# Utilizing nonequilibrium isotope enrichments to dramatically increase turnover measurement ranges in single biopsy samples from humans: Supplemental Information

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**Data included here:**

**Separate Excel Workbook with multiple tabs**

**Supplemental Table S1** – Data about subjects in this study.

**Supplemental Table S2** – Every protein observed in this study. Columns are rates for different subjects calculated from either calculating the average or median of all calculated peptide sequence rates.

**Supplemental Table S3** – summary of peptide sequence rates from serum using all timepoints or only one or two timepoints. Columns indicate if the all calculated peptide sequence rates that passed DeuteRater-H's filters (all) or only those peptide sequences that passed DeuteRater-H's filters and were present in all timepoints (matched).

**Supplemental Table S4** – Serum data for every protein observed, for every condition used in the paper. Average rates and standard deviations are provided for all single timepoints and combinations. Combinations are indicated by a certain number of timepoints (t) being required, and are labeled as require_t_timepoints.

**Supplemental Table S5** – Muscle data for every protein observed, for every condition used in the paper. Average rates and standard deviations are provided for all single timepoints and combinations. Combinations are indicated by a certain number of timepoints (t) being required, and are labeled as require_t_timepoints.

**Supplemental Table S6** – Saliva data for every protein observed, for every condition used in the paper. Average rates and standard deviations are provided for all single timepoints and combinations . Combinations are indicated by a certain number of timepoints (t) being required, and are labeled as require_t_timepoints.

**Supplemental Table S7** – Serum data for every peptide sequence observed, for every condition used in the paper. Calculated rates and mean of all absolute residuals are provided for all single timepoints and combinations. Combinations are indicated by a certain number of timepoints (t) being required, and are labeled as require_t_timepoints.

**Supplemental Table S8** – Muscle data for every peptide sequence observed, for every condition used in the paper. Calculated rates and mean of all absolute residuals are provided for all single timepoints and combinations. Combinations are indicated by a certain number of timepoints (t) being required, and are labeled as require_t_timepoints.

**Supplemental Table S9** – Saliva data for every peptide sequence observed, for every condition used in the paper. Calculated rates and mean of all absolute residuals are provided for all single timepoints and combinations. Combinations are indicated by a certain number of timepoints (t) being required, and are labeled as require_t_timepoints.

**Supplemental Table S10** – Data from mice labeled with a constant enrichment strategy and bolus injection for the purpose of comparing flat vs rising enrichment schemes. Shown in Figure

4C. “Accession” is the Uniprot accession number. “Protein Name” is the common name of the protein. “all data 3 timepoints required” allowed the calculation software to use any available timepoints, but required at least 3 different timepoints to calculate a rate. “day 8 turnover rate” are rates calculated using time 8 days only. “day 32 turnover rate” are rates calculated using time 32 days only. Blank cells indicate a protein was not observed at the relevant time, or there were enough errors with the measurement that the rate was filtered out.

**Supplemental Table S11** – Amino acid specific physical constants used in the DeuteRater algorithm.

Figure S1

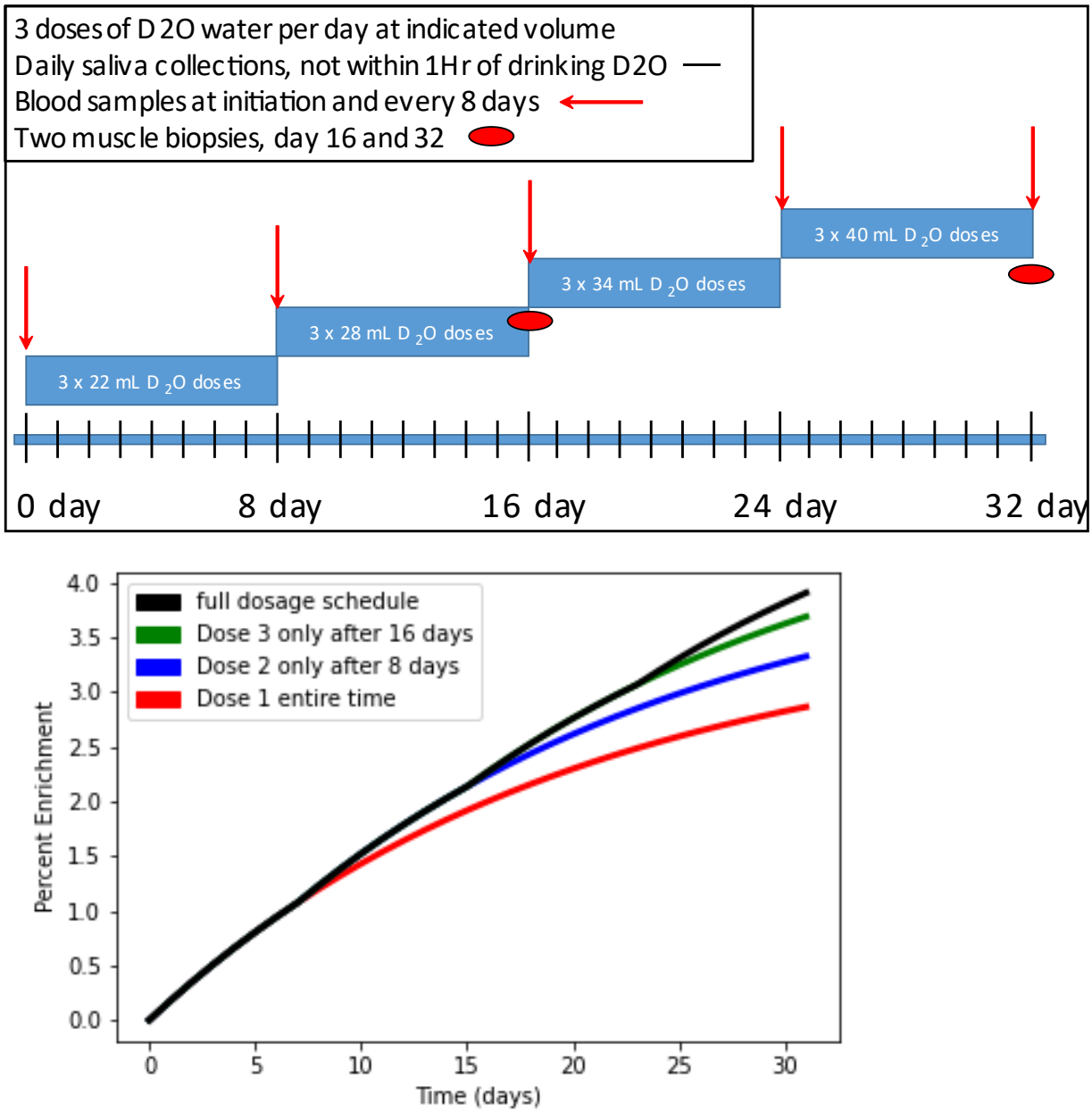


**Figure S1** Experimental Design. (Top) Blue rectangles represent different amounts of Deuterium in daily doses, red ovals represent muscle biopsies, red arrows represent blood draws. (Bottom) Simulations of body water deuterium enrichment for a 100 lb (45Kg) subject assuming 40% of the body mass is water replaced at 5% of the volume each day with the dose schedule shown in the top panel. Four step-wise increases in the dosage was important to maintaining the linearity. Stopping at any of the initial doses would result in plateaus of enrichment. The simulations suggested that with these doses a heavier person would achieve lower total enrichments, but have a similarly linear increase in deuterium. Therefore, a single dosing design was used for all subjects.

Figure S2

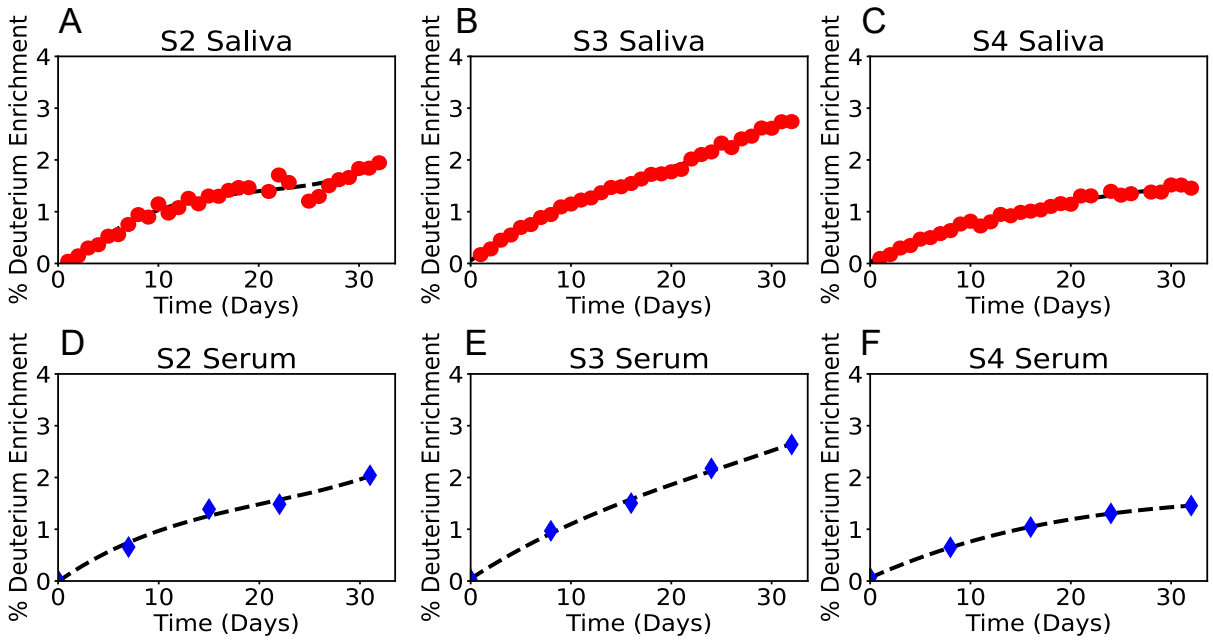


**Figure S2** Saliva and serum enrichments compared. For subjects where $^{2}H_2O$ was measured in both saliva and serum, splines (black dashed lines) were calculated for each. Red dots are saliva measurements, blue diamonds are serum measurements. This supported that the saliva was an accurate predictor of blood plasma enrichment.

Figure S3: Simulations of deuterium ramp

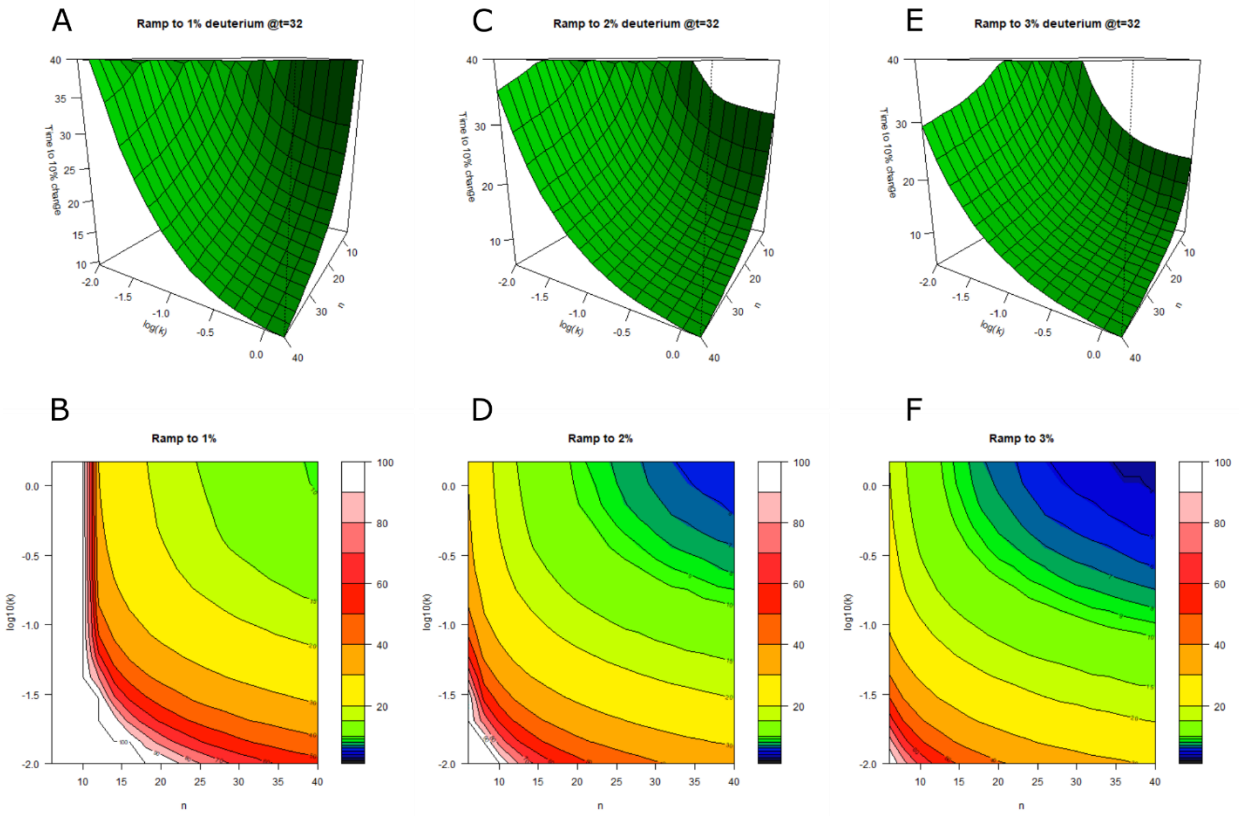


**Figure S3:** Simulation of effect surface. Time required for measurable changes in isotope distributions were simulated for an expected range of peptide turnover rate (***k***, 0.05-1.5), incorporated number of deuteriums (*n-value*, 6-40) at different body water deuterium enrichments (1, 2, or 3 percent final enrichments). The same data is displayed as 3D surface (Top) with time on the z axis or a contour map with the time from 1-100 days displayed as a color gradient from dark blue (1 day) to white (90-100). Based on the general ranges of n-value and rate expected in this data set, the minimum time to detectability is expected to be after 9.0 days for a 1% ramp, 5.0 days for a 2% ramp, and 3.5 days for a 3% ramp for peptides with the largest rate (***k***) and *n-value*.

Figure S4:

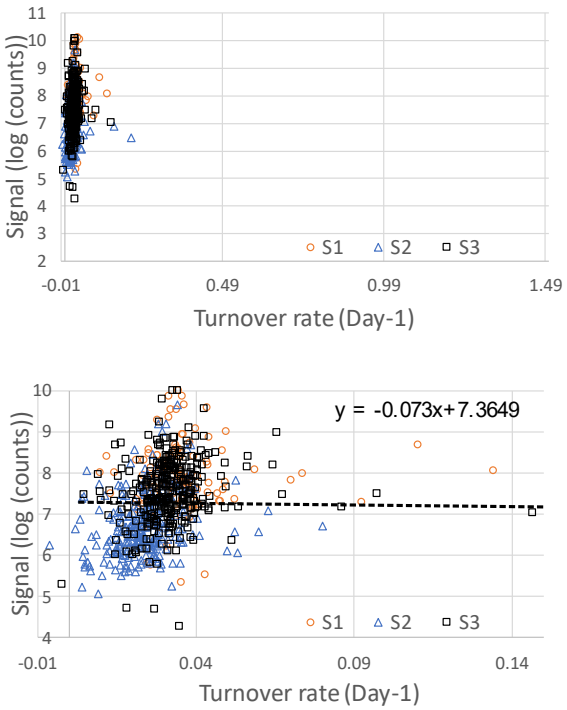


**Figure S4:** Testing for signal-dependent bias. Measurements of Serum Albumin (SA) were used to explore whether signal intensity introduces any bias in the calculation of kinetic rates. Since SA is the most abundant protein in the serum, we had ~ 2300 measured peptide rates across the entire range of signal intensity. There was no statistical support for a signal intensity bias.

Figure S5

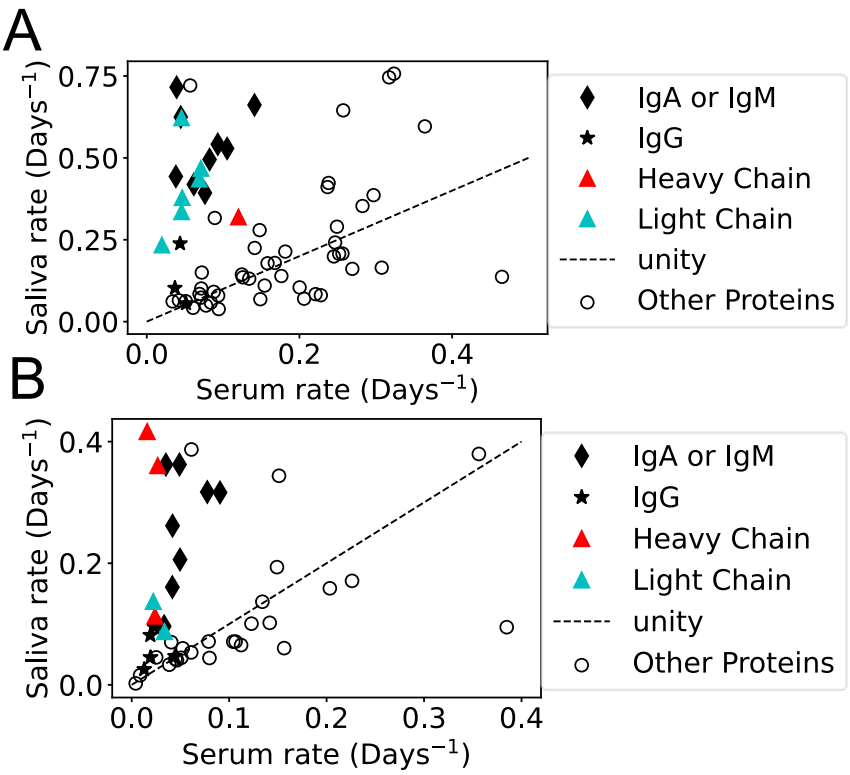


**Figure S5** Timepoint-specific comparison of serum and saliva turnover rates with a focus on immunoglobulins. Only single timepoints were used. Panel A only uses rates calculated from 8-day samples, Panel B only uses rates calculated from 32-day samples. This demonstrates that the individual timepoints at the extreme ends of the experiment still show the biologically relevant pattern that IgA and IgM are produced in the saliva and serum separately with separate turnover rates.