in mature tissues is important when designing therapies that may enhance normal regeneration. Fast renewing tissues, like blood or skin, have an age distribution that is too narrow to warrant modelling; the ^{14}C measurements would correspond to the collection date (or time of death) (2). In non-renewing tissues, such as cortical neurons, the ^{14}C levels correspond to the birth date of soon after (4).

We focused mainly on DNA samples, since most carbon from non-DNA sources turns over quickly in living cells. However, non-DNA material with low turnover is useful in forensic applications to determine the date of birth and could also benefit from the methods presented here. Tooth enamel is formed at distinct times during childhood and there is no turnover once enamel has been formed, allowing precise estimation of birth dates of adults (10). Using an approach similar to the one presented here, it was recently shown that the protein crystalline in the lens eye is produced almost entirely around birth (11), and is never degraded.

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Figure legends

Figure 1 Atmospheric $\Delta^{14}C$ since 1930 (12). The ^{14}C content from fat cell samples versus birth dates of the subjects is plotted (*diamond*: lean, *dots*: overweight, *square*: obese).

Figure 2 Adipocyte turnover in a post-bomb and a pre-bomb subject. (*Filled square*) Subject aged 27 at fat cell collection, BMI=22.8 kg/m^2, $\Delta^{14}C$=107 ‰, bomb date=1997.1, average fat cell age=7.9 years, average birth date=1998, turnover rate=10.4% per year, production=0.32x10^{10} cells/year. (*Open square*) Subject aged 70, BMI=24.4 kg/m^2, $\Delta^{14}C$=133 ‰, bomb dates= 1959 and 1993, average fat cell age=11.77 years, average birth date=1995.2, turnover rate=8.4% per year, production=0.44x10^{10} cells/year. The filled areas show the cell density $n(t,a)$ for each subject (filled: post-bomb, open: pre-bomb).

Figure 1

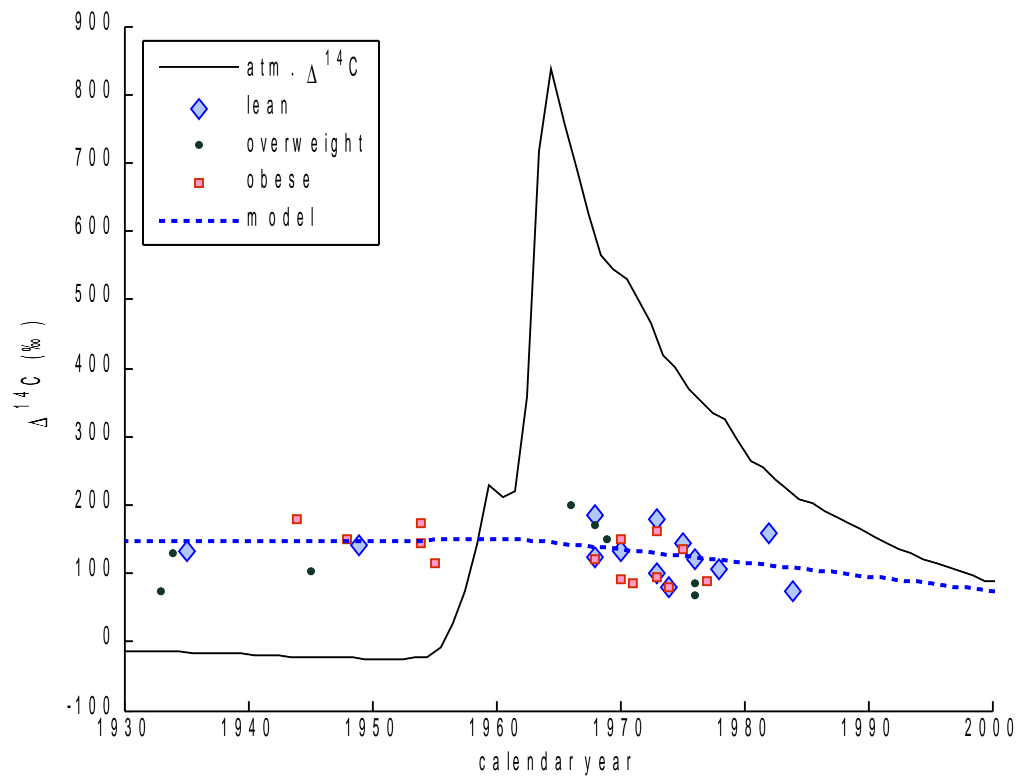

Figure 2