Leiden University Medical Center

Muscle MR spectroscopy in neuromuscular disease

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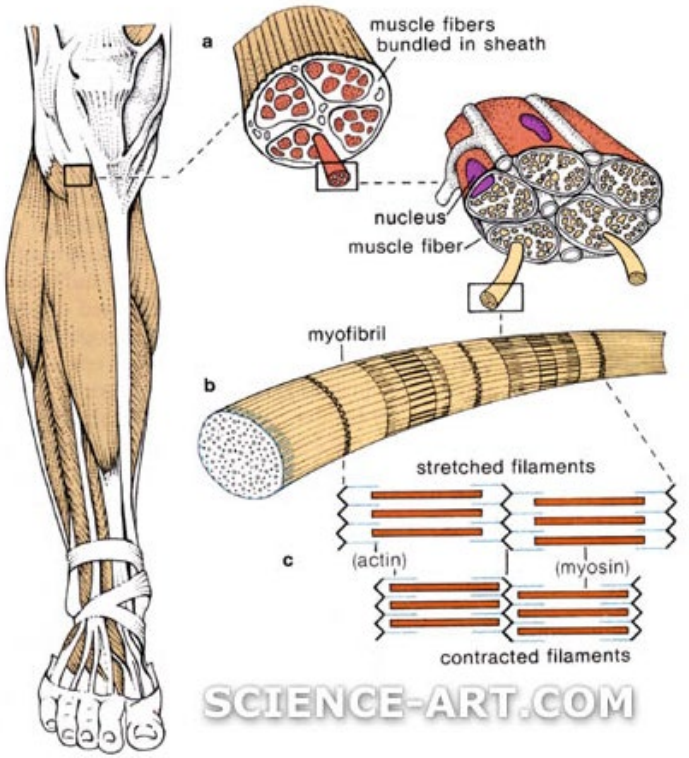
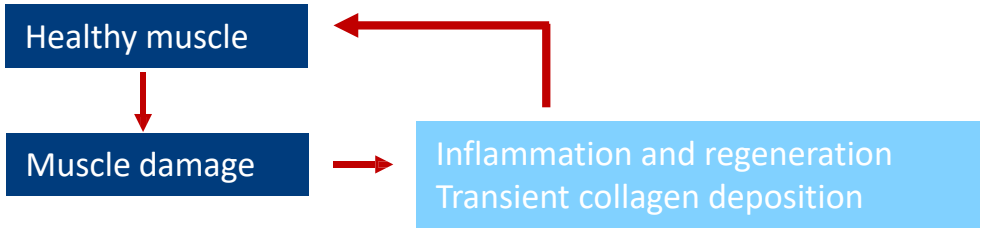
Background neuromuscular disease

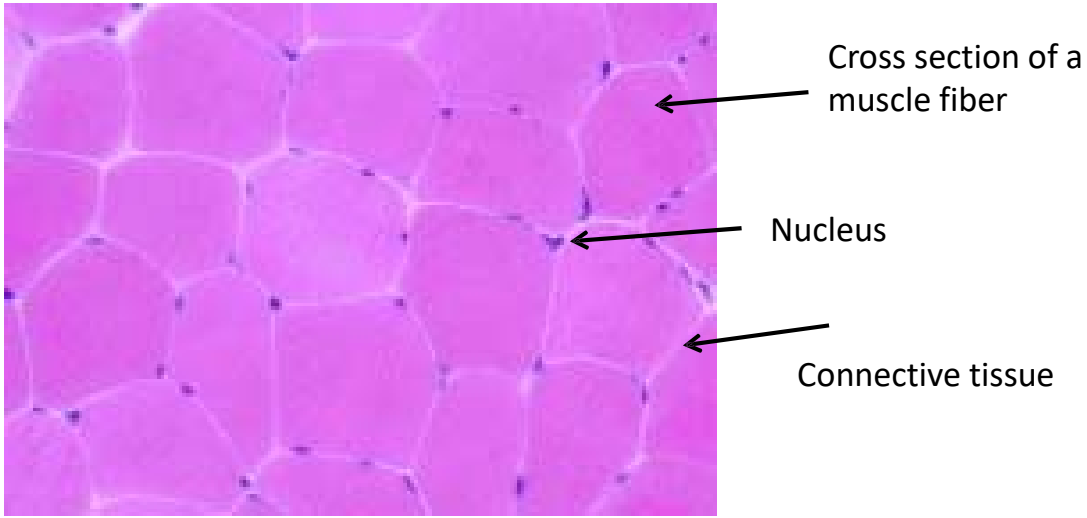
- Clinical symptoms: muscle weakness, stiffness or hypotonia, contractures
- Many different forms: episodic, slowly progressive, fast progressive
- Differential involvement pattern
- Individually rare, common as a group
- Hardly any approved drugs
- Many clinical trials in development



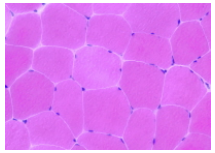
Skeletal muscle damage

- Healthy muscle
- Muscle damage
- Edema/inflammation





Disease cascade



Healthy muscle

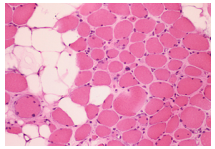
Healthy muscle

Muscle damage

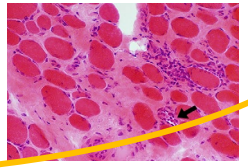
Inflammation and regeneration
Transient collagen deposition

Muscle disease: persistent inflammation

Fibrosis
Fatty replacement
Changes in fiber size



Fatty infiltration
Changes in fiber size



Fibrosis
Inflammation

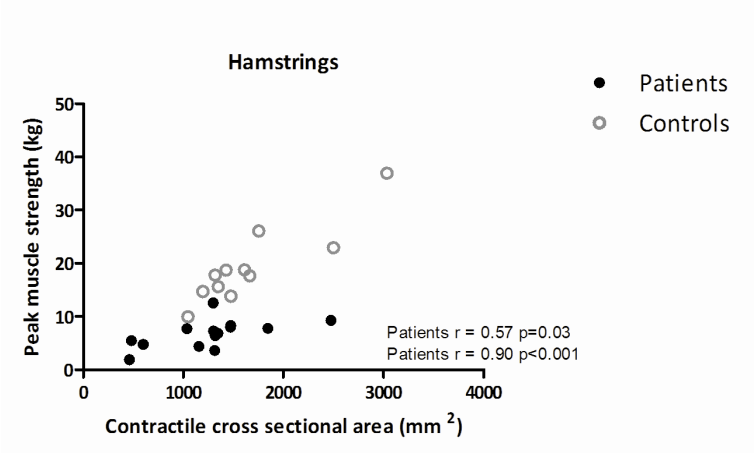
Metabolism

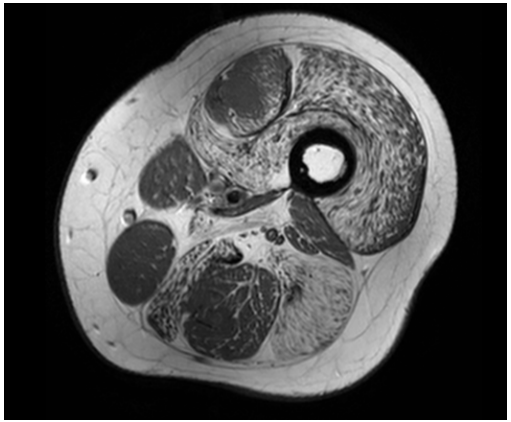
Less muscle recovery

LOSS OF MUSCLE FIBERS AND FUNCTION

There is more to muscle than meets the eye

1. Is metabolism different?
2. Is normal appearing muscle tissue really normal?
3. And what happens during exercise?

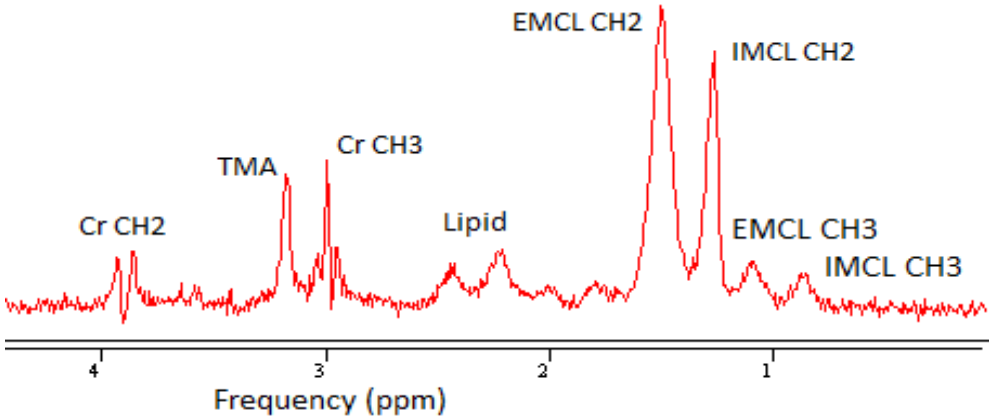




Wokke et al, Neuromusc Disord 2014

Lower abundant metabolite MRS

- Creatine, intra and extra myocellular lipids, carnitine, acetylcarnitine, carnosine
- Challenging in fat replaced muscle
- Conflicting results on decreases in TMA and creatine - age effect?
- Carnosine to asses intracellular pH



Lott et al Neuromusc Disord 2014

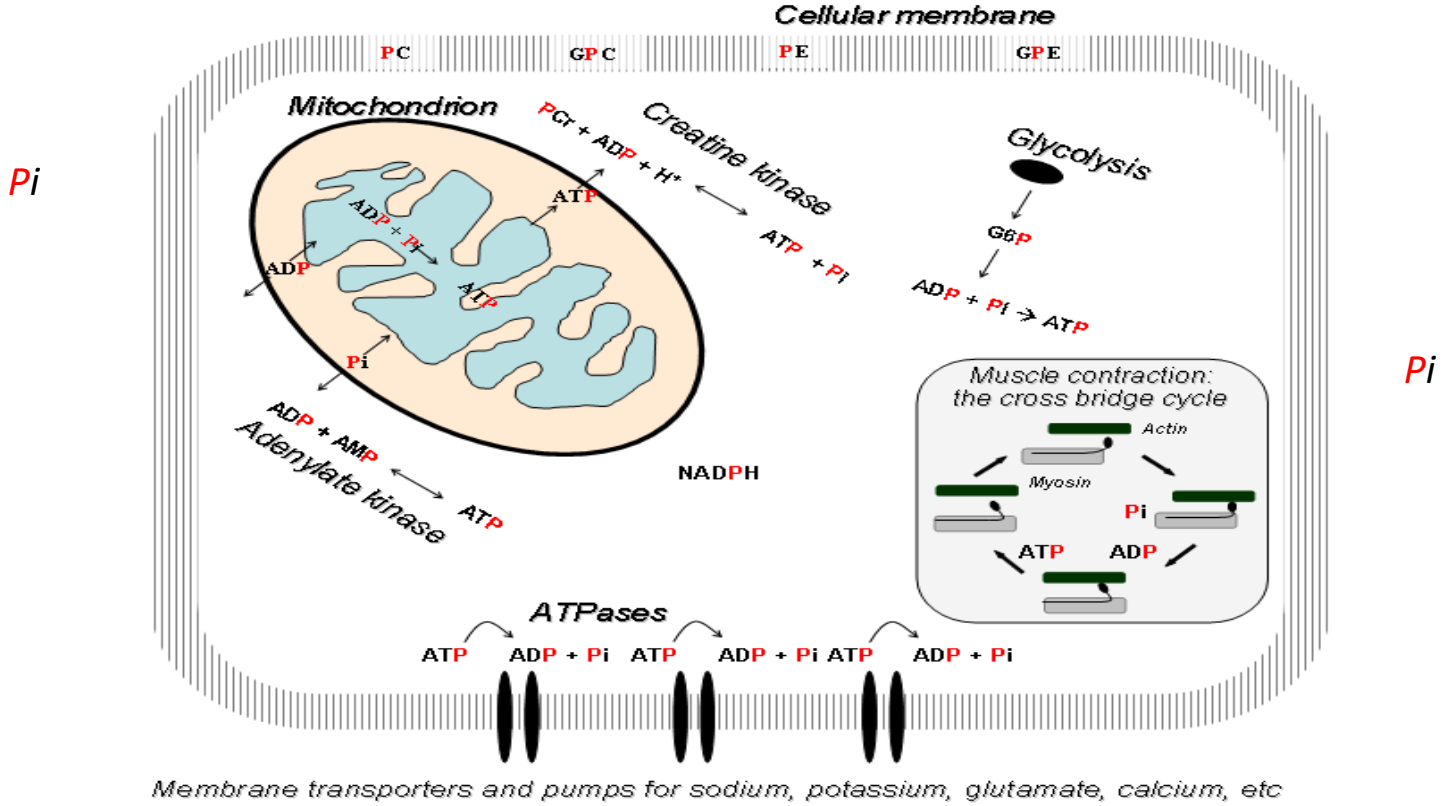
Hsieh et al J Comput Assist Tomogr. 2009

Hsieh et al J Pediatr 2007

Tarnopolsky, Neurology 2004

Reyngoudt et al, NMR in Biomed 2017

^{31}P in skeletal muscle energy metabolism

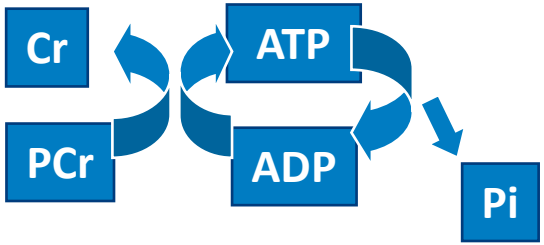


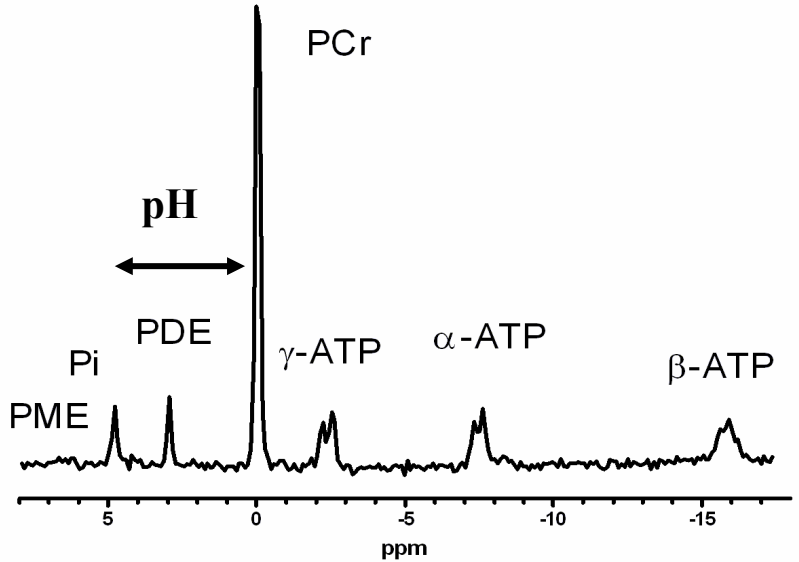
Pi

Pi

Metabolites

PCr – phosphocreatine – energy storage
Pi – inorganic phosphate - pH
PME – phosphomonoesters - glycolysis
PDE – phosphodiesters
ATP – adenosine triphosphate – mg^{2+}
ADP – adenosine diphosphate
pH - tissue pH





BUT: Sensitivity much lower compared to proton

^{31}P MRS in NMD

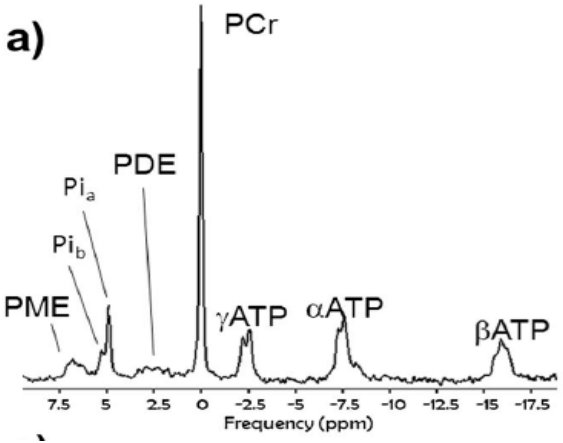
- Applied often, unspecific findings of disturbed metabolism:
  - pH increase: BMD, DMD, myopathies, GSDIII, not in myotonic dystrophy
  - PCr decrease: BMD, DMD, myotonic dystrophy, inflammatory myopathies
  - Pi increase: DMD, BMD, dysferlinopathy, not in FSHD
  - PDE increase: BMD, DMD, dysferlinopathy
  - PME increase: GSDIII

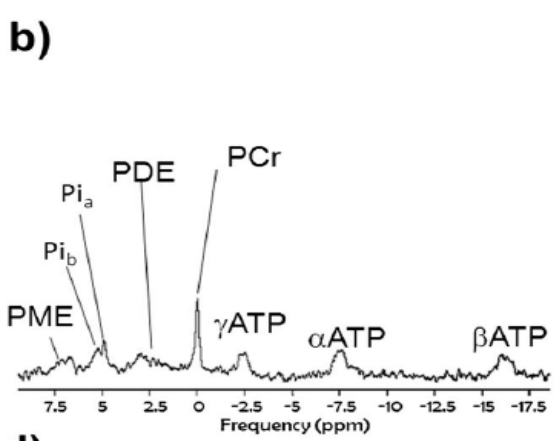
Relatively recent work:

- *Banerjee et al, Magn Res Med 2010: DMD*
- *Tosetti et al, Muscle & Nerve 2011: DMD*
- *Wary et al, NMR in Biomed 2015: DMD*
- *Hogrel et al, Neurology 2016: DMD*
- *Reyngoudt et al, NMR in Biomed 2017: DMD*
- *Hooijmans et al, NMR in Biomed 2016: DMD*
- *Wokke et al, NMR in Biomed 2014: BMD*
- *Torriani et al, Skelet Radiology 2011: BMD*
- *Kan et al, NMR in Biomed 2010: FSHD*
- *Janssen et al, PLosOne 2014: FSHD*
- *Wary et al, Neuromusc Disord 2010: GSD III*
- *Azzabou et al, poster 50: dysferlinopathy*

Alkaline Pi signal in DMD

- ^{31}P MRS in the forearms of DMD boys
- Presence of an alkaline Pi signal: extracellular Pi?
- Increased alkaline Pi signal in non-ambulant boys

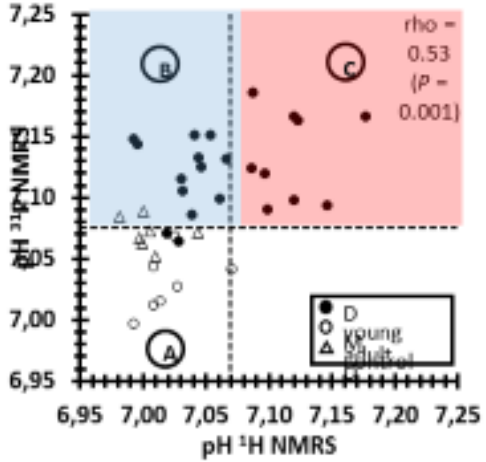




Wary et al, Magma 2012
Wary et al, NMR in Biomed 2015

Combination of ^{1}H MRS and ^{31}P MRS

^{1}H NMR of carnosine combined with ^{31}P NMRS to better characterize skeletal muscle pH dysregulation in Duchenne muscular dystrophy



^{1}H NMRS-based pH determination showed:

- **A majority of dystrophic muscles** displaying an **alkaline pH** in **agreement with the ^{31}P NMRS pH ➔ dystrophic myocytes (C)**
- **In some cases,** dystrophic muscles with a **neutral intracellular pH** indicating that the **alkaline ^{31}P NMRS pH** can arise **from an expanded interstitial volume fraction,** possibly related to **fibrosis (B)**
- As expected, an **alkaline intracellular pH is never associated with a neutral ^{31}P NMRS pH**
- **Excellent agreement with ^{31}P NMRS pH in control subjects (A)**

CONCLUSION:

Because ^{1}H NMRS-derived pH based on carnosine probes the functional impact of lack of dystrophin on DMD myocyte homeostasis, it might be a potential biomarker of successful therapeutic expression of dystrophin that would be able to normalize sarcolemma permeability

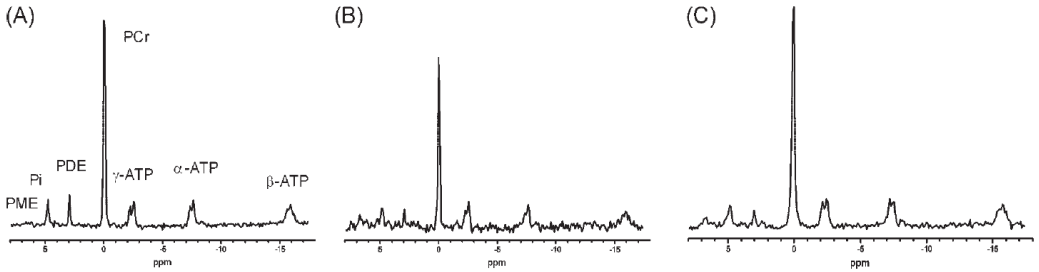
Reyngoudt et al, NMR in Biomed 2017

Slide courtesy of Harmen Reyngoudt, IoM Paris

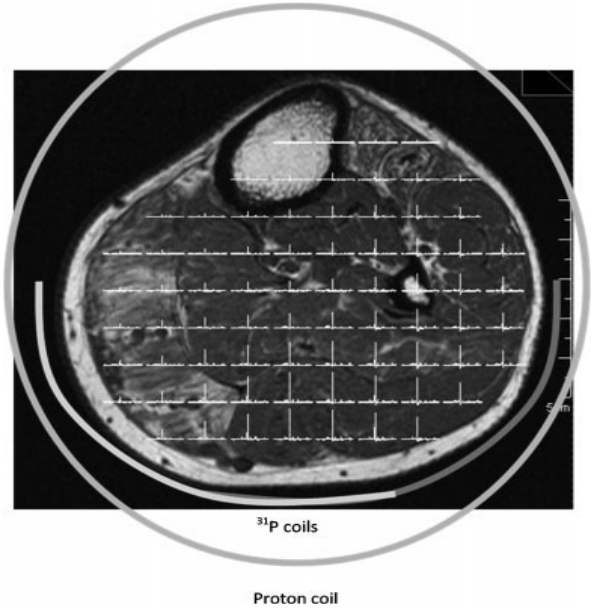
Changes in normal appearing muscle tissue

- Surface coil: sensitivity depends on the shape of the coil and drops further from the coil
- Mixture of tissue types and inter-individual variability
- Group muscles based on their fat percentage

Healthy | Fat replaced FSHD | Non-fat replaced FSHD

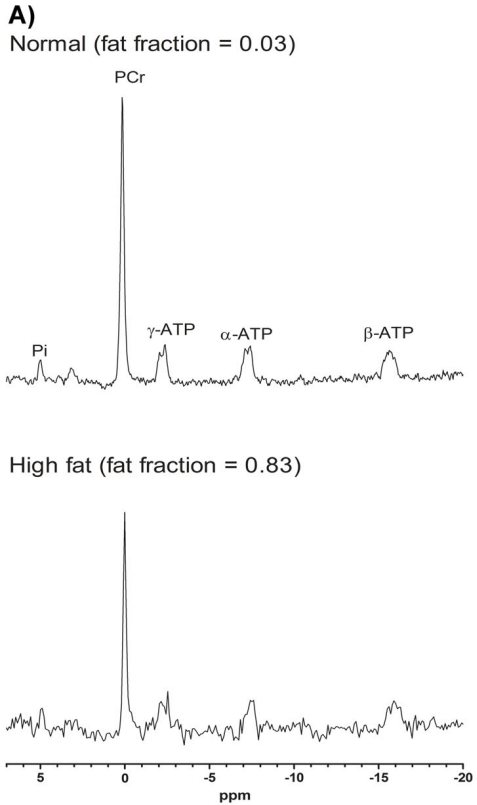


Kan et al, NMR in Biomed, 2010



FSHD

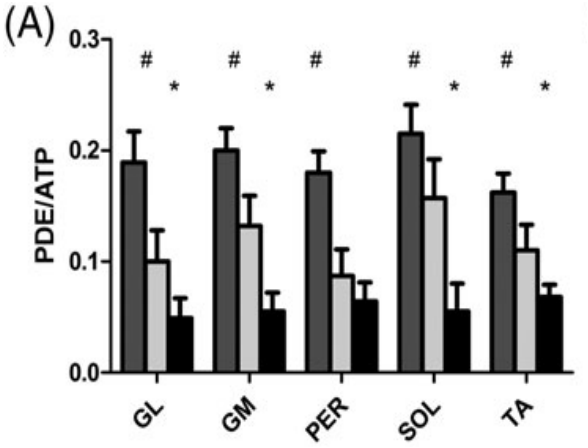
- Increases in tissue pH only in fat replaced muscles[1]
- PCr decreases more in fat replaced muscles[2]



[1]*Kan et al, NMR in Biomed, 2010*
[2]*Janssen et al, PLOS One, 2014*

Often changes stronger in fat replaced muscles

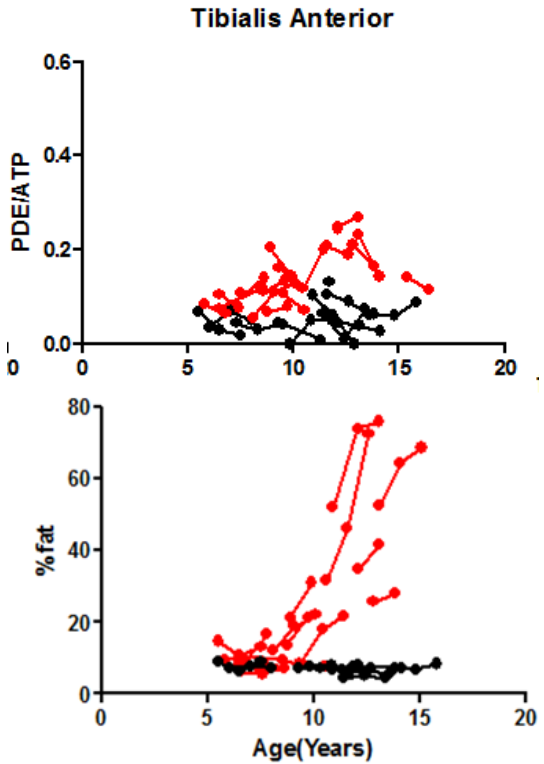
- BMD and DMD: Higher PDE[1,2], especially in muscles which are replaced by fat
- Dysferlinopathy:
  - Higher PDE/ATP and Pi/PCr
  - Splitting of Pi
  - Higher alkaline Pi peak in more fat replaced muscle



[1]*Tosetti et al, Muscle and Nerve 2011*
[2]*Wokke et al, NMR in Biomed, 2014*
Hooijmans et al, NMR in Biomed 2017

Phosphorous changes are slower than fat

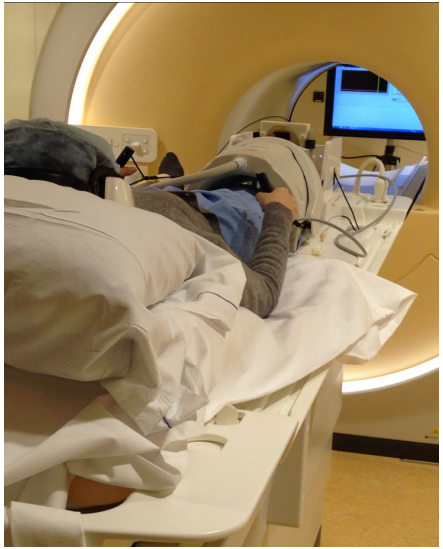
- No changes in PCr or PDE over 1 year
- PDE does not change over 2 years in fat replaced muscle

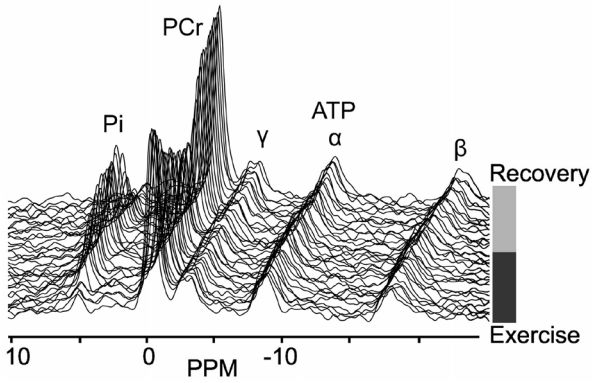


Hooijmans et al, PLosOne 2017

Dynamic measurements

- Many studies in last century in different NMDs
- Usually no reduction in PCr recovery rate
- Differences in acidification or other characteristics
- Important where to assess the muscle

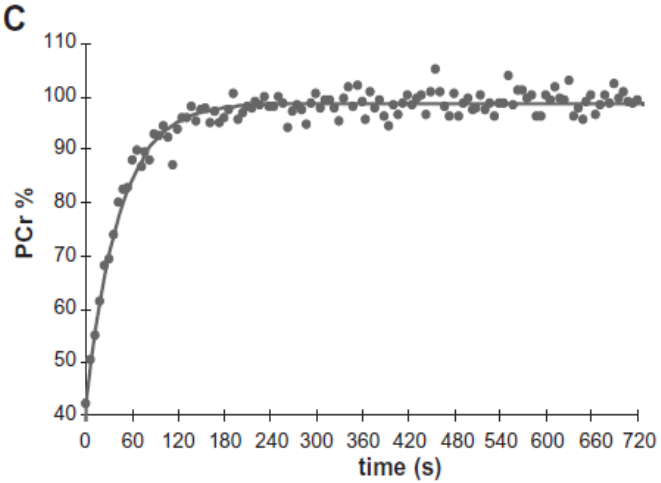


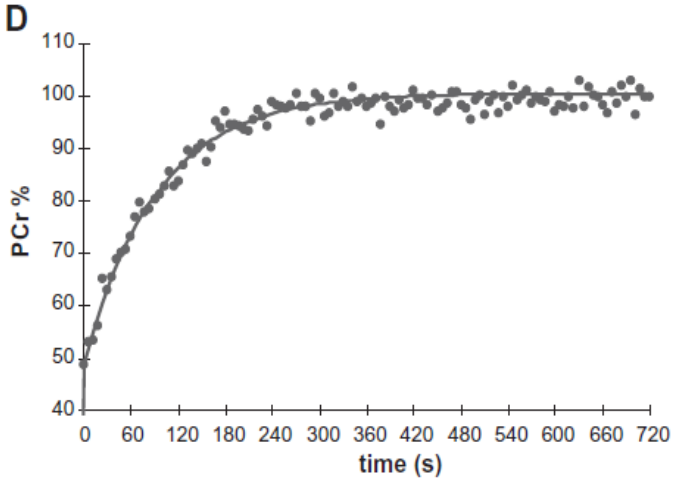


Kemp et al, Journal of Neur Sci 1993
Lodi et al, Brain 1999
Lodi et al, neuromuscular disord 1997
Tosetti et al, Muscle & Nerve 2011

GSD III

- Slower PCr recovery: partly due to impaired perfusion
- Higher PME





Wary et al, Neuromuscular disorders 2010

Summary

- Energy metabolism is different in several NMDs
- MRS can give information beyond structure
  - Possible marker for muscle tissue changes?
  - Changes are slower compared to fat
- Measurements during exercise relatively scarce

Leiden University Medical Center

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