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Characterization of human plasma proteome dynamics using deuterium oxide

Ding Wang^{1,2,*}, David A Liem^{1,2,*}, Edward Lau^{1,2}, Dominic CM Ng¹, Brian J Bleakley¹, Martin Cadeiras³, Mario C Deng³, Maggie PY Lam^{1,2}, and Peipei Ping^{1,2,3,#}

¹The NHLBI Proteomics Center at UCLA, Los Angeles, CA, USA

²Department of Physiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

³Department of Medicine, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Abstract

Purpose—High-throughput quantification of human protein turnover via in vivo administration of deuterium oxide (²H₂O) is a powerful new approach to examine potential disease mechanisms. Its immediate clinical translation is contingent upon characterizations of the safety and hemodynamic effects of in vivo administration of ²H₂O to human subjects.

Experimental design—We recruited 10 healthy human subjects with a broad demographic variety to evaluate the safety, feasibility, efficacy, and reproducibility of ²H₂O intake for studying protein dynamics. We designed a protocol where each subject orally consumed weight-adjusted doses of 70% ²H₂O daily for 14 days to enrich body water and proteins with deuterium. Plasma proteome dynamics was measured using a high-resolution MS method we recently developed.

Results—This protocol was successfully applied in 10 human subjects to characterize the endogenous turnover rates of 542 human plasma proteins, the largest such human dataset to-date. Throughout the study, we did not detect physiological effects or signs of discomfort from ²H₂O consumption.

Conclusions and clinical relevance—Our investigation supports the utility of a ²H₂O intake protocol that is safe, accessible, and effective for clinical investigations of large-scale human protein turnover dynamics. This workflow shows promising clinical translational value for examining plasma protein dynamics in human diseases.

Keywords

deuterium oxide; heavy water; plasma proteome; protein dynamics; protein turnover

#Correspondence to Peipei Ping: 675 Charles E. Young Drive S, MRL Building 1-609, Los Angeles, CA 90095, USA. Tel: 310-267-5624; Fax: 310-267-5623; pping@mednet.ucla.edu.

*These authors contributed equally.

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Non-standard abbreviations: None

1 Introduction

Protein turnover, in parallel to protein abundance and protein modifications, is an essential property to protein function in biological processes. Protein half-life is an integral component of homeostasis that is increasingly implicated in human disorders including cardiovascular diseases [1–4], suggesting it is possible to measure protein dynamics and half-life as a novel source of insights into the underlying mechanisms of human disease development. International efforts from the Human Proteome Organization (HUPO) and others have reported 3,000–10,000+ known protein constituents in the human plasma, including circulating proteins originating from different organs [5, 6]; accordingly, the plasma proteome has become the major source of medically relevant protein biomarkers [7]. Annotation efforts have verified >300 cardiovascular-relevant proteins detectable in the human plasma [8]. A global survey of human plasma protein dynamics therefore has the potential to propel basic cardiovascular research on the regulation of this unique proteome, and will further lay the groundwork for the discovery of novel diagnostics biomarkers that differ between healthy and diseased subjects.

We previously reported an analytical approach designed to investigate the half-life of individual proteins on a proteome scale via the administration of deuterium oxide ($^2\text{H}_2\text{O}$) [9, 10]. Following the introduction of $^2\text{H}_2\text{O}$ to body water, incorporation of deuterium atoms into newly synthesized proteins gradually shifts the protein's isotopic distributions over time, which can be resolved by MS to calculate the turnover half-life of the protein species. A distinguishing feature of our method is that it accommodates the slow and gradual $^2\text{H}_2\text{O}$ administration in human study design by modeling the rise of available deuterium with a first-order kinetics function. This allows the $^2\text{H}_2\text{O}$ enrichment time course to be simply described with two parameters: the first-order enrichment rate constant (k_p) and plateau enrichment (p_{ss}). The resulting unified kinetics model thus corrects for gradual isotope intake in human to calculate protein isotope patterns due to turnover. In conjunction with computational automation, the model could be employed to quantify the in vivo half-life of thousands of proteins, even from protein samples acquired at as few as one time point.

Applying this method, we recently showed that maladaptive cardiac remodeling is associated with widespread changes of plasma protein dynamics in a mouse model of heart failure [9]. These promising developments encouraged us to translate the method to study human heart diseases, but successful clinical translation requires two essential questions to be addressed. Firstly, it is not known what the acute and long-term hemodynamic impacts and safety outcomes of $^2\text{H}_2\text{O}$ consumption are on human subjects. Secondly, it remains to be established a minimal $^2\text{H}_2\text{O}$ administration dosage and duration that is sufficient to characterize the dynamics of cardiovascular disease relevant plasma proteins. Hence the goal of the present study is to address these knowledge gaps and evaluate general safety and efficacy of the $^2\text{H}_2\text{O}$ administration protocol, as an essential step toward translating this strategy to investigate cardiovascular diseases in human patients.

2 Materials and methods

2.1 Reagents

$^2\text{H}_2\text{O}$ (70%) was purchased from Cambridge Isotope Laboratories and filtered through 0.1- μm polyethersulfone membranes (VWR). Chemical reagents were acquired from Sigma-Aldrich unless specified. Milli-Q (Millipore) filtered water (18.2 M Ω) was used for sample processing.

2.2 Human subject recruitment

All experimental procedures involving human subjects were reviewed, approved and overseen by the UCLA Institutional Review Board (IRB#12-000899). A written informed consent was obtained from each subject who entered the study. Ten healthy human subjects (Table 1) were recruited by public advertisement in the Los Angeles area of California and screened by a detailed medical health questionnaire. Exclusion criteria consisted of a known chronic disease or medical condition, including diabetes, liver, lung, cardiovascular, neoplastic or kidney disease, any form of bacterial or viral infection, systemic inflammatory disorders; or inability to give written consent. All subjects were normotensive (<140/90 mmHg) and were not prescribed any medications.

2.3 Measurement of $^2\text{H}_2\text{O}$ enrichment in body fluids

Each human blood specimen was collected in a lithium heparin coated tube (Greiner Bio-one) and centrifuged (800 $\times$ g, 5 min) to separate plasma from formed elements. Plasma was stored frozen at -80°C until use. Biohazard materials were handled and disposed in accordance with the biosafety guidelines at UCLA. To determine the $^2\text{H}_2\text{O}$ enrichment in body fluid of human subject, plasma or saliva specimen was centrifuged (20 min, 4,000 $\times$ g, 4°C); the supernatant was collected and analyzed with GC-MS as described after isotope exchange with acetone [10]. Briefly, 20 μL of plasma was mixed with 2 μL of 10 N NaOH and 4 μL of 5% (v/v) acetone in acetonitrile. The sample mixtures and standard curves (0–20% $^2\text{H}_2\text{O}$) were incubated overnight. After exchange, the acetone was extracted from the sample with 500 μL of chloroform and 0.5 g of anhydrous sodium sulfate, then analyzed on an Agilent 6890/5975 GC mass spectrometer with a J&W DB17-MS capillary column (Agilent, 30 m $\times$ 0.25 mm $\times$ 0.25 μm), operated in the electron impact mode (70 eV) and SIM at m/z 58 and 59 with 10 ms dwell time.

2.4 Measurement of plasma protein dynamics with LC-MS

Quantification of plasma protein dynamics from $^2\text{H}_2\text{O}$ -labeled samples has been previously described in detail [9]. Detailed proteomics methods can be found in the Supplemental Methods. For proteomic characterization, 500 μg of each plasma sample was depleted of 14 top abundance proteins by multiple affinity columns (Agilent Hu14). The eluent was digested on 10-kDa polyethersulfone filters (Pall Life Sciences) after buffer exchange with 100 mM ammonium bicarbonate. The samples were heated at 70°C with 3 mM DTT for 5 min, alkylated with 9 mM iodoacetamide in the dark, and digested on-filter with 50:1 sequencing grade trypsin (Promega) (16 h, 37°C). Proteolytic peptides of $^2\text{H}_2\text{O}$ -labeled plasma proteins were fractionated by 2D RP-RP LC [11], then analyzed on an LTQ Orbitrap

Elite mass spectrometer (Thermo Fisher Scientific) as described [9, 10]. Proteins were identified from mass spectra using ProLuCID [12] against a reverse-decoyed database (Uniprot human reference proteome, reviewed, 02/09/2013, 20,241 entries). Protein abundance and turnover rates were quantified from MS data using the in-house software ProTurn [9]. Details of ProTurn kinetic curve-fitting are in Supplemental Information.

3 Results and discussion

3.1 $^2\text{H}_2\text{O}$ administration regimen for healthy human subjects

Our first objective was to design a standard protocol that ensures reliable establishment and maintenance of $^2\text{H}_2\text{O}$ enrichment in diverse human subjects. We recruited 10 healthy human subjects representing a range of demographics and body types (Table 1). The subjects included 3 Asians, 6 Caucasians and 1 Asian-Indian, 4 females and 6 males. The average age of the subjects was 26 ± 3 (range: 21–51) years old. Body weights averaged 76 ± 5 (range: 51–108) kg, and heights ranged 160–188 cm; body mass index values were 19.9–30.5, altogether indicating a normal distribution of body types.

Given it is relatively proportional to body water content, body weight is a critical parameter in determining the optimal $^2\text{H}_2\text{O}$ dosage. Following two preliminary experiments (data not shown), we optimized a weight-adjusted $^2\text{H}_2\text{O}$ intake amount for the current protocol to target ~2% total body water enrichment, as follows. To enrich body water with $^2\text{H}_2\text{O}$, the subjects were instructed to orally consume 4 boluses of 0.51-mL/kg (body weight) sterile 70% (molar ratio) $^2\text{H}_2\text{O}$ daily at 11:00 am, 2:00 pm, 6:00 pm, and 9:00 pm for the first 7 days of the protocol. In the next 7 days, the participants consumed 2 boluses of 0.56-mL/kg sterile 70% (molar ratio) $^2\text{H}_2\text{O}$ daily at 11:00 am and 9:00 pm (Figure 1A). From day 0 to day 14, we collected 0.5 mL of saliva and 3 mL of venous blood samples in the clinical laboratory of the UCLA Ronald Reagan Medical Center at 12:00 noon for a total of 10 specified time points (day 0, 1, 2, 4, 5, 8, 9, 10, 12, 14 of administration). Compliance with $^2\text{H}_2\text{O}$ intake was ensured by the surveillance of $^2\text{H}_2\text{O}$ enrichment in body fluid and by the return of $^2\text{H}_2\text{O}$ vials for counting. All subjects maintained normal food and fluid intake during the study and routine daily activities.

During the study, we inquired all participants daily with a concise medical health questionnaire for any signs of discomfort. General physical examinations were likewise conducted on a daily basis while vital signs including blood pressure, heart rate, and temperature were recorded. The physical conditions of the participants were continuously monitored for at least 14 days after the completion of intake, while blood and saliva were collected to monitor physiological clearance of $^2\text{H}_2\text{O}$ from the body water of the subjects. Throughout the period, no signs of discomfort were observed or reported by any human subjects. We maintained detailed records of the vital signs of each subject. The records show that average body temperature of the 10 human subjects remained steady ($36.8\text{--}37.1^\circ\text{C}$) during the study, with <1% maximal deviation for each subject (Figure 1B). Likewise, $^2\text{H}_2\text{O}$ had no discernible effects on heart rates (60–95 bpm) (Figure 1C), and the variations in systolic/diastolic blood pressure were within acceptable physiological range (Figure 1D). We continued to monitor a subset of subjects for vital signs and hemodynamic parameters for up to 8 months (~240 days) after completion of $^2\text{H}_2\text{O}$ intake. Overall, these results

strongly suggest that our standard protocol has no observable effects on the subjects regarding blood pressure, body temperature and heart rate. To our knowledge, this is the first systematic documentation to address these parameters associated with $^2\text{H}_2\text{O}$ intake in human.

3.2 Enrichment and subsequent physiological clearance of $^2\text{H}_2\text{O}$ in body fluids

As mentioned above, we previously devised a computational approach to analyze protein dynamics data that models $^2\text{H}_2\text{O}$ enrichment in body water with a steady exponential rise curve to simplify the overall kinetic model. This is based on the assumption that human body behaves as a well-mixed system with respect to total water. To investigate the empirical generalizability of this model, we carefully characterized the enrichment curve of all subjects at 10+ time points to examine whether the enrichment kinetics is broadly replicated in a wide range of individuals. We measured $^2\text{H}_2\text{O}$ enrichment in the collected body fluid samples of each human subject by GC-MS. Upon $^2\text{H}_2\text{O}$ consumption, this protocol successfully built up gradual enrichment towards plateau enrichment level of 1.0–2.2% (Figure 2A; Supplemental Figure 1), at enrichment rates of 0.16–0.30 d^{-1} . Thus after adjusting doses by body weight, we were able to acquire relatively reproducible $^2\text{H}_2\text{O}$ enrichment behaviors over a wide range of individuals to the target enrichment level (<2%). We also observed an impact by the lifestyle of participants. During the study, subject 9 reported a bout of increased physical activities and water intake during the second week of the protocol, which led to an observable drop of enrichment level and deviation from the exponential rise curve. After we omitted the samples collected after this lifestyle change, we were nevertheless able to analyze samples (from day 1–10) in the exponential rise region using LC-MS to derive protein turnover rates from the subject. Since a particular dosage of ~1.5%–2% appears to be optimal for proteomics analyses (vide infra), it is possible that other factors that may also influence enrichment behaviors, including gender and total daily water intake, can be taken into account to further improve target $^2\text{H}_2\text{O}$ dosages in the future.

Taken together, the data affirm that in 10 biological replicates, $^2\text{H}_2\text{O}$ enrichment closely approximates the first-order exponential function across multiple personalized dosages. Fitting the data points to an exponential rise curve therefore allows precise estimates of the empirical rate constant (k_p) and plateau enrichment level (p_{ss}) of body water $^2\text{H}_2\text{O}$ enrichment, which could be employed to predict $^2\text{H}_2\text{O}$ enrichment at any given time point during intake, and to correct for protein ^2H incorporation when calculating protein turnover kinetics.

After completion of intake, the level of body $^2\text{H}_2\text{O}$ in the subjects gradually subsided with a half-life of ~7 days (i.e., at a rate of ~0.1 d^{-1}), following the reported natural turnover of water in human. The participants were further monitored for up to 6 months after intake. By day 50 (~7 half-lives) after the completion of the intake protocol, the $^2\text{H}_2\text{O}$ level of the subjects has largely returned to the baseline (Figure 2A). By day 240, approximately 8 months after the completion of intake, we recalled three subjects (#1, 2, 4) to follow up on their body water $^2\text{H}_2\text{O}$ enrichment level, and detected no trace of deuterium remaining in their body fluids.

In addition to plasma samples, we also collected saliva samples from the subjects to independently quantify $^2\text{H}_2\text{O}$ enrichment rates in body water. The blood and saliva samples were collected concomitantly during the first two weeks of intake. For the following two weeks, daily saliva samples were collected regularly with only a few occasional blood samples. Independent GC-MS analyses of these plasma and saliva samples presented remarkably similar estimates of $^2\text{H}_2\text{O}$ enrichment and clearance profiles in total body water ($R^2=0.996$) (Figure 2B). As a result, the best-fit $^2\text{H}_2\text{O}$ enrichment rates (k_p) and plateau enrichment levels (p_{ss}) measured from saliva and from plasma were virtually identical in every examined human subject, suggesting saliva is a highly reliable source of body fluids to monitor the $^2\text{H}_2\text{O}$ enrichment in protein dynamics studies in an equivalent manner to plasma (Figure 2C). Given that the collection of saliva is a more accessible and less invasive procedure for clinical research than venous blood procurement, we envision the use of saliva collection during $^2\text{H}_2\text{O}$ administration can facilitate more flexible study design, e.g., the $^2\text{H}_2\text{O}$ enrichment curve can be acquired by frequent, non-invasive saliva collection, whilst protein turnover half-life can be determined by a single tissue biopsy taken at a particular time point during the course of $^2\text{H}_2\text{O}$ administration.

3.3 Large-scale characterization of human plasma protein dynamics

We obtained from the 10 human subjects the temporal dynamics of 542 plasma proteins, 325 of which were quantified in 3 subjects (Figure 3A). To our knowledge, this dataset is among the largest collections of human protein dynamics to-date, both in terms of proteome coverage and in the number of biological replicates. The quantified proteins belong to diverse functional categories as evident from GO terms (Supplemental Figure 2) and include proteins of intracellular origins, consistent with the notion that proteins from cell leakage can be accessed and measured in the plasma (Supplemental Figure 1). A germane consideration is whether the quantified proteins are of interest to biomarker discovery or cardiovascular disease study. The total plasma proteome presents a formidable 10^{12} dynamic range of concentration. Recent HUPO Plasma Proteome Project efforts reported the concentration of 1,243 plasma based on spectral count information, spanning over 6 orders of magnitude [6]. Given that a single typical MS experiment can only sample 10^4 – 10^6 dynamic range of protein concentrations, some underrepresentation of the plasma proteome is inevitable in the current investigation. Nevertheless, proteins of interest can be found across the sampled concentration range. Previous HUPO annotations have identified 338 out of ~3,000 plasma proteins with known relevance to cardiovascular diseases. Remarkably, our dataset revealed the turnover of 119 of these proteins, with disease relevance ranging from cardioprotection to myocardial infarction markers (Supplemental Table S1). The quantification of their turnover rates serves as a blueprint for future comparisons with cardiac disease patients.

To determine whether the measured turnover rates were independent from protein abundance as was previously observed, we ranked all quantified proteins by their abundance. Remarkably, protein half-life was almost entirely independent from protein expression over 3+ orders of magnitude of abundance ($\rho=0.109$) (Figure 3B; Supplemental Figure 3A). Consumption of $^2\text{H}_2\text{O}$ had no discernible effect on protein abundance, as measured by spectral counts before and after the $^2\text{H}_2\text{O}$ intake protocol in the 10 subjects

(Benjamini-Hochberg P : 0.49–1.00) (Supplemental Table S1). The proteome dynamics data were generally reproducible among the tested subjects despite their differences in age and lifestyle, with ~25% median CV, and are comparable with other observations [13] and literature values from single-protein studies (Table 2; Supplemental Table S1). Consecutive technical triplicate MS experiments showed that the technical CV of >90% of quantified peptides were 5% (Supplemental Information). The turnover half-life of the detected plasma proteins was diverse and spanned over 50-fold in turnover rates from hemoglobin (HBB; half-life: >50 days) to albumin (ALB1; half-life: 22 days) to insulin-like growth factor 2 (IGF2; half-life: <1 day) (Figure 3C). The dynamic range and reproducibility of the data are promising signs that future biomarkers can be discovered from plasma protein dynamics that are informative for other pathophysiological processes than those reflected by changing expression.

Furthermore, these results serve to affirm that the designed protocol, to a very low level of ~1% $^2\text{H}_2\text{O}$ enrichment, was adequate to monitor large-scale protein turnover in human using our workflow. Nevertheless, there appears to be a linear relationship (R^2 : 0.836) between data quality and subject enrichment between ~1% and ~2% of $^2\text{H}_2\text{O}$, with the platform yielding up to twice as much protein dynamics information from subjects enriched to ~2% (Supplemental Figure 3B). This likely reflects the fact that at low enrichment levels the small amount of isotopes incorporated into the protein pool is technically difficult to discern by MS, especially for long-half-life proteins that are slow to accumulate deuterium. We conclude that our original intended target enrichment of 1.5%–2% approximates a cost-effective trade-off between minimal intake and experimental performance for current technological platforms.

4 Concluding remarks

In summary, we have described here a safe and accessible $^2\text{H}_2\text{O}$ administration protocol for large-scale measurements of plasma protein turnover in human. Since its discovery by Harold Urey in 1931, $^2\text{H}_2\text{O}$ has been used as a stable isotope in biological intake studies (Reviewed in [14]) and has demonstrated a long documented safety record, with $^2\text{H}_2\text{O}$ enrichment of <15% total body water generally held to induce no detectable side effects in animals [14, 15]. In human, consumption of up to 0.5–2% total body water for several months is considered safe [16–18]. The highest reported $^2\text{H}_2\text{O}$ enrichment in human is 23% total body water [19], while the longest reported duration of continuous intake is 130 days [20]. In addition to its safety, $^2\text{H}_2\text{O}$ has several other advantages that make it highly attractive for protein dynamics studies in human, which include its bulk availability at relatively low cost, and the ease of administration into the subject without requiring dietary modifications which may be unpalatable or may lead to unintended physiological consequences [10, 13, 21–23]. $^2\text{H}_2\text{O}$ have been extensively employed to determine physiological water turnover rate and fluid intake [24, 25]. Recent $^2\text{H}_2\text{O}$ experiments further demonstrated the potential of protein dynamics as a source of diagnostic biomarkers in human diseases including psoriasis and neurodegeneration [22, 23]. As these previous studies have largely focused on the turnover of a few targeted proteins or did not discriminate the dynamics of individual protein species, the method presented here should aid in the utility and adoption of protein dynamics studies.

Despite broad applicability in pre-clinical studies, however, currently no FDA-approved protocol exists for the use of $^2\text{H}_2\text{O}$ in clinical research and diagnostic applications. In our opinion, this can in part be attributed to a lack of standardized protocols for $^2\text{H}_2\text{O}$ administration, or detailed documentation of physical parameters relevant to clinical interests and safety precautions. For instance, if $^2\text{H}_2\text{O}$ is to be approved for measuring cardiac protein turnover rate in human heart failure patients, it is imperative to have a detailed understanding of the hemodynamic responses in human subjects, especially those with compromised health conditions. Thus the prospects of clinical research and diagnostics are contingent upon preclinical efforts that meticulously document the potential outcomes of labeling protocols in human subjects. This protocol presented here shows promising translational potential for clinical investigations of protein dynamics in human diseases, e.g., case-control studies can compare the plasma protein turnover of age-matched healthy subjects and early- or late-stage heart failure patients (NYHA Class I–IV) to identify novel biomarkers and to sub-classify patients. Such biomarkers may also be used to detect, analyze, or predict patient response to medical or surgical intervention (e.g., after angiotensin-converting-enzyme inhibitor or left-ventricular-assist-device-mediated mechanical unloading [26]). Furthermore, since the presented method is capable of quantifying protein turnover from as few as one time point, future studies can be envisioned that directly compare the proteome dynamics of healthy and diseased human hearts, such as can be acquired from routine clinical biopsies during surgical intervention or post-transplant allograft rejection surveillance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Clinical relevance

The temporal dynamics of plasma proteins can provide a new avenue for biomarker discoveries and novel insights into the molecular mechanisms of human diseases. In this study, we designed a deuterium oxide ($^2\text{H}_2\text{O}$) intake and sample procurement strategy to examine the plasma protein dynamics in 10 healthy human subjects. In conjunction with high-resolution MS and bioinformatics analyses, the experiments revealed the turnover rates of 542 human plasma proteins from up to 10 individual subjects, including that of many known cardiovascular disease markers. Furthermore, the results provide a comprehensive documentation of the safety and efficacy of protein dynamics investigations via in vivo $^2\text{H}_2\text{O}$ intake. In human protein turnover studies, the rate of protein ^2H appearance needs to be corrected with the time-dependent body water $^2\text{H}_2\text{O}$ enrichment of the subjects in order to accurately account for incorporation due to protein turnover. Daily collection of saliva proves to be a convenient and accurate source of body fluid for measuring $^2\text{H}_2\text{O}$ enrichment kinetics, thus presenting a non-invasive alternative over repeated blood draws. This workflow shows promising clinical translational value for understanding plasma proteome dynamics in a range of human diseases.

Figure 1. A safe and effective $^2\text{H}_2\text{O}$ administration protocol for human subjects

(A) The schema and schedule of $^2\text{H}_2\text{O}$ administration are shown. Ten healthy human subjects were administered with regular doses of 70% $^2\text{H}_2\text{O}$ per os for 14 days. During the first 7 days, each subject consumed 51 mL/kg over 4 daily doses. During the last 7 days, the subjects consumed 0.56 mL/kg over 2 doses. Whole venous blood (3 mL) and saliva (0.5 mL) were collected at 10 time points during the consumption period, and regularly after consumption concludes for up to 8 month (sampling time points marked by orange arrowheads). (B–D) These figures show the vital signs of the 10 subjects throughout the intake period. We monitored the vital signs of all subjects during consumption of $^2\text{H}_2\text{O}$ and up to 14 days after completion. The recorded body temperature (B), heart rate (C), and systolic and diastolic blood pressure (D) from 10 healthy subjects were within normal ranges and did not alter significantly over time for each individual as well as the group average, suggesting the specified dosage and duration had no discernible effect on vital signs and hemodynamics of healthy subjects. Circles: individual reading from a subject. Boxes: interquartile of all subjects at a particular time point; whiskers: 5th–95th percentile of all subjects at a particular time point.

Figure 2. Enrichment kinetics of $^2\text{H}_2\text{O}$ in human blood plasma and saliva following administration

(A) $^2\text{H}_2\text{O}$ enrichment in body water was measured by GC-MS from both plasma and saliva samples in 10 subjects. A gradual increase during the first 7 days of the protocol was observed, followed by the plateau in the next 7 days (red circles: % $^2\text{H}_2\text{O}$ in plasma; open circles: % $^2\text{H}_2\text{O}$ in saliva; line: best-fit exponential rise curve). Cessation of intake is followed by normal physiological clearance of $^2\text{H}_2\text{O}$, with a characteristic half-life of ~7 days. (B) Scatterplot and linear regression of $^2\text{H}_2\text{O}$ level in saliva vs. plasma, as measured with GC-MS in the same subjects during the course of $^2\text{H}_2\text{O}$ intake. $^2\text{H}_2\text{O}$ measurements from blood plasma and saliva were highly correlated ($R^2: 0.985$). The results suggest that

saliva provides a reliable sample source for monitoring $^2\text{H}_2\text{O}$ enrichment. (C) The $^2\text{H}_2\text{O}$ enrichment kinetics measured from saliva showed consistent trends and fitted to a first-order exponential rise equation to return almost identical $^2\text{H}_2\text{O}$ intake and clearance kinetics as in plasma. Data points represent saliva $\% ^2\text{H}_2\text{O}$ level reading from individual subjects according to the subject legends. Crosses and bars represent subject average and standard deviation of all subjects at each time point. The averages and standard deviations at day 5 are due to an outlier.

Figure 3. Characterization of human plasma proteome dynamics using $^2\text{H}_2\text{O}$

(A) The histogram shows the distribution of the number of replicates in which the in vivo turnover rates of proteins were quantified, e.g., 103 proteins were quantified in all 10 subjects, and 46 proteins in any 9 subjects, etc. We quantified the in vivo turnover rates of 542 human plasma proteins; 325 proteins were quantified in 3 or more subjects. (B) The scatterplots show the abundance (top panel) of plasma proteins and their corresponding turnover rate (bottom panel), ranked by descending protein abundance. The relative abundance of proteins was quantified from MS data using spectral counting. In the top panel, the blue circles denote immunodepletion-adjusted concentration, from spectral counting relative quantification data. For reference of protein abundance, the red lines denote the absolute abundance range based on clinical diagnostics data curated in Anderson and Anderson [7], whereas green circles denote estimated abundance based on spectral counts from Farrah et al. [6]. In the bottom panel, red circles denote measured protein turnover in an individual subject. There is a marked independence between abundance and

half-life over 3 orders of magnitude of concentration, indicating the two parameters are effectively orthogonal. **(C)** The scatterplot shows the turnover rates of 325 quantified in 3 subjects. The horizontal axis represents protein rank by median turnover in all subjects. Each data point represents turnover rate quantified from one individual subject. Three proteins of different turnover rates: β -globin (HBB, half-life: >50 days), albumin (ALB, half-life: 22 days), and insulin-like growth factor 2 (IGF2, half-life: <1 day), are marked. The data suggest plasma proteome turnover is diverse and spans >50-fold. **(D)** The scatterplots show the distribution of protein turnover rates within particular GO categories. Horizontal position denotes protein species, ranked by descending turnover rate within a category. Each data point represents a protein turnover rate measurement in one individual. The quantified proteins belong to multiple GO biological processes, and diverse protein dynamics can be observed in each process.

Table 1

Demographics of human subjects.

Subject #	Age (years)	Gender	Weight (kg)	Height (cm)	BMI	Ethnicity
1	51	F	68	175	22.7	Asian
2	39	M	82	178	25.9	Asian
3	25	M	88	175	28.6	Asian-Indian
4	35	M	108	188	30.5	Asian
5	21	M	87	180	26.7	Caucasian
6	21	F	64	168	22.7	Caucasian
7	23	M	84	180	25.8	Caucasian
8	21	F	59	168	20.9	Caucasian
9	21	F	51	160	19.9	Caucasian
10	27	M	82	175	26.6	Caucasian

BMI: body mass index.

Table 2

Turnover rates of example plasma proteins measured from 10 human subjects.

Protein name	Uniprot accession	Gene name	Turnover rate in this study k (d ⁻¹)			Turnover rate in literature	
			Range	Replicates	Average	Range	Reference
Albumin	P02768	ALB	0.02–0.07	6	0.04±0.007	0.03–0.06	[27]
Ceruloplasmin	P00450	CP	0.05–0.14	10	0.09±0.007	0.14–0.20	[28]
Fibrinogen α chain	P02671	FGA	0.10–0.21	10	0.15±0.01	0.14–0.19	[27]
Fibrinogen β chain	P02675	FGB	0.06–0.12	5	0.09±0.01	0.14–0.19	[27]
Fibrinogen γ chain	P02679	FGG	0.07–0.19	6	0.12±0.02	0.14–0.19	[27]
Fibronectin	P02751	FN1	0.12–0.23	10	0.15±0.02	0.30–0.40	[29]
Haptoglobin	P00738	HP	0.23–0.44	10	0.31±0.02	0.17–0.25	[30]
Prothrombin	P00734	F2	0.18–0.28	10	0.20±0.009	0.21–0.30	[31]
Transferrin	P02787	TF	0.04–0.14	8	0.09±0.01	0.07–0.20	[32]
Transferritin	P02766	TTR	0.16–0.31	9	0.21±0.02	0.22–0.31	[33]