Pediatric RESEARCH

CLINICAL RESEARCH ARTICLE

D_3-creatine dilution for the noninvasive measurement of skeletal muscle mass in premature infants

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BACKGROUND: The rate of accrual of muscle mass in neonates has not been assessed. We describe the D_3-creatine (D_3Cr) dilution method, a noninvasive assessment of muscle mass in neonates.

METHODS: A total of 76 neonates >26-week-old corrected gestational age were enrolled and measured at 2-week intervals while admitted to a neonatal intensive care unit (NICU). Additional measures at 6 and 12–20 months after initial measurement were obtained if available. An enteral dose of 2 mg D_3Cr in 0.5 mL 20% ^2H_2O was used to determine muscle mass and total body water (TBW).

RESULTS: Muscle mass by the D_3Cr method was strongly associated with TBW and body weight ($r = 0.9272$, $p < 0.0001$ and $r = 0.9435$, $p < 0.0001$ for all time points and $r = 0.6661$, $p < 0.0001$ and $r = 0.8634$, $p < 0.0001$, respectively, while in the NICU). Change in muscle mass vs. change in body weight, TBW, and length were also strongly correlated.

CONCLUSIONS: The D_3Cr dilution method provides a noninvasive assessment of muscle mass accrual in neonates, which has not been previously possible and may be an important new tool for the evaluation of nutritional status and normal growth patterns.

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IMPACT:

- We describe a noninvasive method for the measurement of skeletal muscle mass neonates.
- At the present time, there is no direct measurement of muscle mass in infants available.
- The D_3Cr dilution method is a direct and noninvasive measurement of muscle mass.
- Using a single enteral dose of D_3Cr in ^2H_2O followed by urine and saliva samples, rapid and substantial accrual of muscle mass and TBW is assessed.
- Assessment of muscle mass accrual in premature infants may be a strong indicator of nutritional status.
- Change in muscle mass is strongly related to change in weight and TBW.

INTRODUCTION

Skeletal muscle is the largest reservoir of amino acids in the body and is in principle the most informative index of nutritional status in health and disease[1] and should be a fundamental element in the assessment of health, nutritional status, and disease in infants and children. Impaired lean tissue accrual in infants is quite often associated with adverse long-term outcomes, including impaired intellectual development;[2] thus, there is a need to develop a simple, accurate, method to measure muscle mass in infants and children.

The D_3-creatine (D_3Cr) dilution method represents a novel way to directly and noninvasively measure muscle mass in infants. The measurement of total body creatine pool size provides an assessment of muscle mass as ~98% of creatine in the body is sequestered in the sarcomere.[3,4] A small, enteral dose of D_3Cr is administered, enters the circulation and is actively transported into the sarcomere and mixes with the body creatine pool. D_3Cr is converted to D_3-creatinine (D_3Crn) in a nonenzymatic, irreversible reaction and excreted. Thus, the enrichment of urine D_3Crn is used to determine the total body creatine pool size. We have validated the method in rats and in adult humans using whole-body magnetic resonance imaging.[3–5] In a cohort of >1300 elderly men (>80 years) D_3Cr muscle mass is strongly associated with health-related outcomes, including risk of falls, disability, and mortality,[6,7] while no such associations were observed using commonly used measurements of fat-free mass (FFM).

Nowhere is the need for safe, noninvasive, easy-to-use, and accurate tools to assess growth more evident than in pre-term neonates: Approximately half of all extremely low birth weight neonates are discharged from the neonatal intensive care unit (NICU) with growth failure[8–10] and the association between extrauterine growth restriction and intellectual impairment[8] is only inferential. Important work by Barker[11] and Godfrey and Barker[12] demonstrated that birth weight is predictive of later life

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risk of noncommunicable diseases. However, there is no information on how components of body composition may contribute to the risk. In the larger pediatric population, the means to measure muscle mass has been identified as an urgent need as "early identification of sarcopenia is crucial to enable targeted treatment and prevention to be carried out across the pediatric clinical populations".[5] The most common methods for determining body composition in infants or children[13–15] use assumptions based on data gathered in adults, assumes that subjects are adequately hydrated, and that FFM has a constant proportion of water. None measured the muscle mass.

The purpose of the present study was to determine if this method to determine muscle mass is feasible in neonates and to compare muscle mass measured by D_3Cr dilution with TBW, body weight, and body length, as well as head, chest, and abdominal circumferences. Multiple repeated measurements were made in many of the infants to assess the effects of growth on muscle mass accrual and other changes in body composition.

METHODS

This study was carried out at two NICUs and approved by the local IRBs. Eligible infants were 26 weeks corrected gestational age (CGA) to post-term infants receiving care in a NICU. Exclusion criteria include the following: lethal chromosomal abnormality; diagnoses otherwise not compatible with life; metabolic disease; neuromuscular disorders, such as spinal muscular atrophy; short gut or intestinal failure; significant renal dysfunction; gastrointestinal illness; chronic diarrhea; and infants who were not being fed enterally. A total of 76 neonates were enrolled after consent. Table 1 shows select characteristics of the study population.

We addressed the following aims: (1) the time after receiving the dose to reach isotopic steady state for the enrichment of urine D_3Crn. (2) Relationships between TBW, total body creatine pool size (skeletal muscle mass), body weight, and other anthropometric indices of growth. (3) Changes in TBW, muscle mass, and body weight during stay in the NICU. (4) After discharge from the NICU, changes in FFM and muscle mass during growth over a 6–12-month period.

Table 1. Infant demographics and characteristics.

Characteristic	Statistic
Neonatal participants	76
Gestational age at birth (weeks), mean (range)	31.3 (24 0/7–41 3/7)
Birth weight (g), mean (range)	1793 (550–4270)
White race (%)	72
Female (%)	54
Twin gestation (%)	10
Birth weight <1000 g, *n* (%)	20 (26)
Birth weight <1500 g, *n* (%)	31 (41)
Intrauterine growth restriction	5 (7)
Small for gestational age, *n* (%)	6 (8)
Large for gestational age, *n* (%)	5 (7)
Infant of a diabetic mother	4 (5)
Age (day of life) at 1st test, mean (range)	28 (4–183)
Corrected gestational age at dosing, mean (range)	35 4/7 (26 3/7–51 0/7)
Weight (g) at 1st test, mean (range)	2378 (980–5635)
Respiratory distress syndrome, *n* (%)	55 (72)
On respiratory support at the time of first measure, *n* (%)	51 (67%)
Congenital heart disease, *n* (%)	6 (8)
Congenital diaphragmatic hernia, *n* (%)	4 (5)
Congenital gastrointestinal disorder	6 (8)
Hypoxic–ischemic encephalopathy	3 (5)

This method requires that the infant receive a 2 mg enteral dose of D_3Cr and later to produce urine samples. A single oral administration of D_3Cr is ingested, absorbed, enters the circulation, and is actively transported into the sarcomere and thus diluted in the endogenous creatine pool. A constant fraction of creatine is converted into creatinine, which is rapidly excreted in urine. The creatine pool size, and thus muscle mass, is then determined from urine D_3Crn enrichment by mass spectrometry: the ratio of labeled to unlabeled creatinine in a single sample of urine. Urine was collected using a strip of filter paper placed in a diaper.

The initial measurement was obtained shortly after receiving consent, which was at various times after birth. After the initial measurement, a subset of infants was re-measured every 14 days until discharge from the NICU. Of the 76 infants enrolled, 68 received one dose, 26 received two doses, 7 received 3 doses, 5 received 4 doses, and 2 received 5 doses while in the NICU, which was the maximum number of measures. Subsequent measurements were made at 6 and 15–20 months after the initial measurements. Anthropometric data for each infant was collected at each D_3Cr dosing, including body weight, length measured by length board and head, chest, and abdomen circumferences. A 2 mg dose of D_3Cr/0.5 mL of 20% ^2H_2O was delivered to all infants to assess creatine dilution and TBW and to determine the enrichment patterns of urine D_3Crn. Prior to each dose, a saliva sample was collected to correct for background enrichment of body water. A 1 mL syringe was used to deliver the dose orally or via an enteral feeding tube, which was weighed after pulling 0.5 mL of the prepared dose and immediately after the dose was delivered to accurately determine the volume delivered and thus the amount of D_3Cr and ^2H_2O delivered. Creatine pool size was calculated with the following formula, where 131.1/134.1 is the ratio of the molecular weights of unlabeled creatine to D_3Cr:

$$\text{Creatine pool size} = \frac{(131.1/134.1) \cdot 0.002\,\text{g}(\text{dose of } D_3\text{Cr to each infant})}{(\text{steady} - \text{state } D_3 - \text{creatinine enrichment ratio in urine})}.$$

The enrichment of ^2H_2O in saliva achieved isotopic steady state (no difference between 1 and 3 h) by 1 h after the dose was provided. Each diaper placed on the infants contained a $1.5 \times 5\,cm^2$ numbered strip of filter paper to collect urine. The urine-saturated filter paper strips were removed at each diaper change and stored at −80 °C for later analysis of D_3Crn, D_3Cr, and creatine/creatinine ratio. To determine when the enrichment of urine D_3Crn achieved isotopic steady state and the presence of any of the oral D_3Cr tracer dose not transported into the muscle ("spilling"), urine samples were collected from diapers in the initial eight infants from the time of the dose through 72 h. Because body creatine turns over at the rate of ~1.7%/day,[4] the initial dose of D_3Cr remains in the total body creatine pool for an extended period of time; therefore, a pre-dose urine sample was collected to correct for the residual D_3Crn present in the urine of participants prior to subsequent measurements of urinary D_3Crn enrichment. Some infants were re-measured after each 14-day period of care in the NICU along with anthropometric measurements. In this way, traditional indices of growth were compared to changes in muscle mass.

Urine sample analysis for D_3Crn enrichment

The filter paper strips provided a sufficient urine sample for analysis, provided that they were well saturated. The strips were cut in half and placed in a 2 mL microcentrifuge tube with 300 µL of 50% acetonitrile (ACN), vortexed, and centrifuged for 5 min at

12,000 r.p.m. One hundred microliters of supernatant was mixed with 100 µL internal standards and 300 µL 100% ACN prior to liquid chromatography/mass spectroscopy analysis, which was performed as described previously.[16] The total body Cr pool size was calculated as the retained (delivered) D_3Cr dose divided by D_3Crn enrichment, and muscle mass is estimated from total body Cr pool size as described previously.[5] Some filter paper strips were contaminated by stool and some of these samples provided spurious enrichment values for a few infants enrolled at the start of the study. We subsequently used urine-saturated filter paper taken from diapers without the presence of stool.

^2H_2O for total body water (TBW)

Saliva samples were collected from each subject using SalivaBio infant swab placed orally until saturated (~30–60 s) at 1–3 h after administration of the D_3Cr in ^2H_2O dose to measure the body distribution of ^2H_2O. Measurement of ^2H_2O was by isotope ratio IR spectroscopy, and TBW was derived as previously described.[17]

RESULTS

A total of 76 infants were enrolled in this study and muscle mass data are available for 68 single measurements, 26 were measured twice (0 and 14 days), 7 were measured 3 times (0, 14, and 28 days), 5 were measured 4 times (0, 14, 28, and 42 days), and 2 were measured 5 times (0, 14, 28, 42, and 56 days) during their stay in NICU. Data are not available on eight infants due to incomplete dosing, and unsaturated or stool contaminated filter paper strips.

Enrichment time course of urine D_3Crn

After dosing with D_3Cr, the enrichment of urine D_3Crn increased and reached a plateau concentration (isotopic steady state). In the initial eight infants enrolled, filter paper strips were obtained from each diaper from immediately after the initial dose of D_3Cr through 75 h. Figure 1 shows the average enrichment of urine D_3Crn for each 5-h period. There was a significant ($p < 0.05$ mixed-effects analysis of variance (ANOVA)) time-dependent increase, and isotopic steady state was achieved by 10 h after the initial dose, which remained at plateau through the entire sampling period. Based on the variance of the D_3Crn measurement, sufficient power (>0.9) was achieved with an $n = 6–9$ at each time point for repeated-measures ANOVA.

Spillage of D_3Cr label

Spillage of the D_3Cr oral label that appeared in urine samples during the first 24 h post dose was measured in 8 subjects, with 4 subjects after 1 dose and 4 subjects after 2 doses, for a total of 12 measurements. While several samples had no measurable D_3Cr, a very low amount of D_3Cr (spillage) was detected in some of the samples. The total milligram of D_3Cr spilled in the first 24 h post dose was 0.025 ± 0.029 mg (mean ± SD, $N = 12$), representing an average loss of 1.25% of the dose.

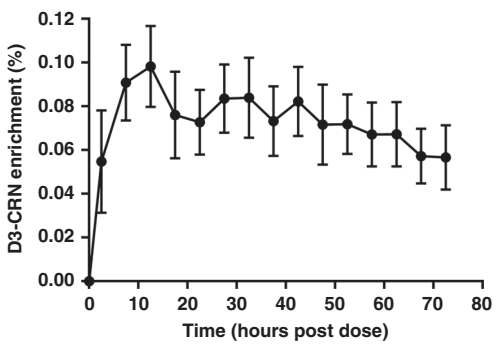

Fig. 1 Average enrichment of urine D_3-creatinine (D_3Crn) enrichment measured from urine-saturated filter paper placed in diapers from immediately after dosing of D_3-creatine through 72 h. Urine D_3Crn enrichment achieved isotopic steady state after ~10 h and remained unchanged through 72 h.

Initial measurements

Infants received the initial dose of D_3Cr and ^2H_2O at varying times after birth and the data from this assessment are presented in Table 1. One-way ANOVA was performed for each of the measurements to compare groups of neonates separated as tertiles of body weight at first dose. Pearson's correlation test was performed to compare the various measurements, as well as longitudinal changes in measurements from first dose. All differences between groups and correlations were considered significant at $p < 0.05$.

Cross-sectional measurements

Table 2 presents cross-sectional data for muscle mass, TBW (relative to body weight), and anthropometrics for the initial measurement for each infant, which was taken at various times after birth when consent was obtained from parents. The data are stratified by body weight at the time of this measurement. Muscle mass, TBW, and anthropometric parameters were greater in each successive tertile of body weight. The relative proportion of muscle mass in the children was not different by weight or age and averaged 24–25% of body weight. Relative TBW tended to decrease with increasing weight, with the lowest % TBW observed in the highest weight tertile. Strong cross-sectional relationships were observed for all measurements between muscle mass vs. body weight, TBW, body length, and head and chest circumferences while in the NICU and after 12 months (Table 3).

Longitudinal measurements

The average increase in muscle mass for those infants measured 14 days apart was 28 ± 17%, or ~2%/day. Figure 2 shows individual values for changes in muscle mass. After discharge from the NICU, 11 infants were measured at ~6 and 15–20 months after birth. The average measured increase in muscle mass from first dose to final dose was 280 ± 113%. Day 14 values for two infants show a decline in muscle mass compared to day 1. These may be spurious values due to stool contamination from the diaper as body weight and TBW values increased in these infants during this period. Changes in muscle mass were strongly correlated and associated with changes in body weight, TBW, and body length (Table 4).

Safety

No adverse events at any dosing time period were observed, which included a diverse population, including neonates as young as 27 weeks CGA and those mechanically ventilated, with congenital heart disease, and post surgery (Table 1).

DISCUSSION

Here, we describe a safe, noninvasive, easy-to-perform method to measure muscle mass in any neonate, including ventilated infants, with a variety of medical and surgical diagnoses. By combining D_3Cr with ^2H_2O, a three-compartment model of body composition consisting of muscle mass, TBW, and fat mass can be determined. The details of sample analysis are described in the "Methods" section; however, for clinicians and researchers, dosing and sample collection is relatively simple. An accurate enteral dose is required (2 mg D_3Cr in 0.5 mL water) and the collection of urine from a diaper uncontaminated with stool can be made at any time between 10 and 70 h after the initial dose. Multiple repeat measurements can be made to assess changes in muscle mass, as long as a pre-dose urine sample is taken to correct for residual enrichment of D_3Crn. We previously demonstrated that in adults,

Table 2. Mean ± SD (*n*) of measurements during 1st test with D_3-creatine in ^2H_2O.

	Body weight 1–2 kg	Body weight 2–3 kg	Body weight >3 kg
Gestational age at birth (weeks)	29 ± 3 (25)	31 ± 3 (26)	36 ± 5 (18)*,**
Age at 1st test (days)	31 ± 26 (25)	35 ± 26 (27)*	52 ± 49 (18)*,**
Body weight (kg)	1.61 ± 0.24 (28)	2.28 ± 0.24 (28)*	4.13 ± 0.76 (18)*,**
Muscle mass (kg)	0.39 ± 0.11 (27)	0.57 ± 0.13 (24)*	0.97 ± 0.24 (16)*,**
Muscle mass/body weight (%)	24 ± 6 (27)	25 ± 6 (24)	24 ± 6 (16)
Total body water	1.44 ± 0.22 (14)	1.90 ± 0.3 (10)*	2.86 ± 0.37 (10)*,**
Total body water/weight (%)	87 ± 8 (14)	84 ± 11 (10)	72 ± 10 (10)*,**
Length (cm)	41.98 ± 3.3 (28)	45.59 ± 4.75 (27)*	54.66 ± 3.75 (18)*,**
Head circumference (cm)	28.06 ± 1.94 (26)	30.35 ± 1.90 (27)*	35.28 ± 1.87 (17)*,**
Chest circumference (cm)	26.28 ± 1.55 (28)	30.31 ± 1.37 (27)*	36.98 ± 3.26 (18)*,**
Waist circumference (cm)	26.79 ± 1.56 (27)	29.79 ± 1.70 (27)*	36.28 ± 3.68 (18)*,**

Initial measurements were made after receiving consent and at various times after birth. The data are presented as tertiles of body weight. Age at the first measurement is presented as gestational age + the amount of time from birth that the measurement was made.
*$P < 0.05$ vs. 1–2 kg.
**$P < 0.05$ vs. 2–3 kg, one-way ANOVA.

Table 3. Correlation matrix showing cross-sectional relationships between body composition variables at each measurement time point.

Correlation matrix of measurements	Body weight	Muscle mass	Total body water	Length	Head circumference	Chest circumference	Waist circumference
Body weight	1.0000	$r = 0.8989$ $p < 0.0001$ $n = 119$	$r = 0.9687$ $p < 0.0001$ $n = 71$	$r = 0.9480$ $p < 0.0001$ $n = 153$	$r = 0.9523$ $p < 0.0001$ $n = 149$	$r = 0.9431$ $p < 0.0001$ $n = 153$	$r = 0.9413$ $p < 0.0001$ $n = 152$
Muscle mass	$r = 0.8989$ $p < 0.0001$ $n = 119$	1.0000	$r = 0.9141$ $p < 0.0001$ $n = 54$	$r = 0.8808$ $p < 0.0001$ $n = 119$	$r = 0.8832$ $p < 0.0001$ $n = 115$	$r = 0.8721$ $p < 0.0001$ $n = 119$	$r = 0.8405$ $p < 0.0001$ $n = 118$
Total body water	$r = 0.9687$ $p < 0.0001$ $n = 71$	$r = 0.9141$ $p < 0.0001$ $n = 54$	1.0000	$r = 0.9464$ $p < 0.0001$ $n = 71$	$r = 0.9468$ $p < 0.0001$ $n = 68$	$r = 0.9374$ $p < 0.0001$ $n = 71$	$r = 0.9307$ $p < 0.0001$ $n = 70$
Length	$r = 0.9480$ $p < 0.0001$ $n = 153$	$r = 0.8808$ $p < 0.0001$ $n = 119$	$r = 0.9464$ $p < 0.0001$ $n = 71$	1.0000	$r = 0.9474$ $p < 0.0001$ $n = 149$	$r = 0.9352$ $p < 0.0001$ $n = 154$	$r = 0.9102$ $p < 0.0001$ $n = 153$
Head circumference	$r = 0.9523$ $p < 0.0001$ $n = 149$	$r = 0.8832$ $p < 0.0001$ $n = 115$	$r = 0.9468$ $p < 0.0001$ $n = 68$	$r = 0.9474$ $p < 0.0001$ $n = 149$	1.0000	$r = 0.9420$ $p < 0.0001$ $n = 149$	$r = 0.9254$ $p < 0.0001$ $n = 148$
Chest circumference	$r = 0.9431$ $p < 0.0001$ $n = 153$	$r = 0.8721$ $p < 0.0001$ $n = 119$	$r = 0.9374$ $p < 0.0001$ $n = 71$	$r = 0.9352$ $p < 0.0001$ $n = 154$	$r = 0.9420$ $p < 0.0001$ $n = 149$	1.0000	$r = 0.9667$ $p < 0.0001$ $n = 153$
Waist circumference	$r = 0.9413$ $p < 0.0001$ $n = 152$	$r = 0.8405$ $p < 0.0001$ $n = 118$	$r = 0.9307$ $p < 0.0001$ $n = 70$	$r = 0.9102$ $p < 0.0001$ $n = 153$	$r = 0.9254$ $p < 0.0001$ $n = 148$	$r = 0.9667$ $p < 0.0001$ $n = 153$	1.0000

n is a variable as not all infants were measured multiple times.

D_3Cr muscle mass was strongly associated with total body magnetic resonance imaging; however, this was not feasible to serve as the comparator in critically ill neonates. D_3Cr muscle and longitudinal accrual of muscle mass was strongly associated with TBW and body weight and longitudinally with changes in each. Muscle mass comprised an average of 24–25% (±6% SD across tertiles) of body weight and remained constant while infants were cared for in the NICU (Table 2).

These results demonstrate a rapid, previously unreported, rate of accrual of muscle mass in neonates. Infants from gestational age of 26–40 weeks CGA increased muscle mass by an average of ~2%/day. The D_3Cr dilution method is sufficiently precise to measure small changes in muscle mass during 14 days of growth.

In some adults, an equivalent dose of D_3Cr results in a small increase of the oral dose in urine (spillage).[16] However, spillage of the ingested D_3Cr was not detectable in urine samples for most infants and minimal in others. This is, perhaps, an indication that the rate of production of endogenous hepatic and renal creatine is closely matched by active transport into the sarcomere, with little to no renal clearance. Isotopic steady state was achieved in these infants rapidly, 10 h after dosing and the urine D_3Crn enrichment remained stable between 10 and 70 h. We have also observed that consumption of food containing creatinine results in a short-term increase in urine creatinine levels that dilutes the enrichment of urine D_3Crn. For this reason, a fasting urine sample is required in adults.[5,16] However, we observed no such dilution from feeding, likely due to the very low amounts of creatinine found in breast milk and the frequent feeding of the infants. Thus, a single, spot urine sample may be obtained at any time between 10 and 70 h after the dose has been provided to the infant with no need for a fasting sample or use of a correction algorithm for urinary spillage. The lack of a requirement for the precise timing of the urine

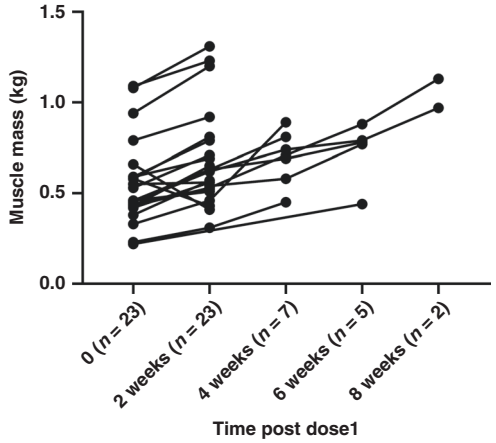

Fig. 2 Individual longitudinal data. While in the NICU, 17 infants were measured two times (0 and 14 days), 5 were measured two times (0, 14, and 28 days), and 2 were measured five times during an 8-week stay in NICU.

Table 4. Correlation matrix of longitudinal changes in body composition values.

Correlation matrix of change from 1st test	Body weight	Muscle mass	Total body water	Length
Body weight	1.0000	$r = 0.8857$ $p < 0.0001$ $n = 41$	$r = 0.9411$ $p < 0.0001$ $n = 11$	$r = 0.8152$ $p < 0.0001$ $n = 40$
Muscle mass	$r = 0.8857$ $p < 0.0001$ $n = 41$	1.0000	$r = 0.8110$ $p < 0.0001$ $n = 18$	$r = 0.8802$ $p < 0.0001$ $n = 76$
Total body water	$r = 0.9411$ $p < 0.0001$ $n = 11$	$r = 0.8110$ $p < 0.0001$ $n = 18$	1.0000	$r = 0.8632$ $p < 0.0001$ $n = 18$
Length	$r = 0.8152$ $p < 0.0001$ $n = 40$	$r = 0.8802$ $p < 0.0001$ $n = 76$	$r = 0.8632$ $p < 0.0001$ $n = 18$	1.0000

Differences in *n* reflects samples that were not obtained, or in the case of TBW, inadequate amount of saliva was obtained for analysis.

sample allows greater flexibility for the use of this method for researchers and clinicians.

TBW is frequently used to measure body composition in infants; however, in neonates, TBW is not a valid estimate of FFM. The formula used to convert TBW to FFM and FM in adults and children assumes a constant hydration of FFM such that $FM = Wt - k_1 \times TBW$, where $k_1 = 0.732 \pm 3\%$. However, Ellis[18] points out that for neonates, FFM hydration is not constant (k_1 varies from 0.9 to 0.77), decreases after birth, and declines throughout infancy. Our data confirm this observation and show that TBW as a percentage of body weight was lower for infants with a higher body mass and older than the infants between 1 and 3 kg. Using the TBW in the present study (excluding bone mass), body fat averaged 13% for the smallest infants and 28% for the infants in the highest tertile for body weight at the initial measurement confirming a previous observation.[19] Muscle mass was strongly associated with TBW cross-sectionally and changes in muscle mass vs. change in TBW was also strongly related. However, the relationship between TBW and muscle mass was not as strong while infants were cared for in the NICU ($r^2 = 0.444$), a reflection of the changing hydration status during this time period. The measurement of muscle mass by D_3Cr dilution is independent of hydration status. While TBW and D_3Cr dilution measure different aspects of body composition (muscle mass and FFM), their comparison provides novel information on the relative content of muscle mass and body water in growing neonates. Muscle mass comprised 27, 30, and 34% (by tertile of body weight) of TBW at the initial measurements. In older men, muscle mass is a highly variable component of LBM (40–60%).[20] The relatively small and variable amount of muscle mass in relation to TBW or LBM suggests that LBM is not a good surrogate measurement for muscle mass.

Approximately half of pre-term infants experience ex utero growth failure when compared to fetal growth, and there is a strong association between very pre-term infant growth failure and neurodevelopmental impairment[8,9] and between FFM accrual and improved neurodevelopmental outcome.[21,22] However, there is no consensus definition of "healthy" neonatal growth. In the present study, we observed a strong relationship between short-term (in the NICU) and long-term (up to 1 year after birth) changes in TBW and changes in muscle mass. Nonmuscle components of FFM consist of both intra- and extracellular water, viscera, and other components of body composition. Importantly, skeletal muscle is directly linked to the CNS through heavily myelinated motor nerves and the measurement of accretion of this component of FFM may better reflect central nervous system (CNS) and neurocognitive development in neonates. Head circumference is a poor measure of brain growth,[23] and while linear growth stunting is associated with neurodevelopmental impairment,[24] accurate length measurement is in practice challenging.[25] We observed strong cross-sectional relationships between anthropometric measures such as length, head and waist circumferences, and muscle mass. However, these relationships were not as strong as those observed between body weight or TBW and muscle mass (Fig. 3). In particular, the relationship between short-term changes in body length vs. muscle mass was not statistically significant ($r^2 = 0.112$, $p = 0.07$). This may reflect the small rate of change in length over 2–4 weeks and the relative inaccuracy common to this measurement.[25] The long-term changes in length were more strongly related ($r^2 = 0.668$, $p < 0.0001$) to changes in muscle mass.

Rapid increases in BMI in low birth weight pre-term infants may increase the risk of childhood obesity. Accrual of skeletal muscle requires an increase in the rate of muscle protein synthesis requiring a substantial amount of energy. Availability of energy and high-quality protein to a rapidly growing pre-term infant will affect the rate of accrual of muscle mass and any limitation may have both short- and long-term health consequences. Barker[26] has shown that low birth weight is strongly associated with increased risk adult chronic diseases, although the etiology of this association is not clear. He suggests that there may be different periods of sensitivity for development of specific tissue, including skeletal muscle. The rapid accrual seen in the present study points to muscle mass as potential indicators of nutritional and/or health status while in the NICU, and for the first time, this major component of FFM can be measured directly.

There are limitations to this study. The D_3Cr dilution method provides a measurement of creatine pool size. In adult humans and in rodents, the average concentration of creatine in muscle is 4.3 g/kg;[4] however, the concentration of creatine in the sarcomere of neonates has not been reported and the conversion of creatine pool size to kilogram of muscle mass may not be exact. However, since this is a monotonic transformation (from Cr pool size to muscle mass), the correlations with other measures (TBW, length) would be similar if the concentration of creatine in the skeletal muscle of infants is different than that of adults. The study population was not homogeneous, not all patients provided more than one measure, and outpatient measures were only possible on a small number of subjects.

The D_3Cr dilution method in neonates offers several potential advantages. In this study, it is safe, facile, allows assessment of a heretofore inaccessible component of FFM, highly correlated with

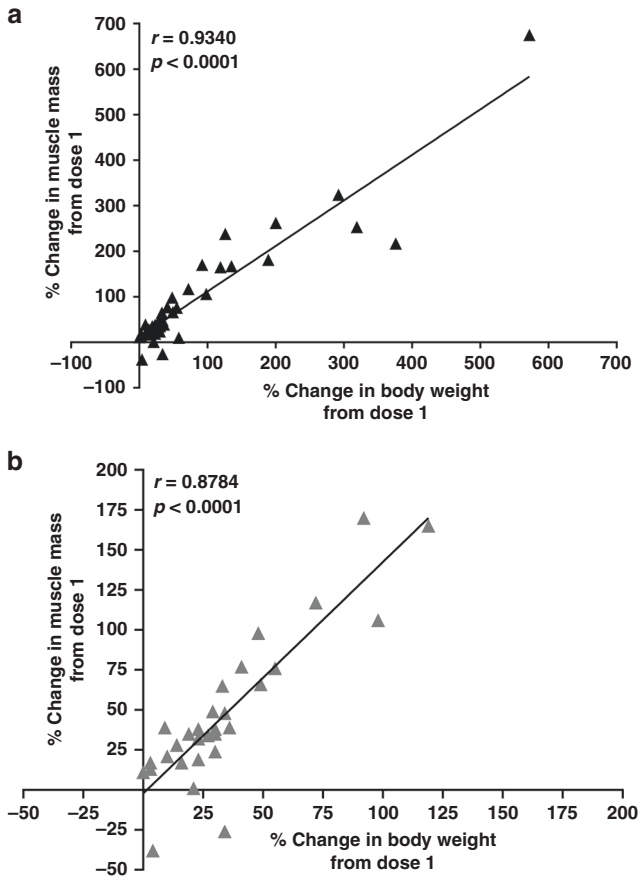

Fig. 3 Correlations between change in muscle mass vs. change in body weight. Correlation while in the NICU (**a**) and all time points including growth at home (**b**).

TBW, and its use is not limited to stable neonates. Obtaining a urine sample with a filter paper strip in a diaper is simple in clinical or field settings. Importantly, multiple repeat measurements can be made using this method. We were able to observe a consistent increase of 100–200 g of muscle during 14 days of growth, indicating that the method may provide a feasible monitor of effects of illness or nutritional status in infants.

There are currently no data on normal or abnormal rates of accrual of muscle mass in pre-term or full-term infants, which is the first step in the practical application of the D_3Cr dilution method. The influence of nutritional support, growth restriction, and acute and chronic disease on accrual of muscle mass with growth in infants is unknown; the availability of the D_3Cr dilution method to determine muscle mass and changes in muscle mass may offer a unique and new window into healthy neonatal growth and nutrition.

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AUTHOR CONTRIBUTIONS

W.J.E., M.H., and M.S. conceptualized and designed the study, interpreted the data, drafted the initial manuscript, and reviewed and revised the manuscript. B.S. and F.I. implemented the study, acquired the data, interpreted the data, and reviewed and revised the manuscript. E.N., K.G., and G.C. acquired the data, interpreted the analysis, and reviewed and revised the manuscript. All authors approved the final manuscript as submitted and agree to be accountable for all aspects of the work.

ADDITIONAL INFORMATION

Competing interests: W.J.E., M.H., and M.S. were employed by Kinemed, Inc. for a period of time during the conduct of this project. W.J.E. and M.H. are listed as co-inventors on the granted patents for the D_3-creatine dilution method, but do not own or derive any income from the intellectual property.

Consent statement: The study was approved by the Oregon Health & Sciences University Medical School and University of California San Francisco Benioff Children's Hospital at Oakland Institutional Review Boards. Parents provided consent for all infants enrolled in the study.

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