Am J Physiol Renal Physiol 319: F115–F124, 2020.
First published June 1, 2020; doi:10.1152/ajprenal.00377.2019.

INNOVATIVE METHODOLOGY

A noninvasive method to study the evolution of extracellular fluid volume in mice using time-domain nuclear magnetic resonance

Luciana Morla,[1,2] Oliver Shore,[3,4] I. Jeanette Lynch,[3,4] Matthew E. Merritt,[5] and Charles S. Wingo[3,4]

[1]*Centre de Recherche des Cordeliers, Institut National de la Santé et de la Recherche Médicale, Sorbonne Université, USPC, Université Paris Descartes, Université Paris Diderot, Paris, France;* [2]*Centre National de la Recherche Scientifique, ERL 8228, Laboratoire de Physiologie Rénale et Tubulopathies, Paris, France;* [3]*North Florida/South Georgia Veterans Health System, Gainesville, Florida;* [4]*Department of Medicine, University of Florida, Gainesville, Florida; and* [5]*Department of Biochemistry and Molecular Biology, University of Florida, Gainesville, Florida*

Submitted 7 August 2019; accepted in final form 21 May 2020

Morla L, Shore O, Lynch IJ, Merritt ME, Wingo CS. A noninvasive method to study the evolution of extracellular fluid volume in mice using time-domain nuclear magnetic resonance. *Am J Physiol Renal Physiol* 319: F115–F124, 2020. First published June 1, 2020; doi:10.1152/ajprenal.00377.2019.—Maintaining water homeostasis is fundamental for cellular function. Many diseases and drugs affect water balance and plasma osmolality. Water homeostasis studies in small animals require the use of invasive or terminal methods that make intracellular fluid volume and extracellular fluid volume (ECF) monitoring over time stressful and time consuming. We examined the feasibility of monitoring mouse ECF by a noninvasive method using time-domain nuclear magnetic resonance (TD-NMR). This technique allows differentiation of protons in a liquid environment (free fluid) from protons in soft tissues containing a majority of either small molecules (lean) or large molecules (fat). Moreover, this apparatus enables rapid, noninvasive, and repeated measurements on the same animal. We assessed the feasibility of coupling TD-NMR analysis to a longitudinal metabolic cage study by monitoring mice daily. We determined the effect of 24-h water deprivation on mouse body parameters and detected a sequential and overlapping decrease in free fluid and lean mass during water deprivation. Finally, we studied the effect of mineralocorticoids that are known to induce a transient increase in ECF but for which no direct measurements have been performed in mice. We showed, for the first time, that mineralocorticoids induced a transient ~15% increase in free fluid in conscious mice. TD-NMR is, therefore, the first method to allow direct measurement of discrete changes in ECF in conscious small animals. This method allows analysis of kinetic changes to stimuli before investigating with terminal methods and will allow further understanding of fluid disorders.

fluid; dehydration; hydration; mice; nuclear magnetic resonance

INTRODUCTION

Water is the most abundant component of the body and represents 45−60% of total body weight in an adult, depending on race, age, and sex (11). Whereas intracellular fluid (ICF) accounts for ~40% of body weight, extracellular fluid (ECF) accounts for ~20% of body weight. ECF is divided into the interstitial fluid and intravascular fluid, or blood volume, accounting for around 16% and 4% of body weight, respectively. Many diseases and drugs can disturb water homeostasis, and, as a result, fluid disorders are one of the most frequent issues in clinical medicine (52).

Dehydration is an abnormal decrease in total body water (TBW) of more than 2% of body weight (1.4 L for 70 kg) (9). Severe dehydration is defined as a loss of water exceeding 5% of body weight and is life threatening due to hyperthermia and hypoperfusion of organs (1). Dehydration occurs when water intake and water formation from metabolism does not compensate for obligatory water loss, e.g., urine, sweat, and stool production. Excessive sweating, diarrhea, diabetes insipidus, diuretic drugs, diabetes mellitus, or age-associated hypodipsia are major causes of dehydration (26, 39, 45, 52). Moreover, dehydration is a common condition (15, 48) and poses a high risk of morbidity and mortality in children, the elderly, and inpatients (24, 51). Hypertonic dehydration occurs when hypotonic fluid is lost, causing an increase in plasma osmolality, e.g., water restriction or excessive sweating. Thus, a 100-mL loss of TBW causes approximately a 10-mL loss of plasma volume, a 30-mL loss of ECF, and a 60-mL loss of ICF (14). Isotonic dehydration occurs when both water and sodium salts are lost at the same rate. Under these conditions, ECF volume depletion accounts for TBW loss, and the plasma volume decrease can reach 20% of TBW loss (9). An increase greater than 2% in plasma osmolality or a decrease greater than 10% in plasma volume trigger compensatory mechanisms through the release of vasopressin from the posterior pituitary, which induces water retention by the kidney, thirst, and vasoconstriction to maintain blood osmolality and blood pressure (9).

In contrast, chronic kidney disease (CKD), mineralocorticoid excess, heart failure, and cirrhosis commonly induce ECF expansion. Typically, CKD impairs salt (NaCl) and water excretion, causing a transient increase in plasma volume after salt intake (19, 29). Acute exogenous mineralocorticoid administration induces renal salt retention and causes a transient increase in ECF and plasma volume in humans (10) and small animals (22, 53). In high mineralocorticoid states and frequently in CKD, renal excretion of excess salt and fluid is impaired, causing a sustained increase in systemic arterial blood pressure. Heart failure, cirrhosis, and nephrotic syndrome induce renal water and salt retention. Derangement of Starling forces will promote fluid accumulation in the interstitial compartment, causing edema and ascites (34, 35, 46, 50). Many of these pathological conditions produce changes in one

Correspondence: C. S. Wingo (cswingo@ufl.edu).

or more fluid compartments. For example, in the mineralocorticoid/salt model of hypertension, there is a transient retention of fluid in the intravascular space, which leads to an increase in cardiac output, and this results in an increase in systemic blood pressure, an attendant increase in systemic vascular resistance, and a pressure natriuresis that restores blood volume to normal but at the expense of an increased systemic blood pressure. The ability to measure changes in ICF and ECF accurately and noninvasively is necessary to understand how pathophysiological states result in fluid retention.

Until recently, measurement of the effects of hormones or drugs on body fluid distribution in small animals has been laborious and frequently requires a terminal procedure, which does not allow serial measurements in the same animal. Moreover, measurement of fluid balance in small animals is difficult because important changes in fluid compartments are close to the limit of resolution of most methods. TBW, ECF, and plasma volume are estimated in animals by bioimpedance (8, 16), the dilution principle (47, 54), or by inference from measurements of hematocrit, osmolality, Na^+ concentration, and renin activity (3). Studies with these techniques have ascertained that, like humans, mouse ECF volume represents ~20% of body weight. Unlike in humans, mouse blood represents ~7.2% of body weight and plasma ~65% of blood (13, 43).

Time-domain nuclear magnetic resonance (TD-NMR) is a relaxometry-based method that uses an array of inversion recovery (encoding T_1 effects) and echo times (encoding T_2 effects) to precisely measure the relative size of the three in vivo reservoirs. ^{1}H-NMR relaxation times [longitudinal (T_1) and transverse (T_2)] vary as a function of the immediate environment, and the magnitude of the NMR signal is proportional to the mass of protons (40). The free induction decay of the magnetic resonance signal, which contains signals from water in different environments, as well as fat, is directly analyzed. Depending on the water environment (fat, meat, and saline), signals will be sufficiently different to be separated in three compartments. The Bruker devices used in this study were calibrated with a batch of animal fat, chicken breast, and physiological serum. The T_1 and T_2 values obtained are used in the calibration curves as corresponding to the mixture of amounts of fat, lean, and saline called "free fluid." Thereafter, when an animal is placed in the machine, the signals obtained are compared with the calibration curve to calculate the amounts of fat, lean, and free fluid. Colucci et al. (12) have mathematically transformed the magnetization decay data to extract the T_2 signal from mice. They showed that, at the operating field of 0.15 T, T_2 of fat-associated water, lean-associated water, and free water is 50, 250, and 1,500–2,000 ms, respectively (7, 12). Moreover, they showed that a saline intraperitoneal injection increases the height of only the slowest decaying signal (1,500–2,000 ms). The method is nondestructive, and, therefore, TD-NMR is a noninvasive and rapid way to measure body composition in awake mice (6, 21, 30, 36, 42). Researchers have used TD-NMR to monitor body composition in rodents with metabolic disorders. Most studies have analyzed lean and fat mass, and only a few studies have assessed free fluid mass (41, 42). Gordon et al. (17) and Colucci et al. (12) showed it was possible to measure differences in free fluid before and after an intraperitoneal saline injection in rats (17) and mice (12), respectively. However, direct measurement of pathophysiological changes in all three compartments in conscious animals by noninvasive methods will allow a more precise understanding of the changes during different physiological or pathophysiological conditions.

Prior to TD-NMR, determining body fluid composition in mice requires invasive and terminal methods, which makes longitudinal studies time consuming and expensive. Here, we show that TD-NMR can be a quantitative tool to estimate water loss or gain by the extracellular compartment after a drug treatment or a change in a diet. We first assessed TD-NMR measurement of body fluid volume variation in whole mice and in phantoms of diverse composition. Second, we compared the free fluid of C57BL/6J mice of different weights and in both sexes and calculated the variability of this parameter in individual male mice over time. We used TD-NMR to assess free fluid under two common mouse husbandry conditions: ventilated rack and metabolic caging, and whether daily TD-NMR measurement in tandem with metabolic caging would induce stress-related body weight loss. Finally, we monitored lean, fat, and free fluid during two longitudinal studies: *1*) the effect of 24-h water deprivation and *2*) the effect of treatment with a long-acting mineralocorticoid [deoxycorticosterone pivalate (DOCP)] and a high-salt diet (HS/DOCP group).

MATERIAL AND METHODS

Ethical approval. All animal use complied with the American Physiological Society's "Guiding Principles in the Care and Use of Laboratory Animals." The Institutional Animal Care and Use Committees of the North Florida/South Georgia Veterans Administration and the University of Florida approved the animal use protocol (Animal Component of Research Project no. 0012). All animal use in France complied with the institutional guidelines and recommendations for the care and use of laboratory animals put forward by Directive 2010/63/EU revising Directive 86/609/EEC on the protection of animals used for scientific purposes. (The project was approved by a user establishment Ethics Committee and received Project Authorization no. 97 2289.01.) Adult male and female mice were studied to test the feasibility of monitoring body compartments in a strain commonly used in biomedical research: the C57BL/6J strain.

Time-domain nuclear magnetic resonance. TD-NMR is a method based on the acquisition of radiofrequency signals generated by hydrogen spins from fluid and soft tissues, such as muscle and adipose. TD-NMR LF90 (Minispec, LF90, Bruker, Billerica, MA) and the TD-NMR LF50 (Minispec, LF50, Bruker) function using the contrast in T_1 and T_2 relaxation times of the hydrogen spins associated with fat, lean tissue, and free water signal to estimate whole body composition. The Minispec LF90 was calibrated for mice and rats weighing up to 800 g with phantom samples made of mixes of oil for "fat," chicken breast for "lean," and saline (0.9 g NaCl/L water) for "free fluid," as explained elsewhere (17). The calibration of the Minispec LF50 was specifically adapted to mice ranging from 5 to 60 g. The magnetic field strength of the Minispec LF90 is 0.15 T, corresponding to a minimum proton frequency of 6.2 MHz, while the magnetic field strength of the Minispec LF50 is 0.17 T, corresponding to a minimum proton frequency of 7.5 MHz.

All mice were habituated to TD-NMR analysis before experimental measurements. Mice were weighed on a precision scale after emptying their bladder by abdominal massage. Mice were placed in a clear plastic cylinder (50-mm diameter) and kept in the smallest possible space allowing free movements by insertion of a tight-fitting plunger into the cylinder. The tube was then inserted into the Minispec chamber for 2 min, the duration of the scan process. If the mice urinated in the cylinder, the cylinder was cleaned and dried before the

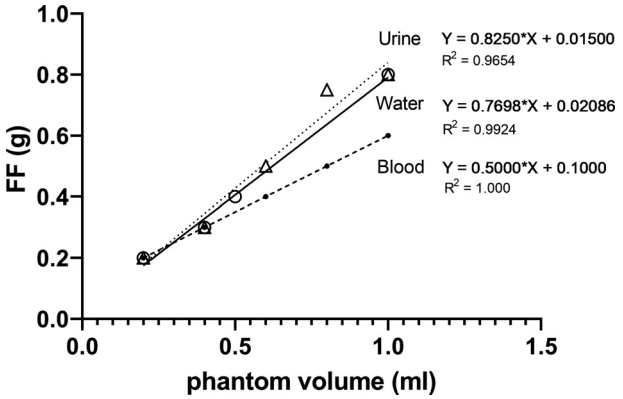

Fig. 1. Linear regression of free fluid (FF) with corresponding phantom water (○ and solid line), urine (△, dotted line), and blood (●, dashed line) volumes (in mL). Plastic tubes containing increasing amounts of 18.2-MΩ water, fresh urine, and heparinized blood were placed in the Minispec LF90 for time-domain nuclear magnetic resonance analysis.

next measurement. Mice that urinated in the tube were allowed to dry for 20−30 min and were reweighed and scanned a second time.

Regression curves from phantoms. Different volumes of deionized water, ranging from 0.2 to 1 mL, were pipetted in plastic Eppendorf tubes and placed in the Minispec LF-90 for analysis. The amount of free fluid detected was used to generate a regression curve. Next, various volumes of urine and heparinized blood, both freshly collected from anesthetized mice, were analyzed as water phantom samples to generate regression curves for free fluid.

Saline injection. TD-NMR measurements were made before and immediately after intraperitoneal injection of normal saline (37°C). To determine the effective amount of saline injected, mouse body weight was measured before and after intraperitoneal injection.

Water restriction. Mice were weighed and analyzed by TD-NMR immediately before and after 5-h and 24-h water deprivation.

DOCP treatments. Mice were habituated to TD-NMR analysis, fed a control gelled diet [45% TD99191 (0.2% Na), 54% water, and 1% agar, Teklad, Madison, WI], and had free access to tap water for 4 days. The next day, mice were treated with a long-lasting mineralocorticoid analog (DOCP, Novartis, 75 μg/g im) and fed ad libitum a high-salt (total of 2% Na) diet prepared as a gelled diet as follows: 45% TD99191 (0.2% Na^+), 54% water, and 1% agar plus an additional 1.8% Na^+ added as NaCl, and the mice were given 0.9% NaCl to drink. We have previously shown that a high-salt diet that included 0.9% NaCl to drink with DOCP treatment induced a transient increase in Na^+ and fluid balance in mice (33). Mice were analyzed daily using the Minispec LF90 for the next 3 days.

DOCP treatment for comparison between Minispecs LF50 and LF90. Mice were habituated to TD-NMR analysis, fed a standard laboratory chow (A04, Safe, Epinay, France, and no. 2918, Teklad, Tampa, FL) and had free access to tap water for 4 days. Mice were analyzed before and 1 day after DOCP treatment (Novartis, 75 μg/g im).

DOCP treatment for comparison between % free fluid, body weight, and hematocrit variations. Additional mice fed a standard laboratory chow (A04, Safe, Epinay, France) with free access to tap water were habituated to TD-NMR Minispec LF50 analysis for 4 days. Mice were randomly separated into two groups: control and DOCP treatment (Novartis, 75 μg/g im). The next day, mice were analyzed by TD-NMR, and a blood sample was collected from the retroorbital sinus in the next hour. Hematocrit was measured on a hematocrit centrifuge (Sigma 201m, Meditech Scientifique).

Statistics. Data are shown as means ± SD. Calculations were made using GraphPad Prism (version 8.1.2 for Windows, GraphPad Software, La Jolla, CA). Unless specified, a paired, two-tailed Student's *t* test was used to determine significance of differences between groups.

RESULTS

TD-NMR regression curve for water, urine, and blood. In an initial study, the Minispec LF90 showed that five C57BL/6J mice weighing 28.53 ± 1.78 g had an average of 2.73 ± 0.30 g of free fluid corresponding to ~10% of their body weight. To clarify what TD-NMR detected as free fluid in a whole animal, we compared free fluid in phantoms of deionized water, urine, and heparinized blood samples ranging from 0.2 to 1 mL. Using the Minispec LF90, we found there was a strong linear correlation between the different fluid volumes and the amount of free fluid measured (Fig. 1). Linear regression of the TD-NMR-generated mass of free fluid versus the phantom volumes of water, urine, and blood was performed. The slopes and intercepts for water and urine were similar (P = not significant), whereas the slope for heparinized blood was significantly different from the slopes of both water and urine ($P < 0.001$). The Minispec LF50 showed five C57BL/6J mice weighing 28.93 ± 0.84 g had an average of 1.57 ± 0.06 g of free fluid corresponding to ~5.4% of their body weight. All of the following TD-NMR measurements were performed with the Minispec LF90 apparatus unless stated otherwise.

Linear regression between small saline intraperitoneal injection and free fluid in mice. We investigated the capacity of TD-NMR to detect a small change in free fluid in vivo. We performed intraperitoneal injections of different amounts of saline ranging from 0.15 to 0.40 mL in mice (Fig. 2). There was a good correlation between the body weight change and TD-NMR-generated mass of free fluid change measured before and after the saline injection ($r^2 = 0.53$). We found lean values did not change significantly before and after injection of saline (P = not significant; data not shown).

Free fluid in C57BL/6J male and female mice. We measured free fluid in 5 female mice at 8 wk of age (body weight: 20.06 ± 0.90 g, free fluid: 2.02 ± 0.13 g), 5 male mice at 6 wk of age (body weight: 20.44 ± 0.68 g, free fluid: 2.13 ± 0.20 g), and 13 male mice at 11 wk of age (body weight: 28.84 ± 1.41 g, free fluid: 2.80 ± 0.23 g; Fig. 3). When taking into account all mice, there was a good correlation between body weight and free fluid ($r^2 = 0.90$). C57BL/6J mice gain weight rapidly,

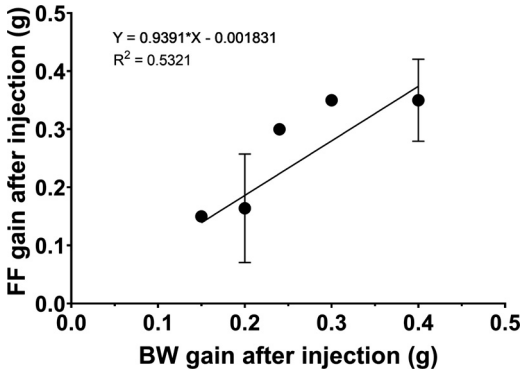

Fig. 2. Linear regression for body weight (BW) gain and free fluid (FF) change after an intraperitoneal injection of saline in male ($n = 6$) and female ($n = 5$) mice. BW was measured before and after injection, and 0.1 g of BW gain was assumed to correspond to 0.1 mL of injected saline. Some animals showed the same change in BW after injection and were pooled. SDs are shown for multiple animals that were injected with the same injection volume (0.2 mL, $n = 5$, and 0.4 mL, $n = 2$). Mice were analyzed by the Minispec LF90 for time-domain nuclear magnetic resonance analysis.

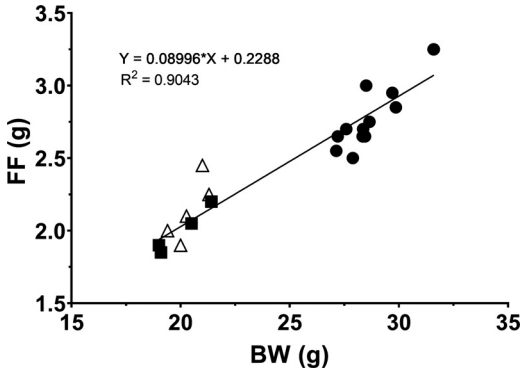

Fig. 3. Body weight (BW) and free fluid mass (FF) in a heterogeneous population of C57BL/6J mice. △, female mice at 8 wk of age ($n = 5$), ■, male mice at 6 wk of age ($n = 5$); ●, male mice at 11 wk of age ($n = 13$).

and male C57BL/6J mice at 6 wk of age were significantly smaller than male mice at 11 wk of age. The larger male mice had a significantly smaller amount of free fluid percentage than smaller male mice (free fluid percentage: 9.71 ± 0.43% in larger male mice and 10.42 ± 0.72% in smaller male mice, $P < 0.03$). These observations are in accordance with others showing that smaller and/or younger animals have larger plasma volume-to-body weight ratios (5). To avoid any bias due to the difference in body mass, we compared weight-matched female and male mice (male mice at 6 wk of age vs. female mice at 8 wk of age). Weight-matched female and male mice had similar amounts of free fluid when adjusted for body weight (free fluid percentage: 10.07 ± 0.24% in female mice and10.42 ± 0.72% in male mice). For simplification, all subsequently described experiments were performed in male mice.

Evolution of body weight and free fluid percentage in mice. The individual variability in free fluid percentage in mice is unknown, as well as the effect of housing or type of feeding on free fluid percentage. We measured the evolution in body weight and free fluid percentage at a 3-day interval in nine male mice (body weight: 28.52 ± 1.34 g, free fluid percentage: 9.52 ± 0.34%) equilibrated to regular housing and feeding. We observed that mouse body weight did not vary significantly over time (Fig. 4*A*), which is normal for male C57BL/6J mice at 13 wk of age (https://www.jax.org), nor did the free fluid percentage change (Fig. 4*B*). Next, we monitored body weight and free fluid percentage in the same mice during the first 3 days of housing in metabolic cages with access to a gel diet and tap water ad libitum. Body weight did not vary significantly during this period (Fig. 4*C*), but free fluid percentage increased significantly from 9.37 ± 0.50% to 10.17 ± 0.59% ($P < 0.0005$, Fig. 4*D*). During the 3 days in normal housing, the intramouse variation was 1.15% for body weight and 3.59% for free fluid. The intermouse variation averaged 4.33% for body weight and 4.45% for free fluid.

Effect of water deprivation on free fluid, lean, and fat in mice. Water deprivation induces hypertonicity of ECF and, as a consequence, a contraction of ICF. Thus, water deprivation causes ICF and ECF water loss (52). We used the Minispec LF90 to follow the effect of water deprivation on the three compartments: *1*) free fluid, which reflects ECF; *2*) lean, which represents protein-rich soft tissue; and *3*) fat, which approximates fat mass. Five male C57BL/6 mice at 13 wk of age (body weight: 29.82 ± 1.76 g, free fluid mass: 2.96 ± 0.30 g, lean

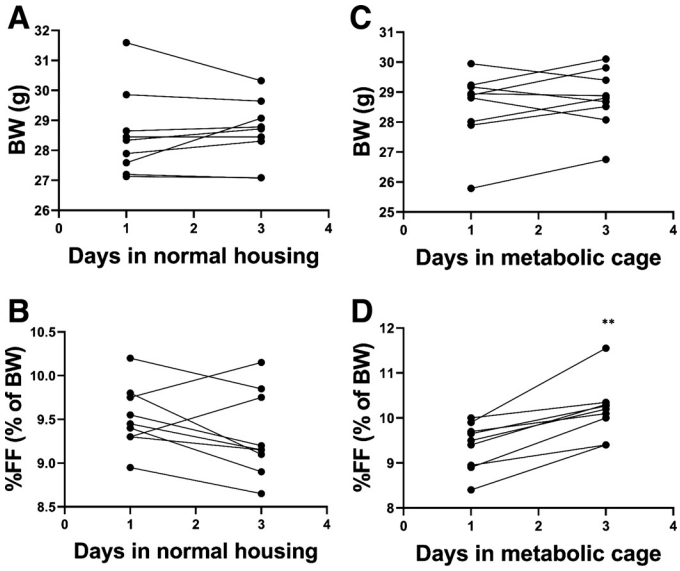

Fig. 4. *A* and *B*: evolution of mouse individual body weight (BW; *A*) and free fluid as a percentage of BW [free fluid percentage (FF%); *B*] during 3 days of normal housing. *C* and *D*: evolution of mouse individual BW (*C*) and %FF (*D*) during 3 days of metabolic cages. $n = 9$. Student's paired t test was used for statistical analysis. $^{**}P < 0.0005$.

mass: 22.30 ± 1.02 g, and fat mass: 1.23 ± 0.53 g) were water deprived for 24 h (Fig. 5, $t = 0$). Our results showed that 5 h of water deprivation did not induce a significant change in mouse body weight, free fluid, lean mass, or fat mass. Nevertheless, when normalized to body weight, there was a tendency for free fluid percentage to decrease during this period ($t = 0$ free fluid percentage: 9.87 ± 0.52% of body weight; 5-h free fluid percentage: 9.50 ± 0.27% of body weight, $P < 0.08$). After 24 h of water deprivation, mice showed a significant decrease in body weight of 2.06 ± 1.03 g ($P < 0.05$), which

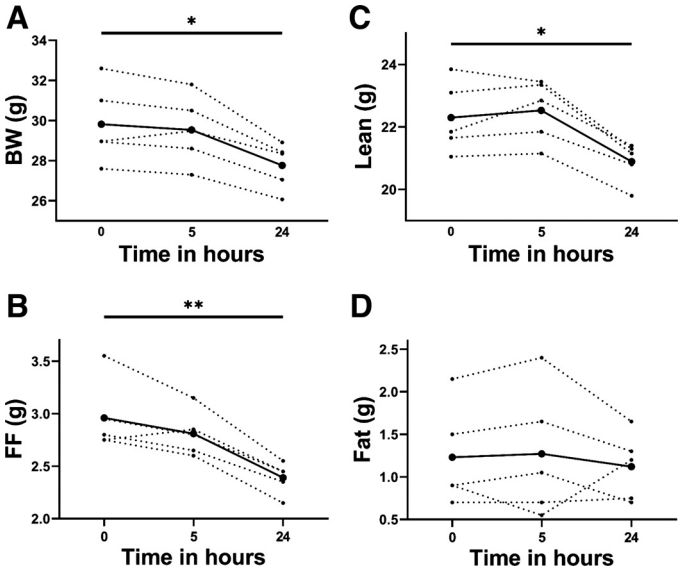

Fig. 5. Effect of water deprivation on body weight (BW; *A*), free fluid mass (FF; *B*), lean mass (*C*), and fat mass (*D*) of mice deprived of water for 24 h. Mice were monitored before [time (t) = 0] and after 5 h ($t = 5$) and 24 h ($t = 24$) of water deprivation. Data are shown as individual values (dotted line) and averages (solid line). $n = 5$. Data were analyzed using a Student's paired t test. $^{*}P < 0.05$ and $^{**}P < 0.01$ between 24 h of water deprivation and control.

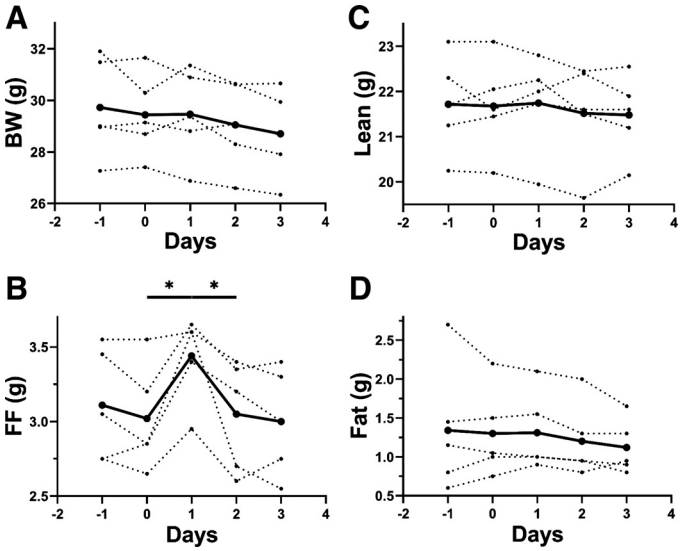

Fig. 6. Effect of long-acting mineralocorticoid treatment and high salt loading on body weight (BW; *A*), free fluid mass (FF; *B*), lean mass (*C*), and fat mass (*D*). Mice were monitored during 2 days on a control gelled diet (control *day -1* and *day 0*). On *day 0*, mice received deoxycorticosterone pivalate (DOCP) and a high-salt diet [2% Na^+ diet and saline (0.9% NaCl) to drink] and were monitored for the next 3 days. Data are shown as individual results (dotted lines) and mean values (full line). $n = 5$. Data were analyzed using a Student's paired *t* test. *$P < 0.03$ compared with *day 1*.

corresponds to ~7% of body weight (Fig. 5*A*). This is in accordance with previous observations (44). Loss in body weight could be attributed to a significant decrease in free fluid of 0.57 ± 0.24 g ($P < 0.01$) or 19% of free fluid (Fig. 5*B*) and a decrease in lean mass of 1.41 ± 0.76 g ($P < 0.05$) or 6% of lean mass (Fig. 5*C*). No significant decrease in fat mass was observed during the experiment (Fig. 5*D*). Thus, dehydration induced a proportional decrease in lean mass compared with body weight (7% body weight loss and 6% lean mass loss) but a relatively greater proportional decrease in free fluid mass (19% of free fluid loss).

Effect of a mineralocorticoid excess and high salt intake on free fluid, lean mass, and fat mass in mice. Mineralocorticoid excess associated with a high salt intake induced a transient increase in Na^+ and water retention and, thereby, a transient increase in ECF (18, 20, 28, 39, 49, 53). We have previously shown that a high-salt diet associated with a DOCP treatment (HS/DOCP) induced a transient increase in Na^+ and fluid balance in mice (33). Here, we determined whether TD-NMR could detect an acute effect of HS/DOCP on mouse ECF. Using the Minispec LF90, we monitored body weight, free fluid mass, fat mass, and lean mass in five male mice (body weight: 29.72 ± 1.73 g, free fluid mass: 3.11 ± 0.34 g, fat mass: 1.34 ± 0.74 g, and lean mass: 21.72 ± 0.96 g) before and after HS/DOCP treatment. One day after DOCP/HS, we observed a significant and striking increase in free fluid mass of 0.42 ± 0.23 g ($P < 0.02$) that corresponded to an increase of 14.48 ± 8.30% in free fluid. The increase in free fluid was very brief, as it returned to baseline on the next day (Fig. 6*B*). In contrast, we did not observe a significant change in body weight (Fig. 6*A*), lean mass (Fig. 6*C*), or fat mass (Fig. 6*D*).

Intramouse and intermouse variation in body weight and free fluid percentage using the Minispec LF50. We measured body weight and free fluid in five male mice (body weight: 31.48 ± 1.95 g, free fluid percentage: 5.58 ± 0.75%) with the Minispec LF50 during 2 consecutive days. We calculated an intramouse variation of 0.68% for body weight and 3.43% for free fluid. These values were equivalent to those observed with the Minispec LF90. The intermouse variation averaged 7.44% for body weight and 12.77% for free fluid. Here, the variation in mouse body weight was greater than in the mice tested with the Minispec LF90 and explains the greater variation observed in free fluid.

Capacity of the Minispec LF50 to detect an increase in ECF induced by mineralocorticoid excess. Next, we compared the effect of DOCP treatment on body weight and free fluid in two groups of weight-matched male mice fed a normal-salt diet ($n = 5$ mice/group, age: 14−39 wk old). The group tested on the Minispec LF90 (body weight: 29.24 ± 1.26 g, free fluid mass: 2.68 ± 0.16 g, fat mass: 0.57 ± 0.36 g, lean mass: 22.65 ± 1.30 g) and the group tested on the Minispec LF50 (body weight: 28.60 ± 1.70 g, free fluid mass: 1.61 ± 0.28 g, fat mass: 3.07 ± 0.60 g, lean mass: 20.34 ± 1.24 g) were analyzed before treatment with DOCP and again the next day. We found that both mouse groups showed no significant change in body weight but a dramatic increase in free fluid mass (Fig. 7, *A* and *B*). The Minispec LF90 group showed a

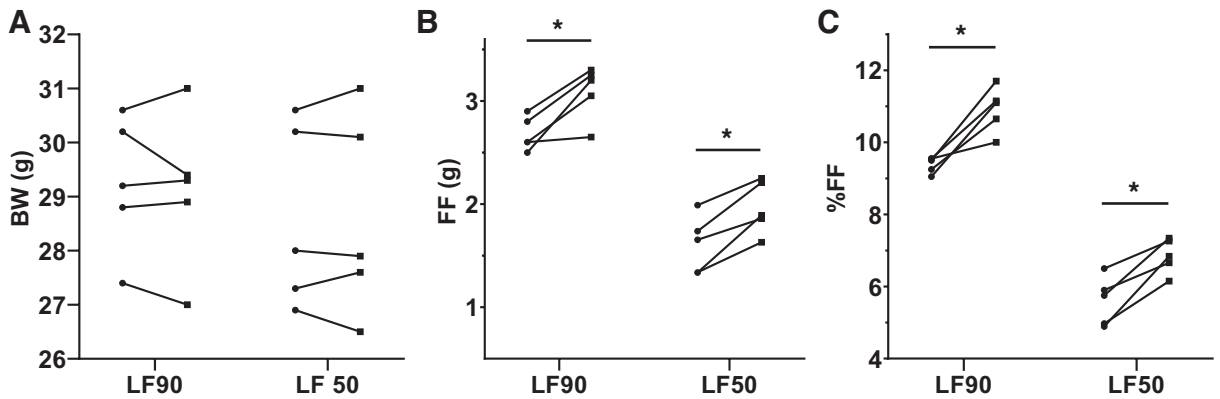

Fig. 7. Effect of long-acting mineralocorticoid treatment [deoxycorticosterone pivalate (DOCP); *day 1*] on body weight (BW; *A*), free fluid mass (FF; *B*), and FF as a percentage of BW (%FF; *C*). Two mouse groups matched for BW were analyzed by Minispec LF90 or LF50 before (circles) and 1 day after DOCP treatment (squares) for time-domain nuclear magnetic resonance analysis. Data are shown as individual results. $n = 5$. Data were analyzed using a Student's paired *t* test. *$P < 0.05$, control vs. *day 1* DOCP.

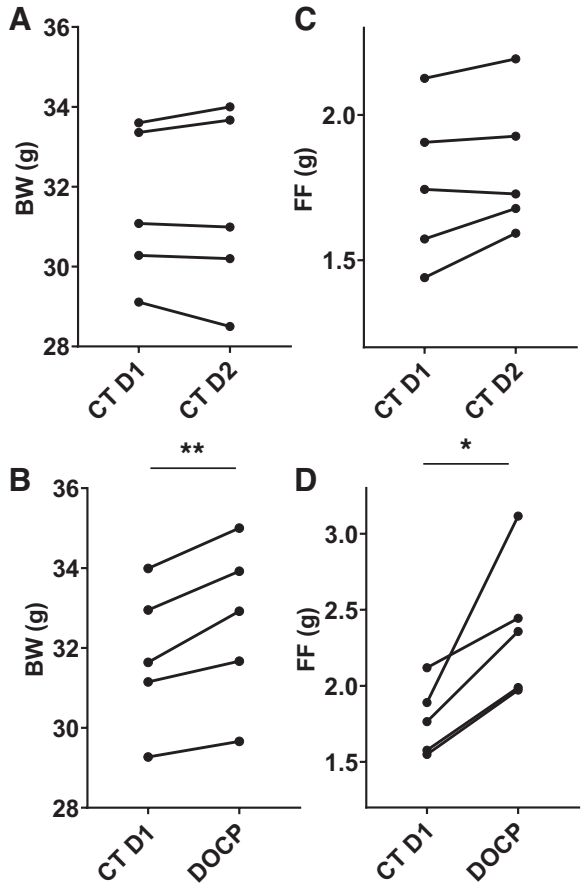

Fig. 8. Indexes of plasma expansion measured with the Minispec LD50 after long-acting mineralocorticoid treatment [deoxycorticosterone pivalate (DOCP)]. *A*–*D*: comparisons of the evolution of body weight (BW; *A*) in the control group ($n = 5$) on *day 1* and *day 2* (CT D1 and CT D2, respectively), BW in the DOCP-treated group ($n = 5$) before injection and 1 day after injection (CT D1 and DOCP, respectively), free fluid mass (FF; *C*) in the control group on *day 1* and *day 2* (CT D1 and CT D2, respectively), and FF (*D*) in the DOCP-treated group before injection and 1 day after (CTD1 and DOCP, respectively). Data are shown as individual results. $n = 5$. Data were analyzed using a Student's paired *t* test. $*P < 0.05$ vs. CT D1; $**P < 0.005$ vs. CT D1.

significant increase of 0.41 ± 0.23 g of free fluid mass ($P < 0.02$) corresponding to a 16.42% significant increase in free fluid percentage ($P < 0.008$; Fig. 7*C*). The Minispec LF50 group showed a significant increase of 0.36 ± 0.15 g, corresponding to a 22.2% increase of free fluid percentage ($P < 0.006$; Fig. 7*C*).

Comparison of different indexes of plasma expansion with TD-NMR free fluid values, body weight, and hematocrit. Hematocrit measurements are used as an index of fluid retention and plasma expansion with treatments that do not affect erythropoiesis (32, 55). Moreover, DOCP-induced body weight gain has been interpreted as fluid and Na^+ retention increase when these observations occurred during comparative studies in mice. Using the Minispec LF50, we compared free fluid percentage, body weight, and hematocrit in two groups of weight-matched male mice (age: 18–27 wk old) during basal or DOCP treatment. One day after DOCP treatment, we observed a significant increase in body weight in the DOCP-treated group of 0.83 ± 0.37 g ($P < 0.007$) when there was no significant change in body weight of the control group (Fig. 8, *A* and *B*). TD-NMR analysis showed that this increase was due primarily to a significant increase in free fluid of 0.60 ± 0.37 g ($P < 0.02$). This corresponded to an increase of 33.44 ± 16.8% in free fluid percentage when there was no significant change in free fluid in the control group (Fig. 8, *C* and *D*). There was no significant change in lean or fat mass in either group (see Supplemental Fig. S1, available online at https://doi.org/10.6084/m9.figshare.12283886.v1).

Hematocrit measurements could not be made 2 days in a row without risking a significant change in mouse plasma volume and/or a stress-induced body weight change (38). Therefore, the groups shown in Fig. 8 were immediately tested for hematocrit by retroorbital puncture. We compared body weight, TD-NMR values, and hematocrit by an unpaired comparison obtained in untreated mice and mice 1 day after DOCP injection. Our results showed that neither body weight nor hematocrit allowed differentiation between the control and DOCP-treated groups (Fig. 9, *A* and *B*), but free fluid values were significantly greater in the DOCP-treated group compared with the control group ($P < 0.022$; Fig. 9*C*). Once normalized to body weight, free fluid percentages were also significantly greater in DOCP-treated mice compared with control mice ($P < 0.03$; Fig. 9*D*). Lean and fat mass were not significantly different between the control and DOCP-treated groups (data not shown).

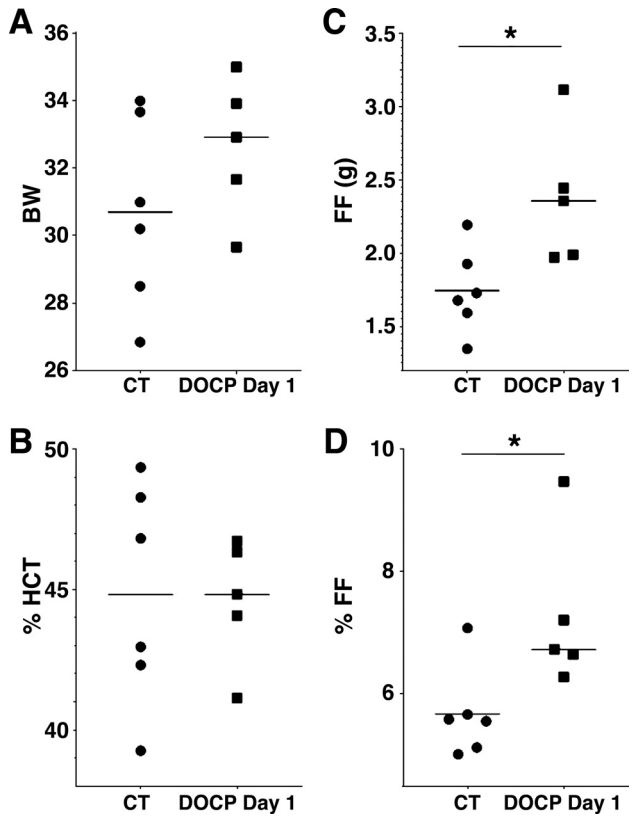

Fig. 9. Effect of long-acting mineralocorticoid treatment [deoxycorticosterone pivalate (DOCP) *day 1*] on body weight (BW; *A*), hematocrit as a percentage of blood volume (%HCT; *B*), free fluid (FF; *C*), free fluid as a percentage of BW (%FF; *D*). Two weight-matched mouse groups were analyzed by the Minispec LF50 for time-domain nuclear magnetic resonance analysis. ●, Control (CT); ■, DOCP treatment. Data are shown as individual results with means shown as bars. Data were analyzed using a Student's unpaired *t* test. $*P < 0.05$, control ($n = 6$) vs. DOCP *day 1* ($n = 5$).

DISCUSSION

Our aim was to determine the ability of TD-NMR models (Minispec LF90 and LF50) to detect a difference in free fluid in mice that would correspond to a physiological change in ECF. Our study shows that TD-NMR can detect small amounts of free fluid without distinction between small volumes (0.15−1.5 mL) of deionized water, urine, or saline and gives the mass of free fluid in heparinized blood samples. We found that the Minispec LF90 detected free fluid averaging 10% of body weight in C57BL/6J mice of both sexes. The Minispec LF50 detected free fluid averaging 5.6% in male C57BL/6J mice. The differential between these two measures could arise from multiple factors, including the calibration of the TD-NMR apparatus with oil, chicken, and saline. Therefore, we conclude that free fluid values correspond to an index of ECF.

To our knowledge, our results are the first to show the change in free fluid in response to mineralocorticoid treatment and that monitoring mice daily by TD-NMR is feasible without causing stress-induced body weight loss, even when mice are placed in individual metabolic cages. Heterogeneity in free fluid mass, lean mass, and fat mass by age and sex could be demonstrated by TD-NMR as well as variability in response to environmental and hormonal changes. We determined that the intramouse variation of free fluid was small (<5%), even when between-subject variations of body weight and free fluid were greater than 5%. By TD-NMR, 24-h water deprivation induced a decrease of 7% of body weight, 6% in lean mass, and 19% in free fluid, whereas fat mass was not affected. Of significant importance was our novel observation that a transient increase in free fluid induced by mineralocorticoids associated with a high-salt diet could be detected when no significant change in body weight was observed. Moreover, we showed that DOCP treatment alone induced a significant increase in free fluid that was consistently detectable. Finally, hematocrit measurements were not sufficient to distinguish mineralocorticoid-treated mice from control mice. Thus, TD-NMR analysis is more accurate than hematocrit measurements when investigating changes in blood volume.

Proton T_1 and T_2 vary as a function of their immediate environment. Water hydrogens close to large molecules have shortened T_2. For instance, hydrogens in water contained in the intracellular and extracellular space of the muscle have T_2 values of 50−800 ms, whereas hydrogens in body water not associated muscle or fat have T_2 values ranging from 1,000 to 3,000 ms. Colucci et al. (12) previously reported that TD-NMR allowed the detection of intraperitoneal injection of saline in mice (2% of body weight or 0.4−0.6 mL for 20-g mice) that mimics ascites. Gordon et al. (17) recently followed the rate of fluid clearance after an intraperitoneal injection of saline or hypertonic solution in different rat strains by TD-NMR by measuring free fluid percentage at multiple time points in the same animal. We determined the capacity of TD-NMR to differentiate fluids containing different concentrations of ions, proteins, and cells. We compared free fluid amounts obtained with small samples (0.15−1.0 mL) of deionized water, urine, and blood. Of note, the slopes of the three linear regressions of free fluid with deionized water phantoms, urine phantoms, or saline injected in the peritoneal space were not significantly different. This indicates that TD-NMR accurately detects water content independent of ionic strength. In contrast, the linear regression curve obtained for free fluid in heparinized blood samples showed an extremely different slope compared with the slopes obtained for deionized water or urine (Fig. 1) and compared with injected saline (Fig. 2). Interestingly, our results showed that 1 mL of blood contained 0.6 g of free fluid. We speculate that the TD-NMR device detects plasma free fluid, which forms 65.6% of total blood and contains 93% water (2).

In the whole mouse, Minispec LF90 detected 10% of body weight as free fluid. This amount is more than the fluid contained in plasma (7.2% of body weight in C57BL/6J mice) and less than total ECF (20% of body weight) as reported by other methods (3, 8, 47, 54). Colucci et al. (12) and Hazlewood et al. (23) reported that free fluid corresponds to water in blood or urine as well as smaller amounts that remain in the gut or lymphatic system. According to Bertram et al. (7), the missing part of ECF could correspond to water in blood vessels and in the interstitial space contained in muscle that would be counted as lean by TD-NMR. The Minispec LF50 detected free fluid averaging 5.6% in male C57BL/6J mice, which is much closer to plasma values reported for that strain. Minispec LF90 and LF50 have nominally the same field strength, which should be readily accounted for by the calibration step. Therefore, we find a difference in hardware unlikely as a source of variance. Instead, we attribute the absolute differences to the use of raw chicken breast as part of the calibration routine. Chicken breast is likely not an optimal solution for "lean" sample calibration, as intracellular deposits of fat cannot be excluded as being present. These results indicate free fluid estimates by these systems is only an index of ECF, and the absolute value measured depends on the calibration curve. In the future, we suggest that an agar phantom of known composition could be used to approximate chicken breast and could then be added to the calibration scheme. This would have the advantage that a calibration phantom could be shared at multiple laboratories with different instrumentation.

We conclude that free fluid is an index of ECF, and its value depends on the calibration curves obtained with phantoms and the TD-NMR software algorithm. Therefore, researchers must be aware that free fluid values correspond to an index of mouse ECF and should be considered as a close approximation of fluid that is not contained in muscle or fat.

Our data showed that TD-NMR analysis can be coupled with metabolic cage experiments and allow for simultaneous monitoring of three body components: free fluid, fat mass, and lean mass. These parameters bring valuable information concerning general health and animal variability. Our experiment showed that mice housed in ventilated cages with access to dry chow had stable free fluid values and that intermouse or intramouse variations were less than 5%. Mice gained free fluid between *day 1* and *day 3* of being housed in individual metabolic cages with access to a gel diet and water. Therefore, investigators should be aware of the existence of differences in mouse ECF volume when comparing the water balance of mice maintained in different environments.

In a separate experiment, mice were water deprived for 24 h. Our results showed that 5 h of water deprivation did not induce a significant change in mouse body weight, free fluid, lean mass, or fat mass. Other experiments have shown that 12 h of water restriction induced an increase in blood osmolality and Na^+ concentration in mice (4). After 24 h of water restriction,

our mice lost 7% of body weight, which TD-NMR revealed to be a decrease of 19% of free fluid and 6% of lean mass. By TD-NMR, Li et al. (31) showed that mouse lean and fat mass decreased significantly during a severe dehydration induced by keeping mice in a hot environment for 7.5 h. They found a body weight loss of 13% but no significant change in free fluid. This severe dehydration could have overlooked ECF contraction, as at this degree of water loss, the intracellular fluid might have been mobilized to keep ECF stable. Li et al. (31) hypothesized that lean weight loss during dehydration was due to water loss and not to catabolism or metabolism. Conversely, Kitada et al. (27) suggested that mice used muscle catabolism to increase the production of urea. Increases in plasma and renal urea concentrations help the body to increase the renal urine concentration ability necessary for water conservation. We did not address this issue in our study. Complementary terminal studies, such as measurements of mouse muscle dry mass or measurement of muscle metabolism, catabolism, or apoptosis frequency could help determine the processes engaged in muscle cells to battle water deprivation in mice.

We monitored fluid in mice subjected to HS/DOCP. Chobanian et al. (10) found treatment of normal human study participants with an excess of mineralocorticoid and a salt-enriched diet induced a transient increase in ECF volume of 14.6%, measured by the dilution technique as $^{35}SO_4^{2-}$ space. Mineralocorticoid treatment induces a transient increase in Na^+ balance in pigs (18), rats (20, 53), and mice (25, 33). The transient positive Na^+ balance precedes the rise in blood pressure and the development of hypertension in hyperaldosteronism (53), as reviewed by Hamlyn et al. (22). In these studies, the authors determined either ECF, blood, or plasma volume by indirect methods, e.g., the dilution technique (inulin space), hematocrit measurements, and Na^+ or water balance. However, the effect of HS/DOCP on free fluid, lean mass, and fat mass was not measured. Our findings are in accordance with Chobanian et al.'s study, showing the HS/DOCP protocol induced an increase of $14.48 \pm 9.28\%$ of free fluid in mice without altering lean or fat mass. Chobanian et al. (10) and others (37) have shown by the dilution method that the increase in ECF induced by mineralocorticoids correlated with an increase in the efficient arterial blood volume and does not induce ascites. In our set of experiments, we were not capable of differentiating between the interstitial milieu and plasma. We would need other methods to determine the compartment involved in the ECF volume expansion. The dilution technique, for instance, uses Evans blue to measure plasma volume in mice (54). This method is terminal and cannot be used repeatedly in small animals. Another technique is bioimpedance spectroscopy, which measures the resistance of the body to an electrical current and allows to dissociate TBW from ECF volume and can calculate the ICF volume by subtracting ECF volume from TBW. Chapman et al. (8) adapted the bioimpedance spectroscopy technique from the rat to the mouse and showed that this technique gave results in accordance with the dilution technique using ^3H for TBW and ^{35}S for ECF volume. Moreover, bioimpedance spectroscopy showed intramouse and intermouse variations of less than 5%. Recently, this method allowed Fu et al. (16) to show that thiazolidinediones (used in the treatment of diabetes) induced an increase in TBW of 8.4% and an increase of ECF of 10% in normal mice. This method requires more technical expertise than TD-NMR, as measurements are performed under anesthesia through electrodes (needles) placed under the shaved skin at very precise places of the mouse body. Therefore, bioimpedance spectroscopy, the dilution technique or other terminal investigations, should be used after a TD-NMR study to draw kinetics of body compartment behaviors following stimuli. In our experiment, the results indicated that the best time to investigate the DOCP effect in mice is 24 h after DOCP injection. In fact, TD-NMR could explore even more precisely the kinetics of DOCP actions by analyzing animals every hour.

We demonstrate that the significant increase in body weight resulting from DOCP treatment corresponded to an increase in free fluid. Furthermore, we showed that TD-NMR measurement provides a more accurate assessment of ECF variation than hematocrit measurement.

In our experiments, DOCP or HS/DOCP treatment did not consistently induce an increase in body weight. However, free fluid was always increased in mouse groups treated with DOCP. One explanation could be that the increase in free fluid is small compared with body weight. In our experiment, the mouse SD for the change in body weight before and after mineralocorticoid injection was greater than the change in free fluid content: 0.80 vs. 0.26 g, respectively. In fact, according to paired-sample size calculators (samplesize.net), one would need to treat 24 mice to see a difference of 0.4-g increase in body weight with such a standard variation. Therefore, we believe that the TD-NMR method is more reliable in detecting small variations in ECF than body weight measurements. Moreover, TD-NMR allows measurement of three components of the body at the same time. Thereby, it is possible to determine how different body compartments and their ratio (fat/lean mass) interfere with the body response to different stresses. Thus, TD-NMR could allow comparison of the response of obese versus lean mice to water balance perturbation.

In summary, TD-NMR allows the detection of not only fat and lean mass in small rodents but also free fluid as an index of ECF. This tool is compatible with longitudinal studies using individual metabolic cages. As such, it provides valuable information on the heterogeneity of body fluid composition. TD-NMR showed low intramouse and intermouse variability for free fluid reported to body weight that permitted the detection of a physiological change in ECF. For the first time, we showed that mineralocorticoids in mice fed a high-salt diet or normal-salt diet induced a transient increase in mouse ECF volume without an obligatory increase in body weight or decrease in hematocrit. TD-NMR can be used before terminal or invasive methods to define the best moment to investigate hormonal or drug-mediated effects. This tool should further our understanding of water homeostasis during states of ECF expansion or contraction that may occur without detectable changes in body weight or total body water. Moreover, TD-NMR will bring valuable data in studies on water balance maintenance during aging and in metabolic disorders. Finally, TD-NMR can reveal new body component changes relevant to fluid disorders.

ACKNOWLEDGMENTS

We are grateful to the laboratory of Dr. Philip Scarpace for the use of the TD-NMR instrument. We thank Dr. Pedro Lameiras (Bruker France) for the advice, documentation, and explanations on the function of the TD-NMR device and the calibration methods.

GRANTS

This work was supported by NF/SGVHS Medical Research and Merit Review Award BX001472-01A1 (to C. S. Wingo) and by the Gatorade Trust through funds distributed by the Department of Medicine, University of Florida.

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

L.M. and C.S.W. conceived and designed research; L.M. and O.S. performed experiments; L.M., O.S., I.J.L., and C.S.W. analyzed data; L.M., O.S., I.J.L., M.E.M., and C.S.W. interpreted results of experiments; L.M., I.J.L., and C.S.W. prepared figures; L.M., I.J.L., M.E.M., and C.S.W. drafted manuscript; L.M., I.J.L., M.E.M., and C.S.W. edited and revised manuscript; L.M., O.S., I.J.L., M.E.M., and C.S.W. approved final version of manuscript.

REFERENCES

1. **Adolf E.** *Heat Exchanges, Sweat Formation, and Water Turnover*. New York: Interscience, 1947.
2. **Albrink MJ, Hald PM, Man EB, Peters JP.** The displacement of serum water by the lipids of hyperlipemic serum: a new method for the rapid determination of serum water. *J Clin Invest* 34: 1483–1488, 1955. doi:10.1172/JCI103199.
3. **Baxmann AC, Ahmed MS, Marques NC, Menon VB, Pereira AB, Kirsztajn GM, Heilberg IP.** Influence of muscle mass and physical activity on serum and urinary creatinine and serum cystatin C. *Clin J Am Soc Nephrol* 3: 348–354, 2008. doi:10.2215/CJN.02870707.
4. **Bekkevold CM, Robertson KL, Reinhard MK, Battles AH, Rowland NE.** Dehydration parameters and standards for laboratory mice. *J Am Assoc Lab Anim Sci* 52: 233–239, 2013.
5. **Belcher EH, Harriss EB.** Studies of plasma volume, red cell volume and total blood volume in young growing rats. *J Physiol* 139: 64–78, 1957. doi:10.1113/jphysiol.1957.sp005875.
6. **Bellantuono I, de Cabo R, Ehninger D, Di Germanio C, Lawrie A, Miller J, Mitchell SJ, Navas-Enamorado I, Potter PK, Tchkonia T, Trejo JL, Lamming DW.** A toolbox for the longitudinal assessment of healthspan in aging mice. *Nat Protoc* 15: 540–574, 2020. doi:10.1038/s41596-019-0256-1.
7. **Bertram HC, Karlsson AH, Rasmussen M, Pedersen OD, Dønstrup S, Andersen HJ.** Origin of multiexponential T(2) relaxation in muscle myowater. *J Agric Food Chem* 49: 3092–3100, 2001. doi:10.1021/jf001402t.
8. **Chapman ME, Hu L, Plato CF, Kohan DE.** Bioimpedance spectroscopy for the estimation of body fluid volumes in mice. *Am J Physiol Renal Physiol* 299: F280–F283, 2010. doi:10.1152/ajprenal.00113.2010.
9. **Cheuvront SN, Kenefick RW, Charkoudian N, Sawka MN.** Physiologic basis for understanding quantitative dehydration assessment. *Am J Clin Nutr* 97: 455–462, 2013. doi:10.3945/ajcn.112.044172.
10. **Chobanian AV, Burrows BA, Hollander W.** Body fluid and electrolyte composition in arterial hypertension. II. Studies in mineralocorticoid hypertension. *J Clin Invest* 40: 416–422, 1961. doi:10.1172/JCI104269.
11. **Chumlea WC, Guo SS, Zeller CM, Reo NV, Baumgartner RN, Garry PJ, Wang J, Pierson RN Jr, Heymsfield SB, Siervogel RM.** Total body water reference values and prediction equations for adults. *Kidney Int* 59: 2250–2258, 2001. doi:10.1046/j.1523-1755.2001.00741.x.
12. **Colucci L, Li M, Cima M.** Quantification of Fluid Accumulation in IP Space of Mice using Whole-Body NMR. ISMRM 23rd Annual Meeting and Exhibition. Toronto, ON, Canada: May 30, 2015.
13. **Diehl KH, Hull R, Morton D, Pfister R, Rabemampianina Y, Smith D, Vidal JM, van de Vorstenbosch C; European Federation of Pharmaceutical Industries Association and European Centre for the Validation of Alternative Methods.** A good practice guide to the administration of substances and removal of blood, including routes and volumes. *J Appl Toxicol* 21: 15–23, 2001. doi:10.1002/jat.727.
14. **Durkot MJ, Martinez O, Brooks-McQuade D, Francesconi R.** Simultaneous determination of fluid shifts during thermal stress in a small-animal model. *J Appl Physiol (1985)* 61: 1031–1034, 1986. doi:10.1152/jappl.1986.61.3.1031.
15. **Ekpenyong CE Akpan I-AM.** High prevalence and associated risk factors of dehydration among college students: implications for health and academic performance. *Int J Commun Med Public Health* 4: 1043, 2017. doi:10.18203/2394-6040.ijcmph20171322.
16. **Fu Y, Gerasimova M, Batz F, Kuczkowski A, Alam Y, Sanders PW, Ronzaud C, Hummler E, Vallon V.** PPARγ agonist-induced fluid retention depends on αENaC expression in connecting tubules. *Nephron* 129: 68–74, 2015. doi:10.1159/000370254.
17. **Gordon CJ, Phillips PM, Johnstone AF.** A noninvasive method to study regulation of extracellular fluid volume in rats using nuclear magnetic resonance. *Am J Physiol Renal Physiol* 310: F426–F431, 2016. doi:10.1152/ajprenal.00405.2015.
18. **Grekin RJ, Terris JM, Bohr DF.** Electrolyte and hormonal effects of deoxycorticosterone acetate in young pigs. *Hypertension* 2: 326–332, 1980. doi:10.1161/01.HYP.2.3.326.
19. **Guyton AC, Coleman TG.** Quantitative analysis of the pathophysiology of hypertension. *Circ Res* 24, *Suppl*: 1–19, 1969.
20. **Haack D, Möhring J, Möhring B, Petri M, Hackenthal E.** Comparative study on development of corticosterone and DOCA hypertension in rats. *Am J Physiol Renal Physiol* 233: F403–F411, 1977. doi:10.1152/ajprenal.1977.233.5.F403.
21. **Halldorsdottir S, Carmody J, Boozer CN, Leduc CA, Leibel RL.** Reproducibility and accuracy of body composition assessments in mice by dual energy x-ray absorptiometry and time domain nuclear magnetic resonance. *Int J Body Compos Res* 7: 147–154, 2009.
22. **Hamlyn JM, Blaustein MP.** Sodium chloride, extracellular fluid volume, and blood pressure regulation. *Am J Physiol Renal Physiol* 251: F563–F575, 1986. doi:10.1152/ajprenal.1986.251.4.F563.
23. **Hazlewood CF, Chang DC, Nichols BL, Woessner DE.** Nuclear magnetic resonance transverse relaxation times of water protons in skeletal muscle. *Biophys J* 14: 583–606, 1974. doi:10.1016/S0006-3495(74)85937-0.
24. **Hughes F, Mythen M, Montgomery H.** The sensitivity of the human thirst response to changes in plasma osmolality: a systematic review. *Perioper Med (Lond)* 7: 1, 2018. doi:10.1186/s13741-017-0081-4.
25. **Jia Z, Aoyagi T, Kohan DE, Yang T.** mPGES-1 deletion impairs aldosterone escape and enhances sodium appetite. *Am J Physiol Renal Physiol* 299: F155–F166, 2010. doi:10.1152/ajprenal.90702.2008.
26. **Kitabchi AE, Umpierrez GE, Miles JM, Fisher JN.** Hyperglycemic crises in adult patients with diabetes. *Diabetes Care* 32: 1335–1343, 2009. doi:10.2337/dc09-9032.
27. **Kitada K, Daub S, Zhang Y, Klein JD, Nakano D, Pedchenko T, Lantier L, LaRocque LM, Marton A, Neubert P, Schröder A, Rakova N, Jantsch J, Dikalova AE, Dikalov SI, Harrison DG, Müller DN, Nishiyama A, Rauh M, Harris RC, Luft FC, Wasserman DH, Sands JM, Titze J.** High salt intake reprioritizes osmolyte and energy metabolism for body fluid conservation. *J Clin Invest* 127: 1944–1959, 2017. doi:10.1172/JCI88532.
28. **Knox FG, Burnett JC Jr, Kohan DE, Spielman WS, Strand JC.** Escape from the sodium-retaining effects of mineralocorticoids. *Kidney Int* 17: 263–276, 1980. doi:10.1038/ki.1980.32.
29. **Koomans HA, Roos JC, Boer P, Geyskes GG, Mees EJ.** Salt sensitivity of blood pressure in chronic renal failure. Evidence for renal control of body fluid distribution in man. *Hypertension* 4: 190–197, 1982. doi:10.1161/01.HYP.4.2.190.
30. **Künnecke B, Verry P, Bénardeau A, von Kienlin M.** Quantitative body composition analysis in awake mice and rats by magnetic resonance relaxometry. *Obes Res* 12: 1604–1615, 2004. doi:10.1038/oby.2004.200.
31. **Li M, Vassiliou CC, Colucci LA, Cima MJ.** ^{1}H nuclear magnetic resonance (NMR) as a tool to measure dehydration in mice. *NMR Biomed* 28: 1031–1039, 2015. doi:10.1002/nbm.3334.
32. **Lumbers ER, Bernasconi C, Burrell JH.** Effects of infusions of ACTH in the chronically catheterized pregnant ewe and her fetus. *Am J Physiol Renal Physiol* 274: R445–R452, 1998. doi:10.1152/ajpregu.1998.274.2.R445.
33. **Lynch IJ, Welch AK, Gumz ML, Kohan DE, Cain BD, Wingo CS.** Effect of mineralocorticoid treatment in mice with collecting duct-specific knockout of endothelin-1. *Am J Physiol Renal Physiol* 309: F1026–F1034, 2015. doi:10.1152/ajprenal.00220.2015.
34. **Lyons KJ, Bischoff MK, Fonarow GC, Horwich TB.** Noninvasive bioelectrical impedance for predicting clinical outcomes in outpatients with heart failure. *Crit Pathw Cardiol* 16: 32–36, 2017. doi:10.1097/HPC.0000000000000105.
35. **Milani GP, Groothoff JW, Vianello FA, Fossali EF, Paglialonga F, Edefonti A, Agostoni C, Consonni D, van Harskamp D, van Goudoever JB, Schierbeek H, Oosterveld MJ.** Bioimpedance and fluid status in

children and adolescents treated with dialysis. *Am J Kidney Dis* 69: 428–435, 2017. doi:10.1053/j.ajkd.2016.10.023.

36. **Mystkowski P, Shankland E, Schreyer SA, LeBoeuf RC, Schwartz RS, Cummings DE, Kushmerick M, Schwartz MW.** Validation of whole-body magnetic resonance spectroscopy as a tool to assess murine body composition. *Int J Obes Relat Metab Disord* 24: 719–724, 2000. doi:10.1038/sj.ijo.0801231.
37. **Palmer B, Alpern R, Seldin D.** Physiology and pathophysiology of sodium retention and wastage. In: *Seldin and Giebisch's The Kidney*. New York: Elsevier, 2013, p. 1005–1049.
38. **Raabe BM, Artwohl JE, Purcell JE, Lovaglio J, Fortman JD.** Effects of weekly blood collection in C57BL/6 mice. *J Am Assoc Lab Anim Sci* 50: 680–685, 2011.
39. **Ragan C, Ferrebee J, Phyfe P, Atchley D, Loeb R.** A syndrome of polydipsia and polyuria induced in normal animals by desoxycorticosterone acetate. *Am J Physiol* 131: 73–78, 1940. doi:10.1152/ajplegacy.1940.131.1.73.
40. **Rahman A-U, Choudhary MI, Choudhary MI.** *Solving Problems with NMR Spectroscopy*. San Diego, CA: Academic, 1996.
41. **Ramanathan L, Siegel JM.** Gender differences between hypocretin/orexin knockout and wild type mice: age, body weight, body composition, metabolic markers, leptin and insulin resistance. *J Neurochem* 131: 615–624, 2014. doi:10.1111/jnc.12840.
42. **Reynolds TH, Dalton A, Calzini L, Tuluca A, Hoyte D, Ives SJ.** The impact of age and sex on body composition and glucose sensitivity in C57BL/6J mice. *Physiol Rep* 7: e13995, 2019. doi:10.14814/phy2.13995.
43. **Riches AC, Sharp JG, Thomas DB, Smith SV.** Blood volume determination in the mouse. *J Physiol* 228: 279–284, 1973. doi:10.1113/jphysiol.1973.sp010086.
44. **Rowland NE.** Food or fluid restriction in common laboratory animals: balancing welfare considerations with scientific inquiry. *Comp Med* 57: 149–160, 2007.
45. **Santosham M, Chandran A, Fitzwater S, Fischer-Walker C, Baqui AH, Black R.** Progress and barriers for the control of diarrhoeal disease. *Lancet* 376: 63–67, 2010. doi:10.1016/S0140-6736(10)60356-X.
46. **Schrier RW.** Body water homeostasis: clinical disorders of urinary dilution and concentration. *J Am Soc Nephrol* 17: 1820–1832, 2006. doi:10.1681/ASN.2006030240.
47. **Staffel J, Valletta D, Federlein A, Ehm K, Volkmann R, Füchsl AM, Witzgall R, Kuhn M, Schweda F.** Natriuretic peptide receptor guanylyl cyclase-A in podocytes is renoprotective but dispensable for physiologic renal function. *J Am Soc Nephrol* 28: 260–277, 2017. doi:10.1681/ASN.2015070731.
48. **Stookey JD.** High prevalence of plasma hypertonicity among community-dwelling older adults: results from NHANES III. *J Am Diet Assoc* 105: 1231–1239, 2005. doi:10.1016/j.jada.2005.05.003.
49. **Titze J, Bauer K, Schafflhuber M, Dietsch P, Lang R, Schwind KH, Luft FC, Eckardt KU, Hilgers KF.** Internal sodium balance in DOCA-salt rats: a body composition study. *Am J Physiol Renal Physiol* 289: F793–F802, 2005. doi:10.1152/ajprenal.00096.2005.
50. **Umar SB, DiBaise JK.** Protein-losing enteropathy: case illustrations and clinical review. *Am J Gastroenterol* 105: 43–49, 2010. doi:10.1038/ajg.2009.561.
51. **Vega R, Avva U.** Pediatric dehydration. In: *StatPearls*. Treasure Island, FL: StatPearls, 2019. https://www.ncbi.nlm.nih.gov/books/NBK436022/.
52. **Verbalis JG.** Disorders of body water homeostasis. *Best Pract Res Clin Endocrinol Metab* 17: 471–503, 2003. doi:10.1016/S1521-690X(03)00049-6.
53. **Villamil MF, Amorena C, Ponce J, Müller A, Taquini AC.** Acute saline expansion lowers blood pressure of early DOCA salt hypertensive rats. *Pflügers Arch* 390: 260–261, 1981. doi:10.1007/BF00658272.
54. **Walter C, Tanfous MB, Igoudjil K, Salhi A, Escher G, Crambert G.** H,K-ATPase type 2 contributes to salt-sensitive hypertension induced by K^+ restriction. *Pflugers Arch* 468: 1673–1683, 2016. doi:10.1007/s00424-016-1872-z.
55. **Zhou L, Liu G, Jia Z, Yang KT, Sun Y, Kakizoe Y, Liu M, Zhou S, Chen R, Yang B, Yang T.** Increased susceptibility of *db/db* mice to rosiglitazone-induced plasma volume expansion: role of dysregulation of renal water transporters. *Am J Physiol Renal Physiol* 305: F1491–F1497, 2013. doi:10.1152/ajprenal.00004.2013.