Supporting Information

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SI Text

Mathematical Modeling. Because the fraction of heavy water in body water is similar to that in urine (1), we model the measured up- and down-labeling of the urine enrichment by the following differential equations for normal water, w, and heavy water, h, in the urine

$$\frac{dw}{dt} = (1-f)s - \delta w \quad \text{and} \quad \frac{dh}{dt} = fs - \delta h$$

during label intake ($t \leq \tau$), and

$$\frac{dw}{dt} = s - \delta w \quad \text{and} \quad \frac{dh}{dt} = -\delta h$$

after label intake ($t > \tau$), where f represents the fraction of ^2H_2O in the drinking water, t represents time in days, and labeling was stopped at $t = \tau = 63$ days. δ represents the turnover rate of body water per day, and s is the amount of water consumed in liters per day. These equations can be solved analytically and rewritten in terms of the fraction, $U(t)$, of ^2H_2O in the urine. The baseline urine enrichment, $U(0) = \beta$, that is attained after the boost of label by the end of day 0 determines the initial conditions, i.e., $w(0) = (1-\beta)s/\delta$ and $h(0) = \beta s/\delta$, such that:

$$U(t) = f(1 - e^{-\delta t}) + \beta e^{-\delta t} \quad \textbf{[1a]}$$

during label intake ($t \leq \tau$), and

$$U(t) = [f(1 - e^{-\delta\tau}) + \beta e^{-\delta\tau}]e^{-\delta(t-\tau)} \quad \textbf{[1b]}$$

after label intake ($t > \tau$).

The parameter estimates of the best fits for the urine curves are given in Table S2.

To model the label enrichment of adenosine in the DNA of cells we assume identical reaction kinetics of hydrogen and deuterium and of labeled and unlabeled adenosines. Further, we extended the model of Asquith *et al.* (2) to include the dependence on the actual enrichment of the body water [as estimated by $U(t)$]. Because the adenosine deoxyribose (dR) moiety contains seven hydrogen atoms that can be replaced by deuterium, one expects an amplification factor, $c > 1$, in the enrichment of dR in DNA relative to the body ^2H_2O enrichment. At the low levels of body ^2H_2O enrichment that one typically achieves [$U(t) < 2\%$], the likelihood of double labeling is very low (3, 4). Theoretically, the fraction of adenosine dR moieties that have exactly one enriched hydrogen atom is expected to be $c \cong \binom{7}{1}U(t)(1 - U(t))^6$. However, because of dilution by the purine nucleoside pathway, one typically measures the amplification factor c from the enrichment in dR in the DNA of cells with a rapid turnover, like granulocytes or monocytes (3, 4). Consistent amplification factors of $c = 3.5$ to $c = 4$ for body water enrichment levels of 2–3% are reported in ref. 4.

Following Asquith *et al.* (2), label enrichment of adenosine in the DNA of a population of cells was modeled by

$$\frac{dl}{dt} = pcU(t)A - dl,$$

where l is the total amount of labeled adenosine in the DNA, p is the average production rate of that population, c is the amplification factor, A is the total amount of adenosine in the DNA of that population, and d is the loss rate of cells carrying labeled adenosine. Basically, one writes that each adenosine residue replicates at rate p and will incorporate a deuterium atom with probability $cU(t)$. For naïve T cells, this replication may occur both in the periphery and the thymus. Scaling this equation by the total amount of adenosine in the DNA, i.e., defining $L = l/A$, yields

$$\frac{dL}{dt} = pcU(t) - dL \quad \textbf{[2]}$$

throughout the labeling and delabeling period, where L represents the fraction of labeled deoxyribose residues of adenosine in DNA. The corresponding analytical solutions for the enrichment of adenosine in DNA are

$$L(t) = \frac{cpf}{\delta - d}\left[\frac{\delta}{d}(1 - e^{-dt}) - (1 - e^{-\delta t}) + \frac{\beta}{f}(e^{-dt} - e^{-\delta t})\right] \quad \textbf{[3a]}$$

during label intake ($t \leq \tau$), and

$$L(t) = \frac{cpf}{\delta - d}\left[\frac{\delta}{d}(e^{-d(t-\tau)} - e^{-dt}) - (e^{-\delta(t-\tau)} - e^{-\delta t}) + \frac{\beta}{f}(e^{-dt} - e^{-\delta t})\right] \quad \textbf{[3b]}$$

after label intake ($t > \tau$). Note that the amplification factor and the division rate always appear together, and that pc can therefore only be estimated as a parameter combination.

Eqs. **3a** and **3b** were fitted to each individual's granulocyte enrichment data, yielding $0.299 \leq pc \leq 0.419$ per day and death rates $0.078 < d < 0.103$ per day (see Table S3). Assuming that the granulocytes are fully turned-over, i.e., assuming that $p = d$ for the granulocytes, we estimate amplification factors of $c = 4.46$, 4.93, 5.15, 3.78 and $c = 4.03$ for the five individuals (which is indeed lower than the theoretical maximum of 7).

Next, Eqs. **3a** and **3b** were applied to calculate the average turnover rate p and the loss rate of labeled cells d in each T cell population. The parameter p in Eq. **2** represents T cell production resulting from both T cell proliferation and thymic output. Because p determines the average T cell turnover rate, pN (the average number of naive cells produced per day) provides an upperbound for the number of T cells exported from the thymus per day. To correct for the body water enrichment as measured by $U(t)$ and the amplification factor, c, of each individual person, all data were normalized by dividing by the maximum cf. Doing the same for the granulocyte data the maximum label enrichment in granulocytes was basically scaled to 100%.

Cells that divided during the first days of the labeling period will have incorporated less deuterium than those that divided later. If such cells were to die earlier than cells that divided later, the loss of label during the chase phase need not be exponential because poorly labeled cells would die earlier. However, because we are assuming an exponential distribution of expected life spans in these models, cells are assumed to have death rates that are independent of their age.

1. Ackermans MT, *et al.* (2001) *J Clin Endocrinol Metab* 86:2220–2226.
2. Asquith B, Debacq C, Macallan DC, Willems L, Bangham CR (2002) *Trends Immunol* 23:596–601.
3. Hellerstein MK, Neese RA (1999) *Am J Physiol* 276:E1146–E1170.
4. Neese RA, *et al.* (2002) *Proc Natl Acad Sci USA* 99:15345–15350.

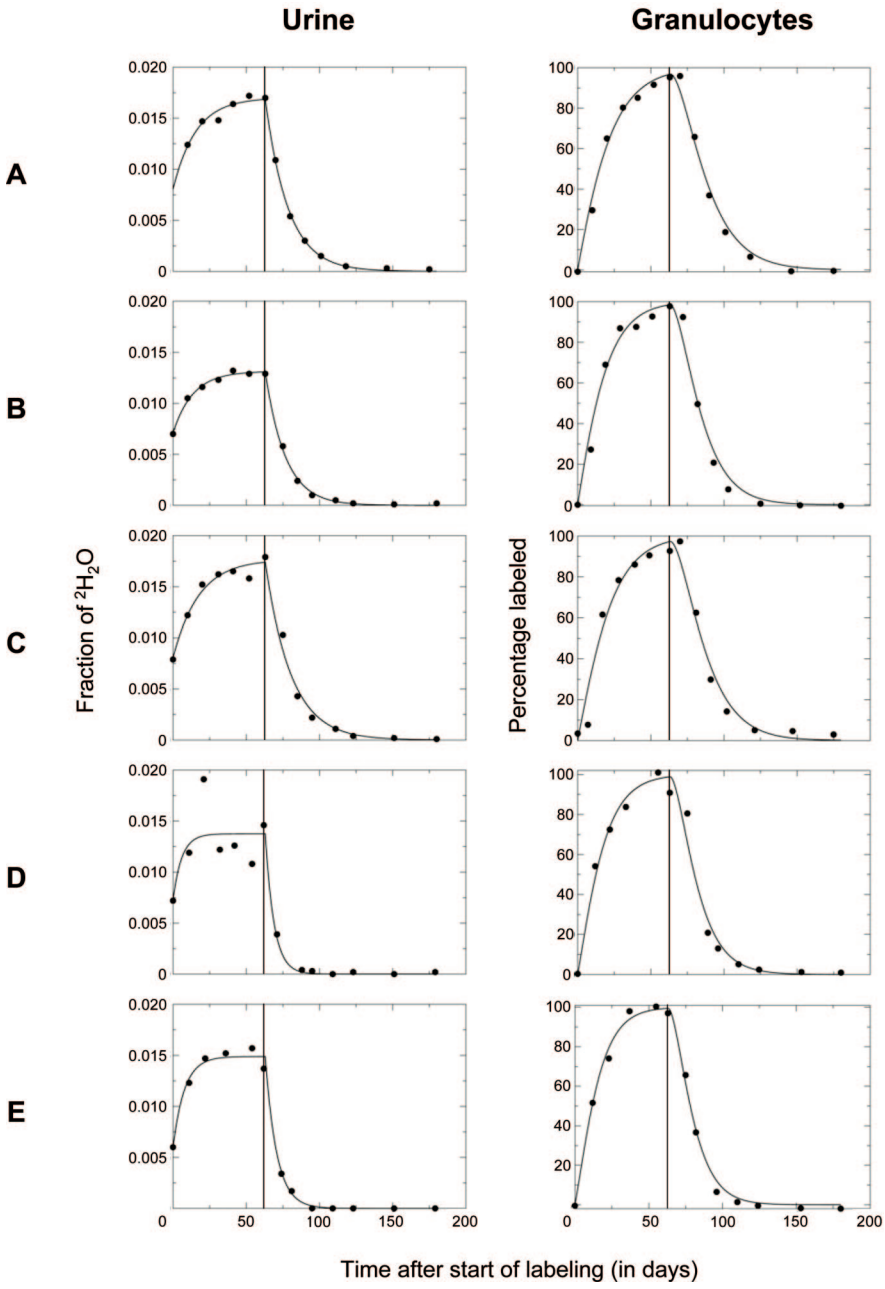

Fig. S1. Best fits of the fraction of ^2H_2O in urine and the percentage of labeled deoxyribose residues of adenosine in granulocytes after scaling by the maximum enrichment level *cf* of the granulocyte population (see *SI Text*). In the graph, the end of the labeling period is marked by a vertical line.

Table S1. Characteristics of healthy volunteers

Characteristic	Individual				
	A	B	C	D	E
Age at start of the protocol	24	22	25	20	22
$CD4^+$ count per μl blood	890 (810–1040)	690 (663–808)	830 (780–990)	1300 (1080–1730)	1320 (1130–1500)
$CD8^+$ count per μl blood	470 (420–550)	355 (320–413)	500 (460–590)	440 (410–530)	820 (660–910)
Naïve $CD4^+$, %	60 (54–66)	68 (65–71)	37 (34–41)	69 (67–73)	68 (63–71)
Memory $CD4^+$, %	40 (34–45)	32 (29–34)	54 (48–56)	31 (27–33)	31 (28–36)
Naïve $CD8^+$, %	54 (50–58)	68 (63–70)	37 (31–41)	65 (62–71)	59 (52–62)
Memory $CD8^+$, %	35 (29–40)	17 (14–18)	18 (15–21)	12 (11–14)	20 (19–21)
$Ki67^+$ within $CD4^+$, %	2.75 (2.33–3.19)	1.86 (1.47–2.25)	2.13 (1.82–2.30)	1.36 (0.59–2.18)	1.61 (1.37–2.12)
$Ki67^+$ within naïve $CD4^+$, %	0.76 (0.39–1.05)	0.91 (0.65–1.24)	0.77 (0.59–0.99)	0.29 (0.21–1.40)	0.43 (0.33–0.73)
$Ki67^+$ within memory $CD4^+$, %	5.00 (4.29–5.02)	3.42 (2.93–4.45)	3.18 (2.87–3.52)	1.82 (1.55–2.50)	3.84 (3.05–4.24)
$Ki67^+$ within $CD8^+$, %	1.65 (1.45–1.88)	1.46 (1.26–1.92)	2.29 (1.68–2.78)	1.26 (0.85–1.72)	1.34 (1.14–1.81)
$Ki67^+$ within naïve $CD8^+$, %	0.94 (0.57–1.12)	0.73 (0.57–1.13)	0.97 (0.63–1.21)	0.47 (0.22–0.68)	0.50 (0.42–0.65)
$Ki67^+$ within memory $CD8^+$, %	1.87 (1.61–2.05)	2.35 (1.98–2.97)	NA	1.51 (1.22–2.51)	3.54 (2.46–4.01)

Depicted are median values and interquartile ranges over follow-up.

Table S2. Parameter estimates of the urine enrichment curves, where f represents the fraction of ^2H_2O in the drinking water, δ is the turnover rate of body water per day, and β represents the baseline urine enrichment attained after the boost of label by the end of day 0

Individual	f	δ	β
A	0.0170	0.064	0.0080
B	0.0129	0.088	0.0070
C	0.0173	0.075	0.0082
D	0.0138	0.128	0.0074
E	0.0150	0.119	0.0059

Table S3. Parameter estimates of the granulocyte enrichment curves (before scaling), where *d* represents the loss rate of labeled granulocytes, *p* is the average production rate of granulocytes, and *c* the amplification factor

Individual	*pc*	*d*
A	0.384	0.086
B	0.419	0.085
C	0.402	0.078
D	0.299	0.079
E	0.415	0.103

Other Supporting Information Files

Dataset S1