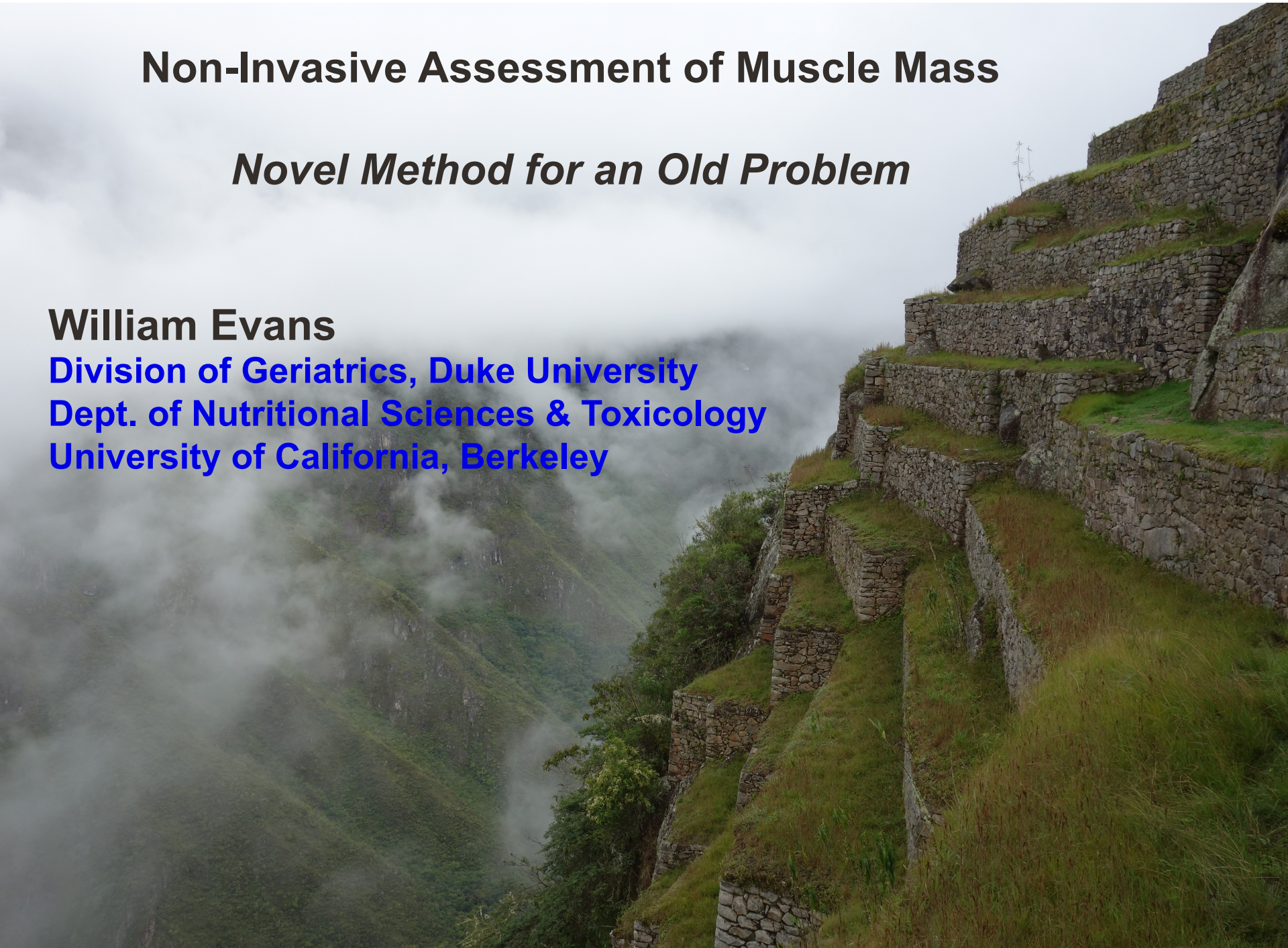
Non-Invasive Assessment of Muscle Mass

Novel Method for an Old Problem



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Editor's Note: This article was originally presented by Dr. Evans as the Keynote Address at the Workshop on Sarcopenia, which took place September 19–21, 1994 at the Airlie House Conference Center, Virginia.

What Is Sarcopenia?

William J. Evans

Age related loss of skeletal muscle mass

Evans, W What is Sarcopenia?, J. Gerontol., 50A: 5-8, 1995.
Evans, W Sarcopenia and age-related changes in body composition and functional capacity, J. Nutr., 123: 465-468, 1993.
Evans and Rosenberg, *Biomarkers*, Simon & Schuster, 1991

Cachexia: A new definition

William J. Evans*, John E. Morley [1], Josep Argilés [1], Connie Bales [1], Vickie Baracos [1], Denis Guttridge [1], Aminah Jatoi [1], Kamyar Kalantar-Zadeh [1], Herbert Lochs [1], Giovanni Mantovani [1], Daniel Marks [1], William E. Mitch [1], Maurizio Muscaritoli [1], Armine Najand [1], Piotr Ponikowski [1], Filippo Rossi Fanelli [1], Morrie Schambelan [1], Annemie Schols [1], Michael Schuster [1], David Thomas [1], Robert Wolfe [1], Stefan D. Anker [1]

"Cachexia is a complex metabolic syndrome associated with underlying illness and characterized by loss of muscle with or without loss of fat."

"Although malnutrition is often present in cachexia, the clinical characteristic of cachexia is that it cannot be successfully treated with nutrition alone."

Clin Nutr. 2008 Dec;27(6):793-9.

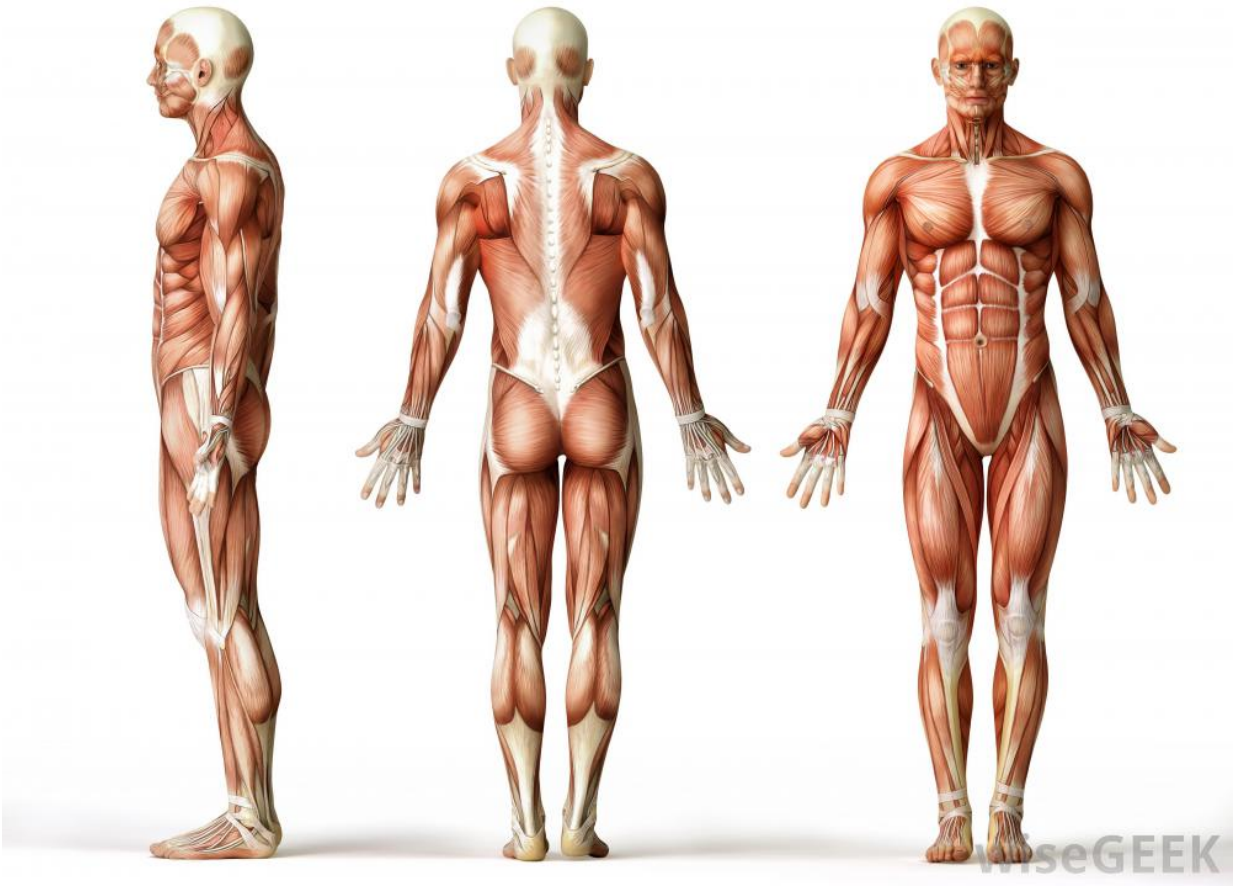
Muscle Wasting and Cancer

". . . so does the amazing weight loss that the (feeding) tube seems unable to combat. I have now lost almost a third of my body mass since the cancer was diagnosed: it may not kill me, but the atrophy of muscle makes it harder to take even the simple exercises without which I'll become more enfeebled still."

Christopher Hitchins

But . . .

How can we measure (only) skeletal muscle mass?



How to measure skeletal muscle mass

- Lean Body Mass  Skeletal Muscle Mass
  - Viscera, Heart, Bone, Blood
  - Assessment of Lean Body Mass provides an inaccurate measure of muscle
  - Changes in LBM (dehydration) may obscure important changes in muscle mass
  - Imaging of skeletal muscle: MRI, DEXA, CT – precise, inaccurate and expensive.
    - Muscle contains lipid and fibrosis (increases with advancing age). Changes in body water reduces accuracy
  - Anthropometrics
    - Skinfold, body circumference (mid-arm circumference)

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Total body skeletal muscle mass: estimation by creatine (*methyl*-d_3) dilution in humans

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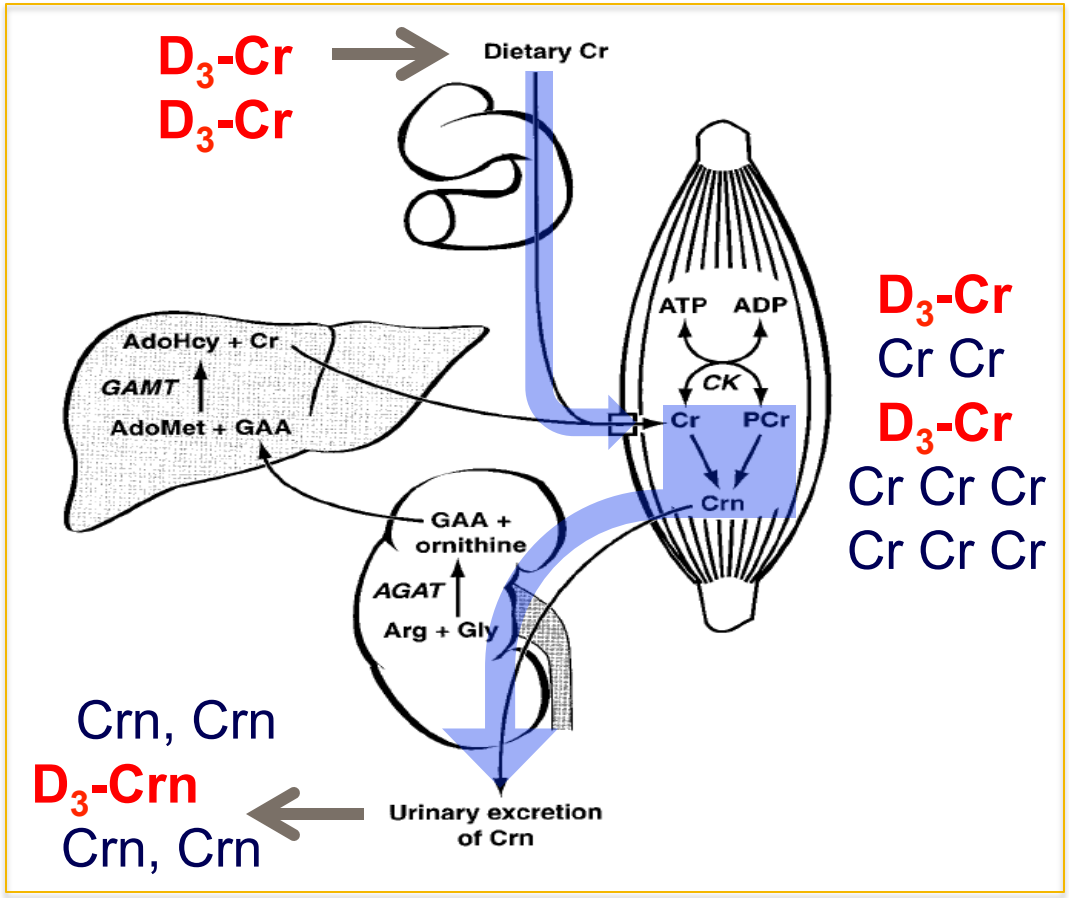
- **Current clinical tools to measure skeletal muscle mass are problematic**
  - DEXA measures LBM, MRI - specialized research, 24hr urine creatinine – unreliable
  - **NONE – directly measure skeletal muscle mass**
  - No method is currently approved for clinical assessment of muscle mass
- **Deuterated creatine:**
  - Non-radioactive, naturally occurring, stable isotope with deuterium
  - Cr is taken up from blood against a large concentration gradient (membrane-bound Cr transporter).
  - **Oral Cr is distributed to all skeletal muscles**

Total-body skeletal muscle mass by D_3-creatine dilution—a novel method

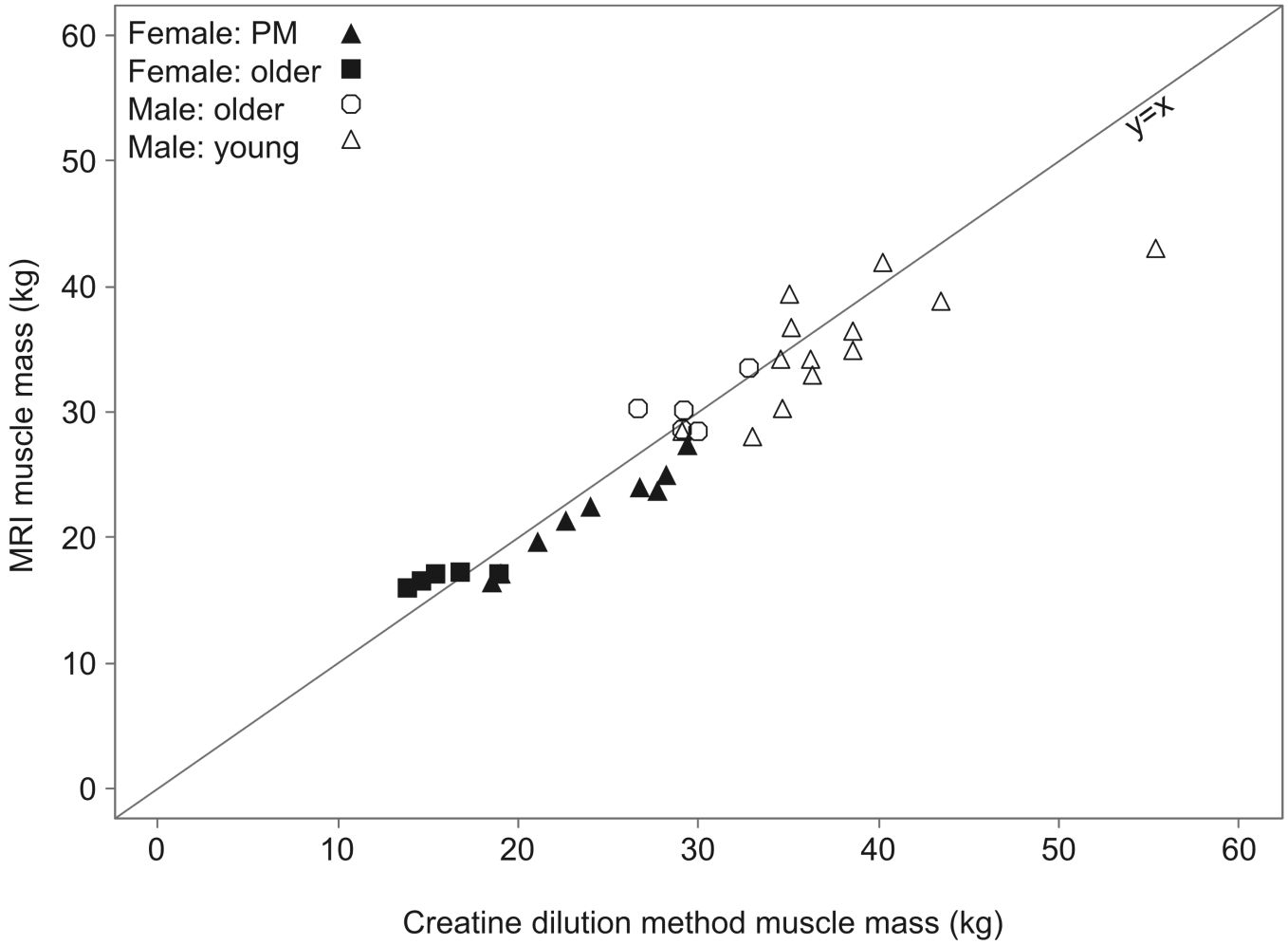
- Single oral tracer dose of D_3-creatine (dose range in humans 30-60mg)
- Absorption, distribution, active uptake and dilution in the skeletal muscle creatine pool
- Conversion to creatinine (~ 1.7% of total creatine per day; non-enzymatic reaction; irreversible *in vivo*)
- Single urine sample at isotopic steady state (2-4 days in humans)
- Analysis of D_3-creatinine enrichment (initially by IRMS) now by LCMS, ratio of D_3 creatinine/total creatinine (TTR)



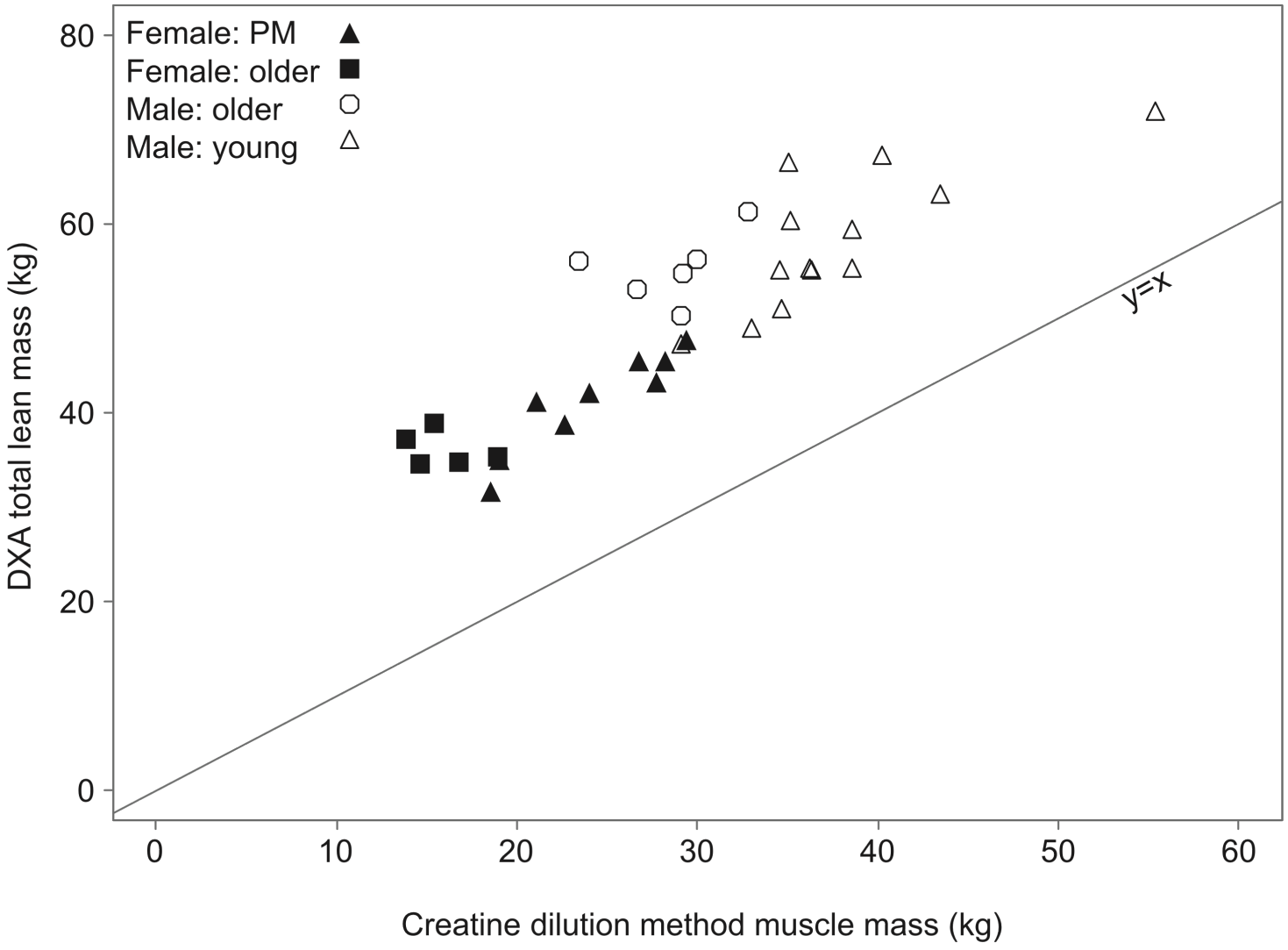
$$\text{Total-body creatine pool (mg)} = \frac{D_3\text{-crn dose (mg)} - D_3\text{-crn urine}}{\text{Enrichment ratio - TTR}}$$

$$\text{Muscle mass (g)} = \frac{\text{Total-body creatine pool (mg)}}{\text{Muscle creatine content (~4.3 mg/kg in humans)}}$$

Creatine dilution vs whole body MRI



Creatine dilution vs whole body DEXA



Children and Infants -

Extremely limited data on changes in body composition with growth in healthy or malnourished infants and children

- Tools for assessment of body composition are limited
- Skeletal Muscle: Highly variable and highly responsive to changes in nutritional status

Changes in muscle mass with growth and feeding provides an important indicator of nutritional status and health

Operational Details for muscle mass

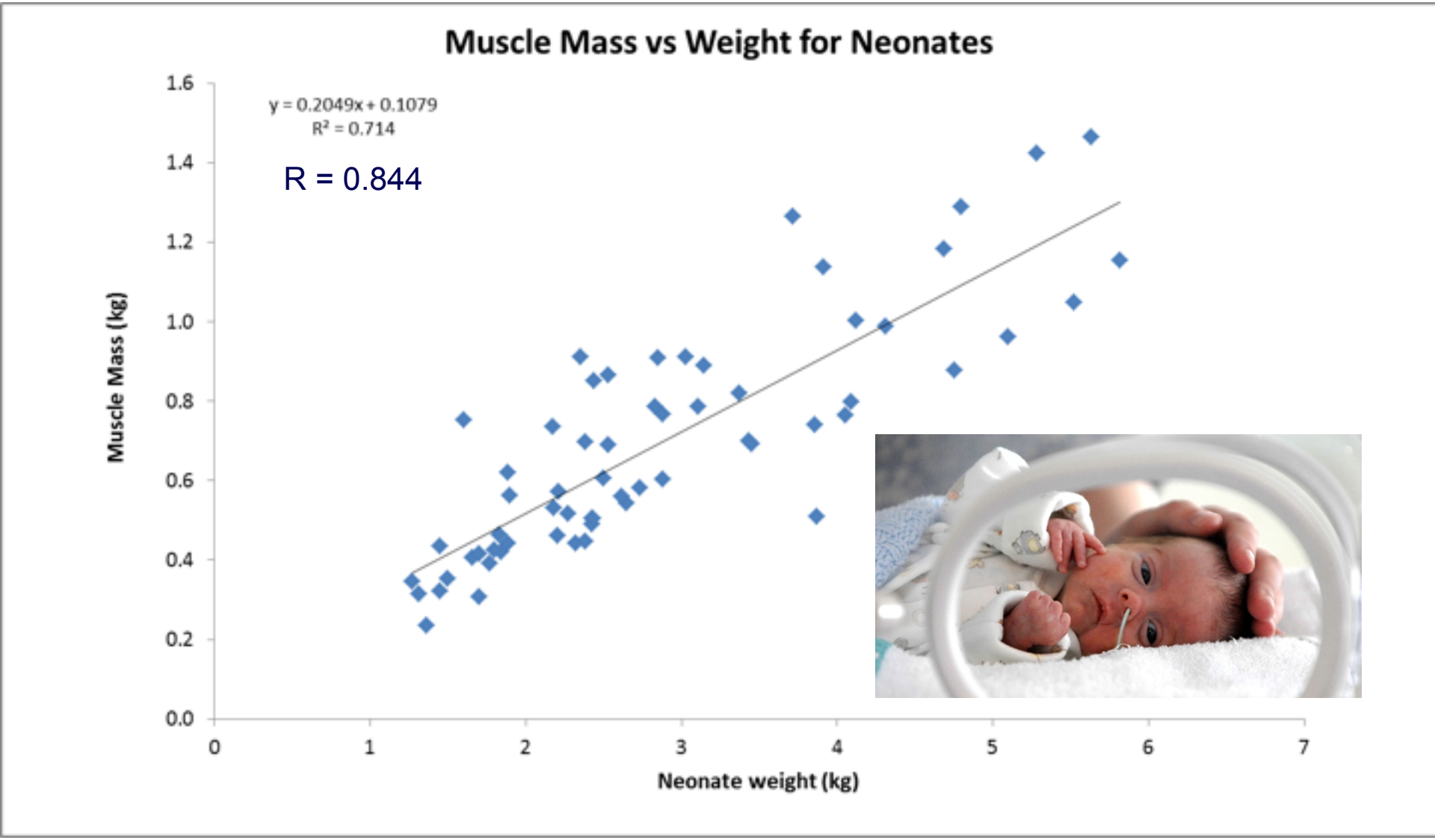
- Adults:
  - Single oral dose of 30 mg D_3-creatine
  - Single, spot, fasting urine sample – can be collected on a strip of filter paper.
- For naïve infants:
  - Single oral dose of 2mg D_3-creatine dissolved in ^2H_2O
  - No requirement for fasting at the time of dose
- For previously dosed subjects
  - Pre-fasting urine sample to correct for background D_3-creatine
- Sample: urine sample collected for 48 hours after dose
  - Sample is from filter paper in diaper and will be collected continuously for 48 hours (with each diaper change)
  - Analysis: enrichment of D_3-creatinine and creatine/creatinine ration (to correct for spillage of label)
- **Total body creatine pool size = muscle mass**
- **Dose of ^2H_2O to determine total body water as an estimate of lean body mass**
  - Saliva sample is collected 1 hour after dose

Subjects for validation study

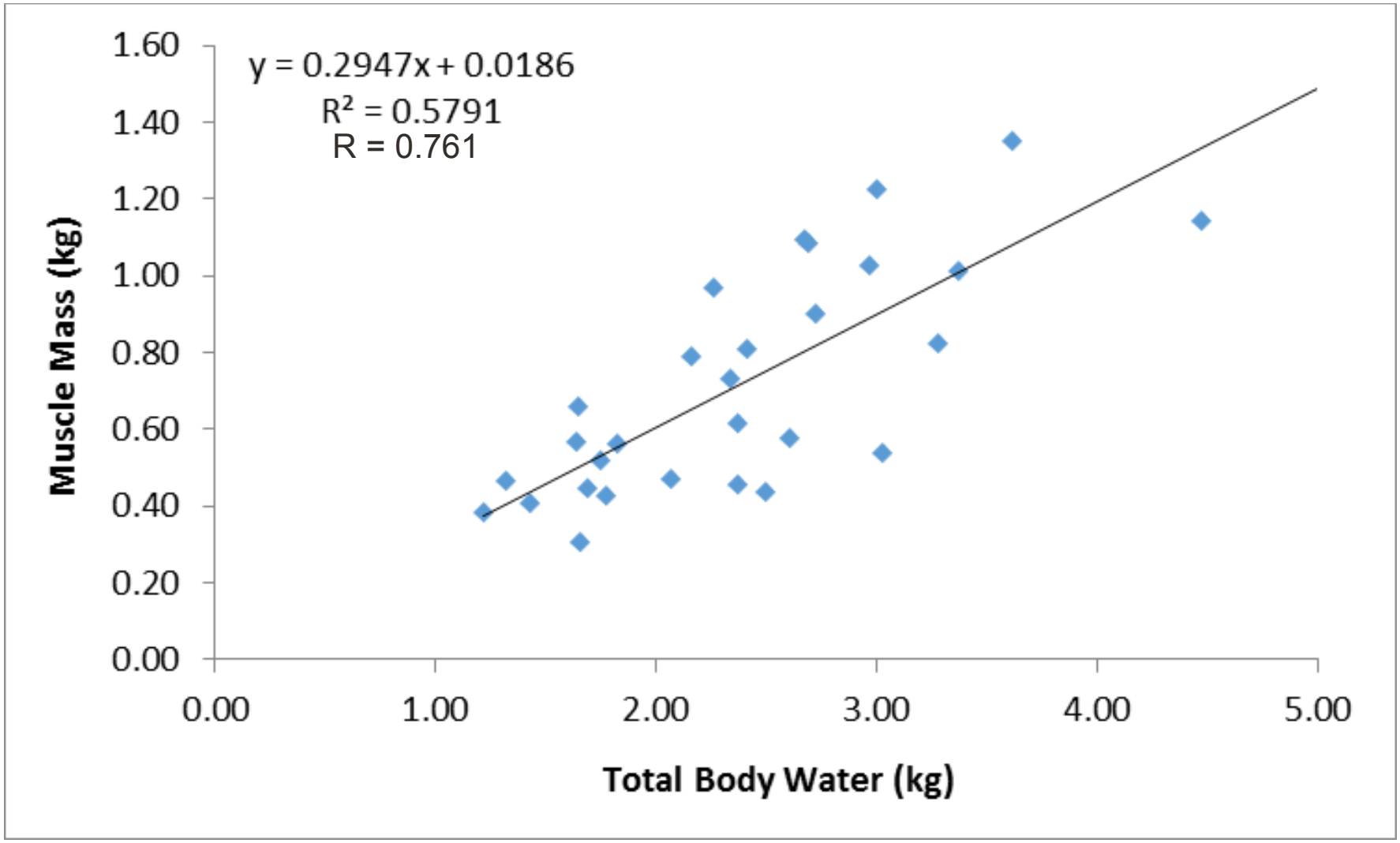
- 80 neonates stratified by gestational age
  - youngest gestational age: 27 weeks with repeat measure every 14 days during residence in the NICU
    - 27-29 wk (4-5 repeat measures)
    - 30-33 wk (3-4 repeat measures)
    - 34-37 wk (2-3 repeat measures)
    - 38-40 wk (0-2 repeat measures)
  - Repeat measures: 6 and 12 months after release from NICU
- 50 infant boys and girls (age 0 – 6 months)
  - All measured 0, 6, and 12 mo
- 50 children (5 – 11 yr)
  - All are measured 3 times: 0, 6, and 12 mo

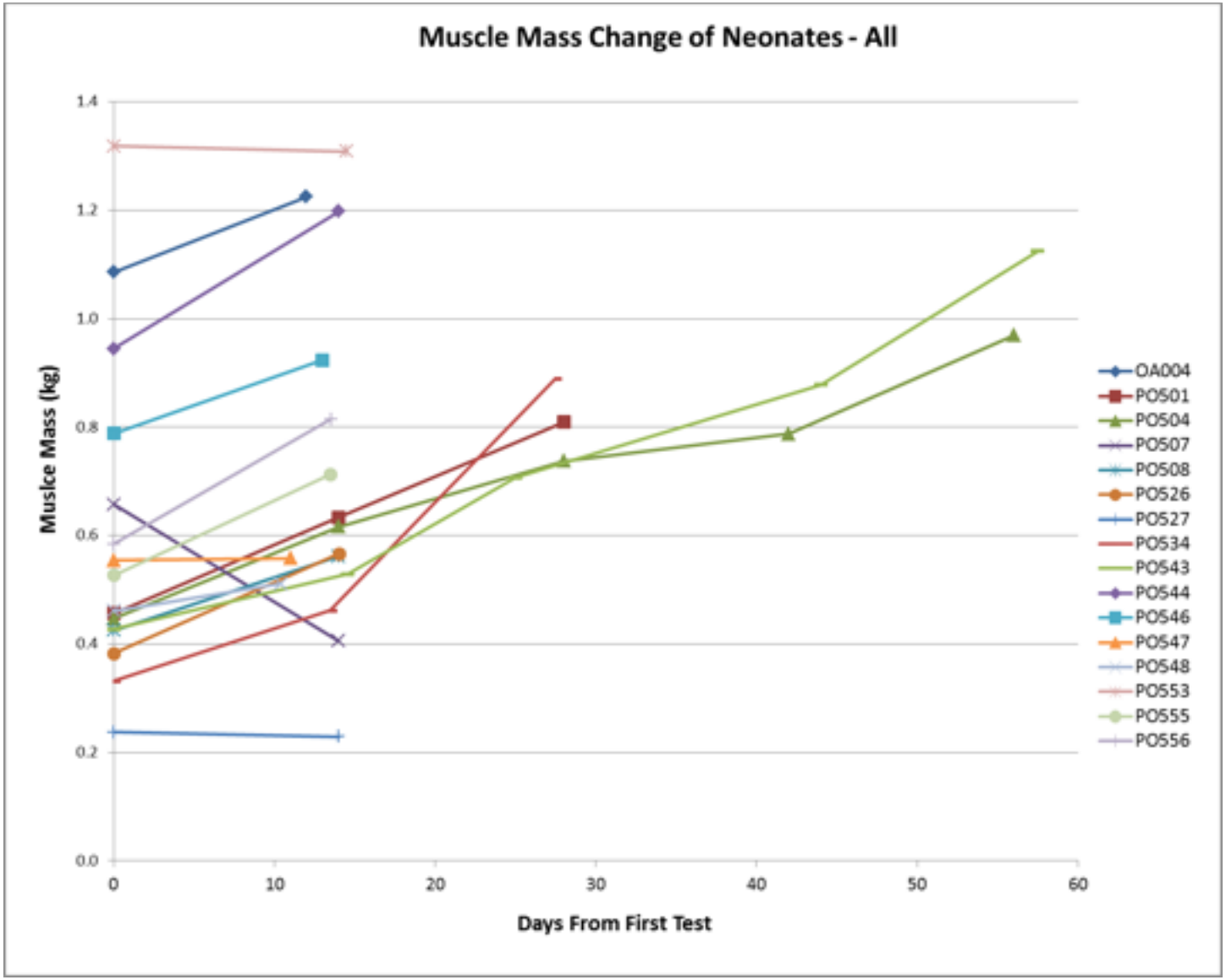
Funded by Bill & Melinda Gates Foundation

2 mg dissolved in 0.5 ml $^{2}H_{2}O$ delivered orally

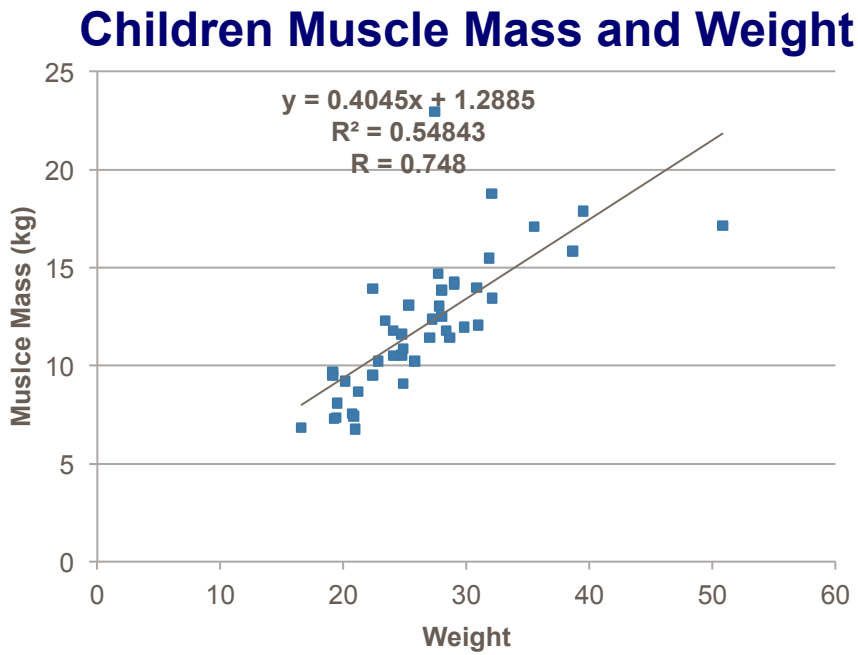


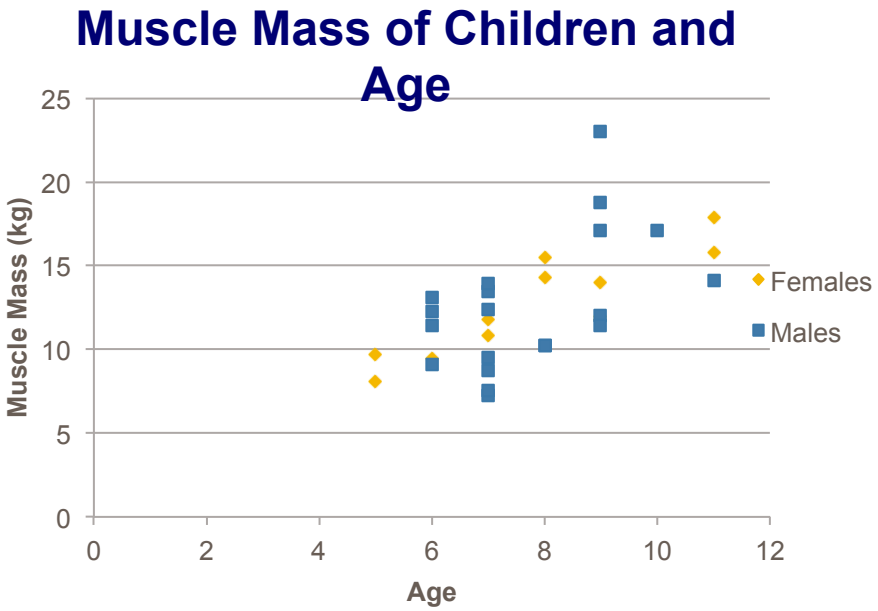
Muscle Mass vs. Total Body Water in Neonates

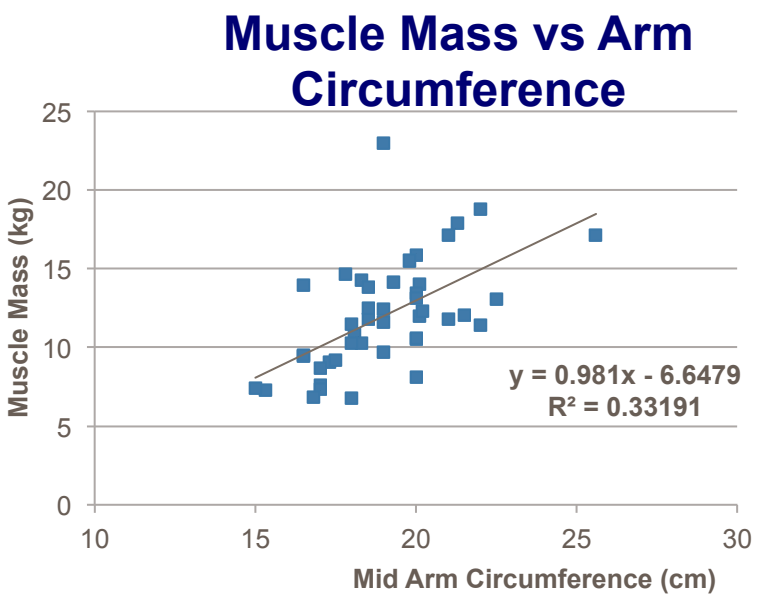



Muscle Mass Change of Neonates - All
Muslce Mass (kg)
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
10
20
30
40
50
60
Days From First Test
OA004
PO501
PO504
PO507
PO508
PO526
PO527
PO534
PO543
PO544
PO546
PO547
PO548
PO553
PO555
PO556

Muscle Mass and Children's Weight







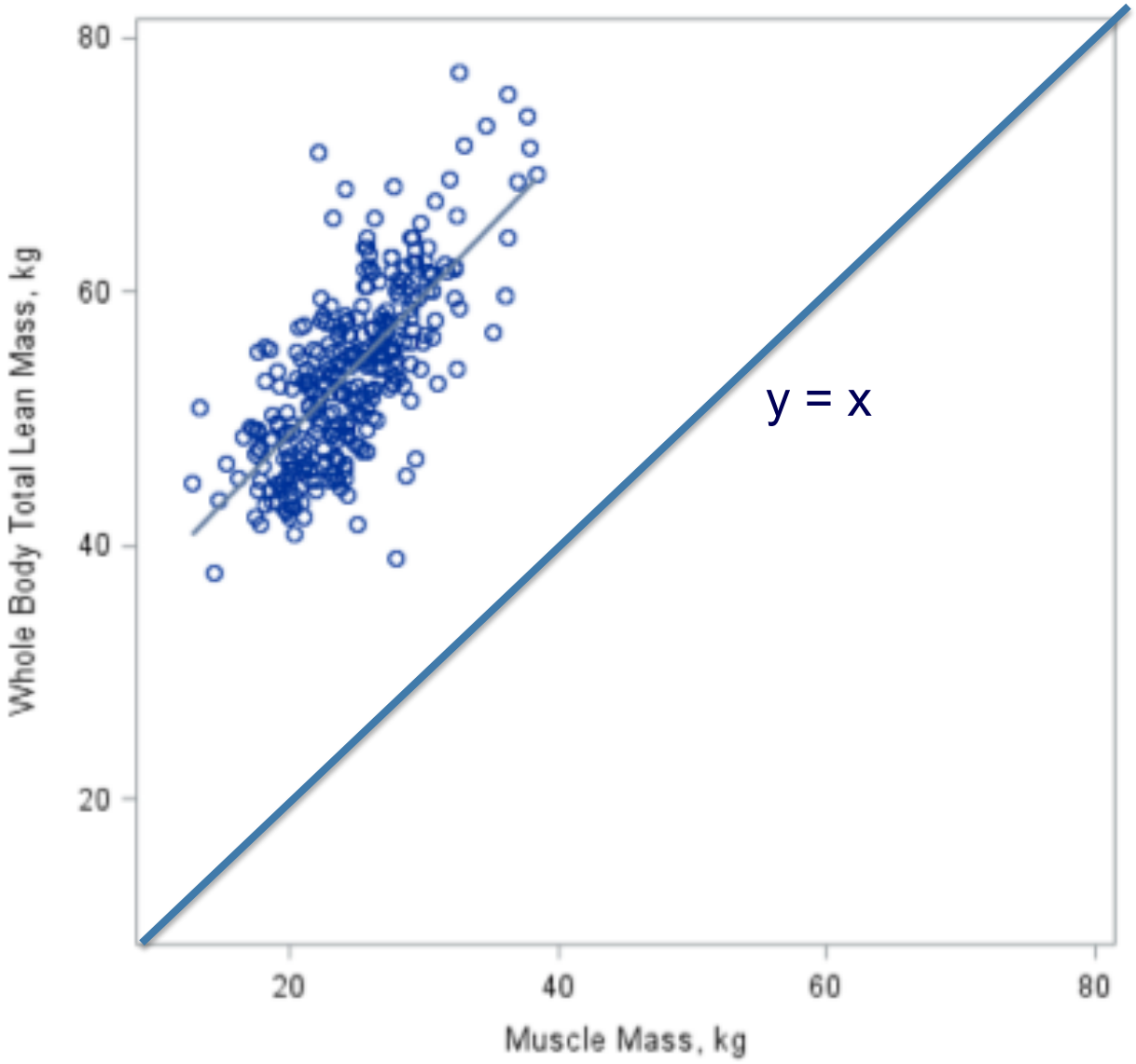
The D_3-creatine dilution method for assessment of total body skeletal muscle mass: implementation in the Osteoporotic Fractures in Men (MrOS) Study:

- Osteoporotic Fractures in Men (MrOS) study: 5994 men initially aged ≥65 years at 6 U.S. clinics at baseline (2000-2002)

- Purpose: To describe the successes and challenges of implementing the D_3-creatine dilution measure in a multi-site study of community-dwelling older men

- **Visit 4 measures include** (completion takes 3-5 hours):
  - D_3-creatine dilution method
  - Imaging: whole body, hip and spine dual energy x-ray absorptiometry (DXA); high resolution peripheral quantitative computed tomography (HRpQCT);
  - 5 day objective activity monitoring and physical performance (short physical performance battery; 400 m walk, force plate for lower extremity power)
  - Stool collection (for gut microbiome); serum and urine collection
  - Height; weight; questionnaires and interviews
- ~2200 participants anticipated for in-clinic measures (70% of survivors)

D_3-creatine vs total body lean mass by DEXA
N = 314, r = 0.71, p < 0.001 (DEXA overestimates muscle mass)



MrOs – D_3-creatine

–*Acceptance and usable samples*

- Thus far in Visit 4 recruitment, 84% of those with a clinic visit have a useable urine sample (1320 of 1568)
  - 134 men did not consent to measure (8.5%)
- **92% of those who consented to the measure have a useable urine sample (1320 of 1434)**
  - Among samples that were unusable, about half were unusable because of dosing problems, and half due to problems with urine collection
- Similar acceptance and usable data as for other complex measures at Visit 4
  - 80% have useable 5 day activity data; 71% have usable stool collection; 83% have useable HRpQCT data

	Can walk 2-3 blocks	Cannot walk 2-3 blocks	P-value
Muscle mass (kg)	24.7	23.3	0.04
ALM (kg)	22.5	22.7	0.74

	Muscle mass (d3-creatine)	Appendicular lean mass (DXA)
With grip strength	0.44*	0.41*
With gait speed	0.25*	0.08

*P < 0.001

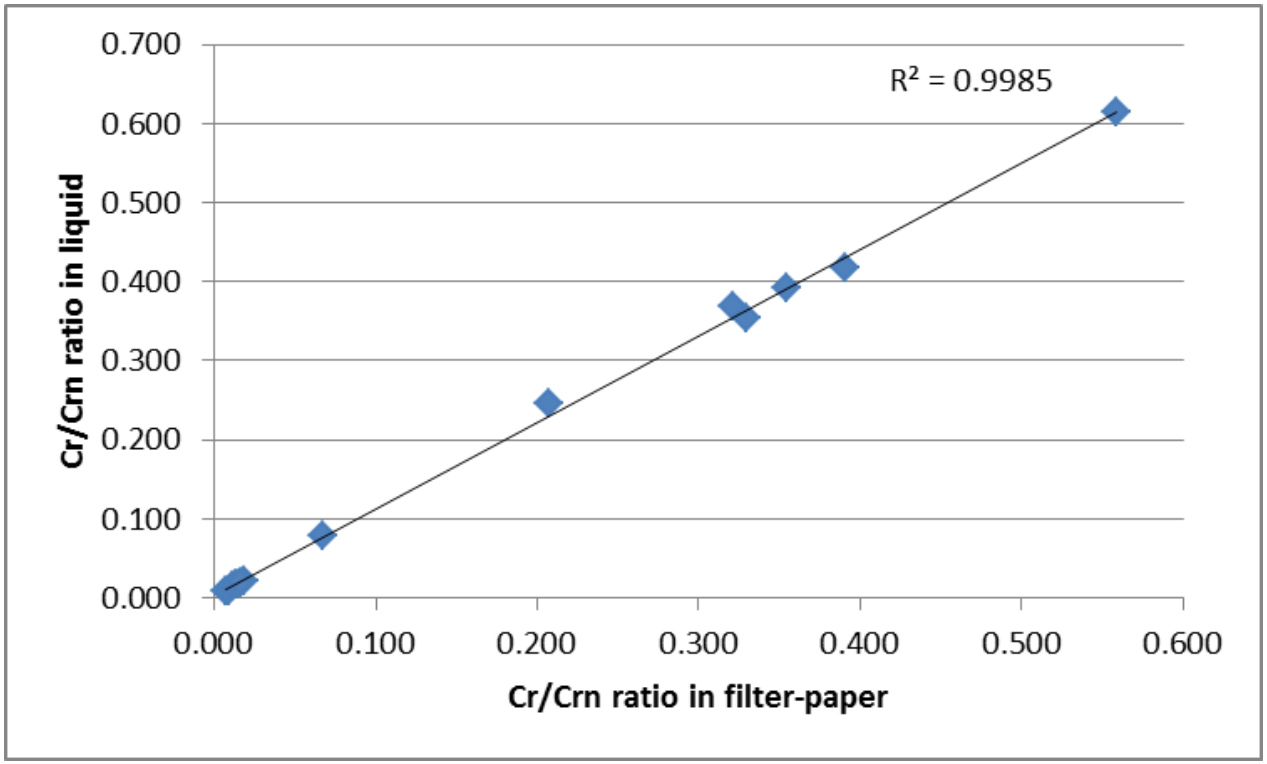
CONCLUSIONS

D_3-creatine dilution method can be successfully implemented in complex studies of very old community dwelling adults

Muscle mass by D_3-creatine dilution is correlated with DXA total body lean mass values, but not along line of identity. DXA lean mass is measuring more than just muscle, as expected, and modest relation was seen between these measures.

Associations between muscle mass by D_3-creatine and mobility appear to be stronger than associations between ALM and mobility. Does this explain equivocal associations between DXA lean mass and outcomes?

Sample may be collected on filter paper strip:



Conclusions

- **D_3creatine dilution method appears to be a valid measurement for muscle mass in adults, infants, and children**
- **Combined with dosing of ^2H_2O dosing, lean body mass and muscle mass can be measured non-invasively.**
  - Urine sample 2 – 3 days after dose for muscle mass.
  - Saliva sample collected one - two hours after dose.
- Accurate, non-invasive tool to assess body composition (FFM and skeletal muscle mass). Not reliant on renal function (determination of creatinine enrichment – not amount of creatinine in urine) – single spot urine and saliva
- Currently muscle mass by creatine dilution:
  - NIH funded longitudinal, observational study, MrOs: 1,200 men > 80 yr
  - NIH Japan: Changes in muscle mass in elderly men and women in Japan
  - Changes in muscle mass in children with Crohn's Disease (pending)
  - Dept. of Defense: Soldiers in extreme environments
- Incorporation of this method into your project:

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