Use of Skeletal Muscle Ultrasound to Measure Skeletal Muscle Glycogen

2015 Rocky Mountain Chapter- American College of Sports Medicine

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MuscleSound, LLC; US Patents 8,512,247, 8,517,942 and additional patents pending
Objectives

- Describe the importance of skeletal muscle glycogen content in sports
- Discuss a new application of ultrasound which can quantify muscle glycogen content and the results of recent validation study.
- Discuss its applications to individualize nutrition, training workloads as well as the prevention of overtraining and skeletal muscle injuries.
What is Glycogen?

- Glycogen is the storage form of glucose.
- Glucose is the most important form of carbohydrate used by humans, and is an essential fuel source for the body.
- Fat and glucose are the predominant fuels for humans.
- During exercise and competition, glucose is the most important fuel for the muscles.
What is Glycogen?

• Fat storage is unlimited….glycogen storage is VERY LIMITED!

• Glycogen is stored in muscles (~85%) and liver (~15%).

• Glycogen storage capacity in muscles and liver is limited to ~400-500g (~1Lb)

• Maintaining adequate Glycogen content is crucial, especially in athletic performance.
Glycogen is the storage form of glucose in liver and skeletal muscle.
Importance of Glycogen Storage

During exercise, skeletal muscle uses glucose delivered from the liver as well as from the glycogen storage in muscle.
Glycogen Depletion During Exercise (Muscle Biopsy)

Before
Optimal Levels

After
Glycogen Depletion
Glycogen Will Be Utilized During Exercise Very Rapidly!

As soon as 80 minutes!

Multiple scientific studies show the direct relationship between glycogen levels and performance

Glycogen and Performance

The relationship of muscle glycogen content, work time and dietary carbohydrate intake (adapted from Borgström et al. 1967).

Glycogen Intake and Performance

The graph shows the muscle glycogen concentration (in mmol/kg body weight) over time (in hours) for individuals on high carbohydrate and low carbohydrate diets, as well as during training periods. The graph indicates that muscle glycogen levels decrease over time, particularly during the early stages of exercise or training. The high carbohydrate diet helps maintain higher glycogen levels compared to the low carbohydrate diet.
The Importance of CHO and Glycogen Storage

Muscle with plenty of glycogen

Muscle Glycogen depleted
The Importance of CHO and Glycogen Storage

During High Exercise intensities (Competition, hard training)

Muscle Protein (BCAA’s)

Muscle Glycogen depleted

glycogen

FAT
The Importance of CHO and Glycogen Storage

Catabolism

Muscle Glycogen depleted
Muscle Damage

Low glycogen levels during hard training and competition will not only decrease performance but will also cause muscle damage!!!
Muscle Damage

Damaged muscle impairs proper muscle glycogen storage capacity!!... This may create a vicious cycle and lead to overtraining!.


- Z-Lines
- Sarcomere

Figure 4.11  (a) An electron micrograph showing the normal arrangement of the actin and myosin filaments and Z disk configuration in the muscle of a runner before a marathon race. (b) A muscle sample taken immediately after a marathon race shows Z disk streaming caused by the eccentric actions of running. Reprinted from Hagerman et al. (1984).
To maintain glycogen storages it is recommended to have a daily intake of CHO of 6-9g/CHO/Kg.

However...Glycogen content can vary among individuals.
INDIRECT ASSESSMENT OF GLYCOGEN STATUS IN COMPETITIVE ATHLETES

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INTRODUCTION: Proper glycogen storage is of great importance for athletic performance. Multiple studies show the positive correlation between glycogen storage and performance. Nevertheless, glycogen assessment is difficult to determine due to the invasive and impractical nature of muscle biopsies. Therefore, it is difficult to identify suboptimal glycogen levels in athletes. Throughout the measurements of maximal blood lactate levels ([La]ₘₐₓ) and maximal carbohydrate oxidation rates (CHOox max) it could be possible to indirectly estimate muscle glycogen status in competitive athletes and identify suboptimal glycogen levels. The purpose of this study was to assess indirectly muscle glycogen status through measurement of [La]ₘₐₓ and CHOox max.

METHODS: 82 competitive men (28 professionals and 54 non-professionals) and 17 competitive carried out a bicycle ergometer test, starting at 2 W·kg⁻¹ with increments of 0.5 W·kg⁻¹ until exhaustion, the duration of three first steps was 5 min, and then 10 min. Oxygen uptake (VO₂) and carbon dioxide (CO₂) were measured (ParvoMedics TrueOne 2400, Sandy, UT) throughout the test and blood lactate concentration ([La]ₘₐₓ) (YSI 1500, Yellow Springs Instruments, Ohio) at the end of each step. [La]ₘₐₓ max was considered the value at the end of last step of exercise. Fat and carbohydrate oxidation rates (FATox and CHOox) were estimated by means of Frayn's equations. A cutoff of 1 SD respect to the ([La]ₘₐₓ) max was suggested in order to classify the subjects in two groups: GO (Optimal [La]ₘₐₓ max) and GS (Suboptimal maximal [La]ₘₐₓ max) with [La]ₘₐₓ max of <5.27 mM in men and <4.00 mM in women respectively as the cutoff. A Student t-test for independent data was used to compare groups, the determination of the Pearson correlation coefficient was used to verify the existence of relationships between variables, level of statistical significance was set at p<0.05.

RESULTS: The results of the present study sowed that 30% for men and 24% for women showed suboptimal [La]ₘₐₓ max (GS). The correlation between [La]ₘₐₓ max and CHOox max was high in men (r=0.771, p<0.05) and low in women (r=0.373). In men, [La]ₘₐₓ max, CHOox max, and RER max were significantly higher in GO vs. GS, whereas FATox max was significantly lower in GO vs. GS. In women, there were not found significant differences neither in CHOox max nor in FATox max. Nevertheless [La]ₘₐₓ max, and RER max were significantly higher in GO vs. GS.

CONCLUSION: The measurement of [La]ₘₐₓ max and CHOox max in competitive athletes could be a good and practical approach to indirectly evaluate glycogen status as well as to identify suboptimal glycogen storages that can ultimately affect athletic performance.

ACKNOWLEDGEMENTS: The authors would like to express their gratitude to all subjects for their cooperation in this study.
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We measure the level of gasoline in the tanks of our cars all the time, right?

Glycogen is the Gasoline of our Muscles
Why Don’t We Measure the Gasoline Levels in Our Muscles?

What if we could?
It is possible...But...up until now the only way to measure glycogen levels was by a MUSCLE BIOPSY
Traditional Muscle Biopsy Techniques

**Borgström Technique**

*Invasive and aggressive*

Impractical and not clinically applicable

Background

- Skeletal muscle ultrasound has been used for years for injury diagnosis and treatment.
Through high frequency ultrasound we can assess muscle glycogen content in a rapid, non-invasive method in about 15 seconds!

Dr. John Hill measures the glycogen levels of Garmin-Transitions Tour de France cyclist Christian Van de Velde.
Glycogen Measurement: Performance Advantages

- If an athlete’s glycogen levels could be tested before, during and after competition, you can ensure the athlete is performing with a full energy stores.

- It would be possible to control the nutrition of the athlete to a level that has never been done before.

- If the athlete does not respond to diet changes, this could be an early method for detecting over training.
Measuring Muscle Glycogen: Does it Work?

- **January 2010** – Dr’s. John Hill and Inigo San Millan developed an ultrasound technique to detect changes in muscle glycogen content with ultrasound.

- In June 2014, Dr. Hill and I completed the validation study comparing muscle biopsy to ultrasound. This is the first report of that study.

Non-Invasive Glycogen Measurement: The Next Frontier of Sports Performance

Quantification of glycogen content in a non-invasive way

How does it Work?
Graphic representation of Scan
Graphic Views of Muscle Glycogen Content with Ultrasound

Glycogen stores empty
When glycogen leaves the tissue
It takes water with it

Glycogen stores full
Graphic Views of Muscle Glycogen Content with Ultrasound

Numerical values for each box

Numerical values glycogen partially depleted
Graphic Views of Muscle Glycogen Content with Ultrasound

Further depletion of glycogen

Glycogen stores almost empty
Graphic Views of Muscle Glycogen Content with Ultrasound

Over time total numerical value Drops as glycogen is depleted

Software design can process just the Key elements and exclude the artifact
These past slides demonstrate graphically how the subjective ultrasound image is converted to an objective numerical value which anyone can understand.

Connective tissue is a critical artifact which can be digitally subtracted.
How are the Images Processed?

Baseline Rectus Femoris SA  Post-Exercise Rectus Femoris SA

Same exact muscle after 90 minutes of moderately intense exertion on cyclergometer
How are the Images Processed?

MuscleSound, LLC; US Patents 8,512,247, 8,517,942 and additional patents pending
How are the Images Processed?

Baseline RF LA view

Post-Exercise Rectus Femoris SA

Chrome is cropped, leaving only the ultrasound image
How are the Images Processed?

Baseline RF LA view

Post-Exercise Rectus Femoris SA

-Sides are cropped, image is smoothed using Gaussian blur
How are the Images Processed?

- Thresholding; converting to binary image (black and white)

Baseline RF LA view

Post-Exercise Rectus Femoris SA
How are the Images Processed?

Baseline RF LA view

Post-Exercise Rectus Femoris SA

-Morphing technique to fill in holes which connects all muscle
-Image is returned to Gray Scale
How are the Images Processed?

- Image extraction performed which removes skin and fat
- Bottom crop removes connective tissue and artifact
- Pixel intensity of all connective tissue is 255
- Muscle glycogen score is determined from Pixels with 1-254 intensities
How are the Images Processed?

- Combined SA & LA, Rectus Femoris
- 3 Different Scans of RF SA view

- Pixels are averaged and compared across linked images,
- This allows individual scans to be tested for consistency and reliability
- Pixel intensity is changed from 0-255 to 0-100 which simplifies the interpretation

(Entire process takes <15 seconds)
Purpose of the Study

- To validate the utilization of MSK U/S for muscle glycogen assessment
- Correlate U/S results with histochemical glycogen quantification through muscle biopsy

Methods

- 22 male competitive cyclists, Pro, Cat 1-4

<table>
<thead>
<tr>
<th>Age</th>
<th>Height</th>
<th>Weight</th>
<th>% Body Fat*</th>
<th>BMI</th>
<th># Races/year</th>
</tr>
</thead>
<tbody>
<tr>
<td>31.3</td>
<td>+/- 183.7</td>
<td>+/- 76.8</td>
<td>12.1 +/- 2.4%</td>
<td>22.7</td>
<td>+/- 27.1</td>
</tr>
<tr>
<td>5.1</td>
<td>+/- 4.9 cm</td>
<td>+/- 7.8 kg</td>
<td></td>
<td>1.8</td>
<td>+/- 19.7</td>
</tr>
</tbody>
</table>

- Perform steady state cyclergometer test for 90 minutes at mod-high exercise intensity
  - CHOox of 2-3 gm/min
  - Lactate levels, 2-3 mM
Methods

- Pre- and Post-exercise glycogen content of right and left Rectus Femoris was measured using:
  - Histochemical analysis through muscle biopsy
  - High frequency ultrasound scans using MuscleSound® technology to measure and quantify glycogen
Muscle Biopsy Technique

Why rectus femoris muscle rather than the vastus lateralis?

- Less connective tissue reduces potential artifact in the ultrasound images
- Less connective tissue in the muscle biopsy samples
  - Reducing potential artifacts may improve both sensitivity and specificity
  - More precisely detect changes in muscle glycogen content
- Many competitive cyclists believe the RF region of the quadriceps feels empty and fatigued before VL region
Muscle Biopsy Technique

- Ultrasound guided muscle biopsy
  - Precisely directs biopsy
- Color flow Doppler
  - Avoid vascular structures
- No complications were encountered with 88 consecutive biopsies
  - No hematoma
  - No hemorrhages

Muscle Biopsy Technique

- Bard Monopty Disposable Core Biopsy Instrument (Liver biopsy)
  - 12 gauge X 10 cm length biopsy needle
  - 2 passes made at Baseline and Post-exercise
Correlation Results

Correlation Results

Individual Subjects Change

Muscle Biopsy Glycogen Change

Ultrasound Glycogen Change

Correlation of Different Muscles

Correlation of Different Muscles

Conclusions of Validation Study

- Pre- and post-exercise ultrasound scans using MuscleSound® technology were highly correlated with histochemical glycogen assessment through muscle biopsy.
- Changes in glycogen content from pre- and post-exercise were also highly correlated between MuscleSound® technology and muscle biopsy histochemical analysis.

Conclusions of Validation Study

- Results show that the use of high-frequency ultrasound through MuscleSound® technology
  - Accurate and reliable method to measure skeletal muscle glycogen
  - It is practical, rapid and non-invasive.

Applications

Rider 3

Glycogen content in the muscle

RFLA

RFSA
Glycogen Content Pre-PostGame

San Millan and Hill. *Data not published yet*
Glycogen Content Regulates Muscle Contraction

Figure 17-32b
Molecular Cell Biology, Sixth Edition
© 2008 W.H. Freeman and Company
A 25% decrease in Glycogen in the legs will decrease Ca++ release from SR by a 10!

Glycogen Content Regulates Muscle Contraction

Glycogen Content Pre-PostGame

San Millan and Hill. *Data not published yet*
Application to Evaluate Muscle Damage

When Muscle Damage, Glycogen Cannot be Stored Properly
It should be possible to track muscle damage by assessing glycogen content


Measurement of Skeletal Muscle Glycogen in Critically Ill Patients

Introduction

Critically ill patients experience hypermetabolism increasing substrate utilization, especially glucose oxidation. Glycogen is the main source of glucose in the body being 85% and 15% stored in skeletal muscle and liver respectively. Since glycogen stores are limited we evaluated the hypothesis that critical illness could be associated with glycogen depletion leading to skeletal muscle catabolism for gluconeogenesis and eventually resulting in cachexia, an important determinant of future ICU survival and ICU-acquired weakness (ICU-AW).

Methods

9 critically ill patients (58.75 ± 25-75 y/o) with an ICU stay from 1 to 5 weeks were evaluated for skeletal muscle glycogen content using a rapid, non-invasive high frequency ultrasound methodology (MuscleSound®, Denver, CO). Muscle scans were performed with a 12 MHz linear transducer and a standard diagnostic high resolution ultrasound machine, Terson and were obtained from the rectus femoris and vastus lateralis muscles. Glycogen content was measured with possible scores from 0-100 according to MuscleSound® scale. Patients had a variety of primary diagnoses including septic shock (n=3), hemorrhagic shock/abdominal hypertension (n=1), hypovolemic shock/post-major oncologic surgery (n=1), trauma (n=3), and burn injury (n=1).

Results

6 out of 9 patients had no glycogen present in their muscle (Score=0). Even 24 hours post-ICU admission we recorded a score of 0, indicating no muscle glycogen. The other 3 patients (all of whom were late in their ICU stay) had glycogen scores between 5-15 which are well below scores of healthy sedentary individuals (reference 50-70). As a comparison we have collected, pre and post-competition levels in athletes who significantly decrease their glycogen stores by ~50% or more, but are well above those of most critically ill patients we have studied.

Conclusion

This is the first time that muscle glycogen stores have been evaluated in critical illness. Our data shows severe glycogen depletion in ICU patients which likely leads to muscle catabolism necessary for gluconeogenesis, eventually resulting in cachexia. This finding poses severe metabolic challenges for ICU patients which likely interferes with recovery and contribute to poor survival. In light of our findings, re-evaluation of nutritional protocols and potential anabolic/anti-catabolic therapy to decrease muscle catabolism may improve survival. Different therapeutics that may prevent hypermetabolism (like beta-blockers) should be re-evaluated along with anabolic agents (i.e. oxandrolone) which could counteract the severe catabolic response in critical illness. When the muscle glycogen score begins to recover in ICU patients this may predict the transition from the “acute phase” of critical illness to the “recovery phase” and may signal when more aggressive nutrition support and anti-catabolic/anabolic therapies could be safely initiated.

References

Summary

- The use of high-frequency skeletal muscle ultrasound through to measure glycogen content is practical, accurate, rapid and non-invasive.
- It has enormous potential in sports performance to dial in and individualize nutrition as well as workload.
- It is a promising method to diagnose muscle damage and prevent skeletal muscle injuries.
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