Protein:
Nutrient Timing & Distribution

MATT CARLIN & MELANIE MARSHALL
Introduction: Nutrient Timing

- **Strategy:** maximize exercise-induced muscular adaptations and facilitate repair of damaged tissue

- Pre- vs. post-exercise period

- Anabolic window of opportunity?
  - Window of time existing after training to optimize training-related muscular adaptations
Effects of Supplement Timing and Resistance Exercise on Skeletal Muscle Hypertrophy

Paul J. Cribb & Alan Hayes

Medicine & Science in Sports & Exercise (2006):38(11);1918-1925
1. Oral supplementation with whole proteins or EAAs immediately before and/or after resistance exercise (RE) shown to promote better anabolic response compared with placebo treatments (Tipton et al. 2003 & 2004)
   ▶ Presence of CHO enhances response

2. Consumption with PRO-CHO supplement immediately before and after RE MAY provide ideal anabolic conditions for muscle growth
   ▶ Observed effects: muscle hypertrophy and greater gains in lean body mass (LBM)

3. Supplementation with creatine monohydrate (CrM) shown to promote greater gains in LBM and strength during RE training compared with placebo treated groups
Limitations of Previous Studies

- Normal eating patterns inhibited
- Lacking control group to assess effects of consuming supplement at other times during the day
- Limited research examining whether taking CrM in hours surrounding RE may result in greater accumulation of Cr within muscle or provide greater adaptations compared with supplementation at other times of the day
Examine the effects of supplement timing during RE training with a CrM-containing PRO/CHO supplement in comparison with supplementation at times not close to the immediate pre- and post-workout periods

Hypothesis:

Supplement timing would provide greater chronic adaptations (greater increases in LBM, strength, and muscle-fiber hypertrophy) compared with supplementation in the hours not close to RE
Methods

- Single-blind, randomized protocol

- **Participants:** 23 recreational male bodybuilders

- **Supplementation:** 4x/wk for 10 wks
  - **PRE-POST:**
    1) Just before commencing workout
    2) Straight after finishing workout
  - **MOR-EVE**
    1) Morning before breakfast
    2) Late evening before sleep

- **Supplement (per 100 g):**
  - 40 g PRO (whey isolate)
  - 43 g CHO (glucose)
  - <0.5 g FAT
  - 7 g CrM
  - \( \approx \) 267 kcals

- Maintained habitual daily diet during trial
  - 3 diet records (before, 1st wk of study, final wk of study)

- Wt. measured (before, 1st wk, & final wk of the study)
Methods cont.

**Resistance-Training Protocol**

- Questionnaires used to determine training history (3-5 days/wk for at least 6 mos)
- Structured training program 8-12 wk before commencing trial
- 10 wk RE program
  - Designed to increase strength and muscle size
  - High-intensity overload workout (compound exercises using free weights)
  - Training intensity – RM from strength tests
- 3 phases of progressive overload program
  - Preparatory (70-75% 1RM)
  - Overload phase 1 (80-85% 1RM)
  - Overload phase 2 (90-95% 1RM)
- Training diaries
  - Exercises, sets, reps performed, and wt. used throughout the program
Following assessment done in the week before and after 10-wk RE program:

- **Strength** – 1RM (barbell bench press, deadlift, squat)

- **Body composition (DEXA)** –
  - Lean mass
  - Fat mass
  - Body fat %

- **Muscle analysis** –
  - Muscle fiber type (I, IIA, IIX)
  - Cross-sectional area (CSA)
  - Contractile protein content
  - Metabolite concentrations (PCr, Cr, & glycogen)
Results

17 participants completed RE program

- **Baseline characteristics** – Ø differences btwn groups in any variables

- **Dietary analysis**
  - Ø differences btwn groups

### TABLE 2. Dietary analyses.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRE-POST</th>
<th>MOR-EVE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal·kg⁻¹·d⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>43.7 ± 6.6</td>
<td>44.4 ± 4.8</td>
</tr>
<tr>
<td>Week 1</td>
<td>44.1 ± 6.9</td>
<td>42.9 ± 4.1</td>
</tr>
<tr>
<td>Week 10</td>
<td>42.8 ± 6.6</td>
<td>42.2 ± 2.8</td>
</tr>
<tr>
<td>Carbohydrate (g·kg⁻¹·d⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>4.88 ± 1.3</td>
<td>4.63 ± 0.7</td>
</tr>
<tr>
<td>Week 1</td>
<td>4.86 ± 1.0</td>
<td>4.62 ± 0.8</td>
</tr>
<tr>
<td>Week 10</td>
<td>4.79 ± 0.9</td>
<td>4.50 ± 0.7</td>
</tr>
<tr>
<td>Protein (g·kg⁻¹·d⁻¹)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before</td>
<td>1.84 ± 0.4</td>
<td>2.08 ± 0.4</td>
</tr>
<tr>
<td>Week 1</td>
<td>1.91 ± 0.4</td>
<td>2.17 ± 0.3</td>
</tr>
<tr>
<td>Week 10</td>
<td>1.92 ± 0.4</td>
<td>2.11 ± 0.3</td>
</tr>
</tbody>
</table>

No differences between groups at any time point (mean ± SD).
TABLE 3. Body mass, composition, and 1RM strength.

<table>
<thead>
<tr>
<th>Variable</th>
<th>PRE-POST (N = 8)</th>
<th>MOR-EVE (N = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Endpoint</td>
<td>Baseline</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>81.8 ± 3.2</td>
<td>84.3 ± 3.2*</td>
<td>78.2 ± 1.8</td>
</tr>
<tr>
<td>Lean mass (kg)</td>
<td>69.5 ± 2.3</td>
<td>72.3 ± 2.3*</td>
<td>65.2 ± 1.5</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>12.1 ± 1.5</td>
<td>11.9 ± 1.4</td>
<td>12.9 ± 1.2</td>
</tr>
<tr>
<td>% body fat</td>
<td>13.7 ± 1.4</td>
<td>12.6 ± 1.3*</td>
<td>15.7 ± 1.4</td>
</tr>
<tr>
<td>1RM squat (kg)</td>
<td>144.4 ± 8.2</td>
<td>164.8 ± 8.6*</td>
<td>138.3 ± 8.5</td>
</tr>
<tr>
<td>1RM bench press (kg)</td>
<td>126.9 ± 6.9</td>
<td>139.1 ± 6.8*</td>
<td>121.9 ± 4.7</td>
</tr>
<tr>
<td>1RM dead lift (kg)</td>
<td>149.7 ± 6.5</td>
<td>168.1 ± 7.7</td>
<td>141.9 ± 6.4</td>
</tr>
</tbody>
</table>

* Greater change compared with MOR-EVE (P < 0.05) (mean ± SE).

FIGURE 1—Body composition changes. * Greater change compared with MOR-EVE (P < 0.05).

FIGURE 2—1RM strength changes. * Greater change compared with MOR-EVE (P < 0.05).
Ø differences btwn groups for fiber proportions

PRE-POST > increases in CSA of type IIa & IIx fibers and contractile proteins
PRE-POST group ↑ PCr and total Cr concentrations and ↑ muscle glycogen concentrations after RE program

<table>
<thead>
<tr>
<th></th>
<th>PRE-POST (N = 8)</th>
<th>MOR-EVE (N = 9)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>Endpoint</td>
<td>Baseline</td>
</tr>
<tr>
<td>PCr (mmol·kg⁻¹ d.w.)</td>
<td>78.1 ± 1.5</td>
<td>91.2 ± 1.4*</td>
<td>79.7 ± 2.6</td>
</tr>
<tr>
<td>Total Cr (PCr + Cr) (mmol·kg⁻¹ d.w.)</td>
<td>123.0 ± 2.3</td>
<td>153.2 ± 1.5*</td>
<td>129.0 ± 3.9</td>
</tr>
<tr>
<td>Glycogen (mmol·kg⁻¹ d.w.)</td>
<td>235.1 ± 12.4</td>
<td>294.0 ± 8.0*</td>
<td>234.0 ± 4.3</td>
</tr>
</tbody>
</table>

* Greater increase compared with MOR-EVE (P < 0.05) (mean ± SE).
Discussion

- Post 10 wk RE program
  - Supplementation before and after each workout resulted in significantly > improvements in 1RM strength and body composition (i.e. ↑ LBM and ↓ body fat %) compared w/ matched group who had consumed the same supplement at times outside of the pre- and post-workout time frames

- Significantly > muscle hypertrophy response from supplement timing at 3 different levels
  - PRE-POST > increases in:
    - LBM
    - Hypertrophy of type IIa & IIx fibers
    - Contractile proteins
Acute-response investigations:

- Supplementation w/ protein (or EAA) before and/or after RE enhances anabolic response by:
  1) Increasing muscle protein-synthesis rates
  2) Decreasing protein degradation
  3) Providing a higher net protein balance

Longitudinal studies

- Protein supplementation before and/or after RE enhances:
  1) Chronic adaptations desired from training (i.e. muscle hypertrophy and strength)
Discussion cont.

1st novel finding

- Beneficial effects of supplement timing on strength and muscle hypertrophy were obtained when participants followed normal eating patterns

- Effects likely not attributed simply to the presence or absence of certain macronutrients in the hours surrounding RE

- Adaptations reflect specific interactive effect between high-intensity muscle contraction and the presence of an abundance of nutritional material
  - EAA
  - Cr
  - CHO
Mechanisms

- **EAA** - ↑ acute stimulation of protein synthesis in muscle during RE and to provide > positive net protein balance over a 24-h assessment period

- **CHO** –
  - Enhance anabolic stimulus by ↑ plasma insulin concentrations (serves to ↑ protein-synthesis rates when EAA are present)
  - Reducing myofibrillar protein breakdown after RE

- **Beneficial effects of supplement timing on muscle hypertrophy may be attributed to abundance of EAA and glucose during high-intensity muscle contraction**
Supplementation consistently shown to augment LBM and strength development during RE

- CrM + whey PRO = > gain in lean mass vs. whey PRO + CHO

CrM likely contributed to improvements in strength and hypertrophy observed in both groups

- **Interesting finding:** PRE-POST > muscle Cr concentrations after trial
Discussion cont.

**Mechanisms**

- Enhanced cellular bioenergetics
- Greater expression of hypertrophy-related genes
  - Higher working capacity during RE

**Speculation:**

- Supplement timing promotes more efficient Cr accumulation within muscle and, therefore, greater strength gains and muscle hypertrophy during RE training
2nd novel finding

- PRE-POST group: significantly > muscle glycogen concentrations at the end of the study

- High levels attributed to increased availability of CHO and Cr from supplementation on day of the biopsy?

- Levels from weeks 1 and 10 should have been higher for PRE-POST group
  - Levels from week 1 Ø significantly different from MOR-EVE group
PRE-POST supplement timing:
- Promoted more efficient CrM accumulation within muscle
- Promoted more efficient muscle glycogen restoration

Enabled greater work capacity during workouts = greater strength improvements and muscly hypertrophy
Limitations

- Supplementation PRE & POST workout
- Addition of CrM to supplement
- Personalized coaching/supervision
PRE-POST supplement timing results in greater strength and body composition improvements as well as muscle hypertrophy compared with supplementation at times outside of the workout period.

Supplement timing represents a simple but effective strategy to enhance the adaptations desired from RE training.
Nutrient timing revisited: is there a post-exercise anabolic window?

Alan Albert Aragon & Brad Jon Schoenfeld
Journal of the International Society of Sports Nutrition (2013):10(5);1-11
Goals of Post-Exercise Nutrition

- Attenuation of muscle protein breakdown
  - ↑ insulin: anti-catabolic
- Potentiates increases in muscle protein synthesis (MPS)
- Muscle hypertrophy

Do benefits extend into practice?
Insulinogenic Effect

- In presence of elevated plasma amino acids, effect of insulin elevation on net muscle protein balance plateaus within a range of 15-30 mU/L (~3-4x normal fasting levels)

- Accomplished w/ typical mixed meals
  - ~1-2 h for circulating substrate levels to peak
  - ~3-6 h (or more) for a complete return to basal levels depending on the meal size

- Recommendation for lifters to spike insulin post-exercise may be trivial
  - Classical post-exercise objective to quickly reverse catabolic processes to promote recovery and growth may only be applicable in absence of a properly constructed pre-exercise meal
Muscle Protein Synthesis (MPS)

- Stimulatory effects of hyperaminoacidemia on MPS, especially from EAAs, are potentiated by previous exercise.

- Maximizing MPS - evidence to support the superiority of post-exercise free amino acids and/or protein compared to solely CHO or non-caloric placebo.

- **However** → evidence lacking for recommendation to consume protein as soon as possible post-exercise.
Protein synthesis of legs and whole body ↑ threefold when supplement (10 g PRO, 8 g CHO, 3 g FAT) ingested immediately post-exercise compared to just 12% when consumption was delayed (Levenhagen et al. 2001)

Ø significant difference in leg net amino acid balance btwn 6 g EAA coingested with 35 g CHO taken 1 h vs. 3 h post-exercise (Rasmussen et al. 2000)

Immediate pre-exercise ingestion of the same EAA-CHO solution resulted in significantly > and more sustained MPS response compared to the immediate post-exercise ingestion (Tipton et al. 2001 & 2007)
  Ø sign. difference in net MPS btwn the ingestion of 20 g whey immediately pre- vs. the same solution consumed 1 h post-exercise
Utility of acute studies is limited to providing clues and generating hypotheses regarding hypertrophic adaptations

- Measures of MPS assessed following an acute bout of RE are not necessarily predictive of long-term hypertrophic responses to regimented RE

**CONSENSUS:**

- Available data lack any consistent indication of an ideal post-exercise timing scheme for maximizing MPS
Results from studies looking at long-term hypertrophic effects of post-exercise protein consumption are conflicting due to varied study designs and methodology.

- Major limitation: use of both pre- and post-workout supplementation

Difficult to draw relevant conclusions as to the validity of an ‘anabolic window’
<table>
<thead>
<tr>
<th>Study</th>
<th>Subjects</th>
<th>Supplementation</th>
<th>Protein matched with Control?</th>
<th>Measurement instrument</th>
<th>Training protocol</th>
<th>Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esmarck et al. [69]</td>
<td>13 untrained elderly males</td>
<td>10 g milk/soy protein combo consumed either immediately or 2 hours after exercise</td>
<td>Yes</td>
<td>MRI and muscle biopsy</td>
<td>Progressive resistance training consisting of multiple sets of lat pulldown, leg press and knee extension performed 3 days/wk for 12 wk</td>
<td>Significant increase in muscle CSA with immediate vs. delayed supplementation</td>
</tr>
<tr>
<td>Cribb and Hayes [70]</td>
<td>23 young recreational male bodybuilders</td>
<td>1 g/kg of a supplement containing 40 g whey isolate, 43 g glucose, and 7 g creatine monohydrate consumed either immediately before and after exercise or in the early morning and late evening</td>
<td>Yes</td>
<td>DXA and muscle biopsy</td>
<td>Progressive resistance training consisting of exercises for the major muscle groups performed 3 days/wk for 10 wks</td>
<td>Significant increases in lean body mass and muscle CSA of type II fibers in immediate vs. delayed supplementation</td>
</tr>
<tr>
<td>Willoughby et al. [71]</td>
<td>19 untrained young males</td>
<td>20 g protein or 20 g dextrose consumed 1 hour before and after exercise</td>
<td>No</td>
<td>Hydrostatic weighing, muscle biopsy, surface measurements</td>
<td>Progressive resistance training consisting of 3 sets of 6–8 repetitions for all the major muscles performed 4 days/wk for 10 wks</td>
<td>Significant increase in total body mass, fat-free mass, and thigh mass with protein vs. carb supplementation</td>
</tr>
<tr>
<td>Hulmi et al. [72]</td>
<td>31 untrained young males</td>
<td>15 g whey isolate or placebo consumed immediately before and after exercise</td>
<td>No</td>
<td>MRI, muscle biopsy</td>
<td>Progressive, periodicized total body resistance training consisting of 2–5 sets of 5–20 repetitions performed 2 days/wk for 21 wks.</td>
<td>Significant increase in CSA of the vastus lateralis but not of the other quadriceps muscles in supplemented group versus placebo.</td>
</tr>
<tr>
<td>Verdijk et al. [73]</td>
<td>28 untrained elderly males</td>
<td>10 g casein hydrolysate or placebo consumed immediately before and after exercise</td>
<td>No</td>
<td>DXA, CT, and muscle biopsy</td>
<td>Progressive resistance training consisting of multiple sets of leg press and knee extension performed 3 days/wk for 12 wks</td>
<td>No significant differences in muscle CSA between groups</td>
</tr>
<tr>
<td>Hoffman et al. [74]</td>
<td>33 well-trained young males</td>
<td>Supplement containing 42 g protein (milk/collagen blend) and 2 g carbohydrate consumed either immediately before and after exercise or in the early morning and late evening</td>
<td>Yes</td>
<td>DXA</td>
<td>Progressive resistance training consisting of 3–4 sets of 6–10 repetitions of multiple exercises for the entire body performed 4 days/wk for 10 weeks.</td>
<td>No significant differences in total body mass or lean body mass between groups.</td>
</tr>
<tr>
<td>Erskine et al. [75]</td>
<td>33 untrained young males</td>
<td>20 g high quality protein or placebo consumed immediately before and after exercise</td>
<td>No</td>
<td>MRI</td>
<td>4-6 sets of elbow flexion performed 3 days/wk for 12 weeks</td>
<td>No significant differences in muscle CSA between groups</td>
</tr>
</tbody>
</table>
Discussion

- Evidenced-based support for an ‘anabolic window of opportunity’ far from definitive

- Hypothesis based on pre-supposition that training is carried out in a fasted state
  - How might pre-exercise nutrition influence the urgency or effectiveness of post-exercise nutrition

- Primary goal → increasing muscle size and/or strength
  - Consuming pre-exercise meal 1-2 h prior in attempt to maximize training performance
  - Potentially act as pre- and post-exercise meal as the time course of digestion/absorption can persist into the recovery period

- If training initiated more than ~3-4 h after preceding meal, classical recommendation of consuming protein (~20-30g) as soon as possible MAY be warranted in order to reverse the catabolic state
Discussion

**Generalizability of recommendations**

- Trained vs. untrained:
  - Less global response of protein synthesis in advanced trainees
  - May warrant closer attention to protein timing and type (i.e. high leucine sources) in order to optimize rate of muscular adaptation

- Elderly population:
  - ‘Anabolic resistance’ = lower receptivity to amino acids and resistance training
  - Likely require higher individual PRO doses to optimize the anabolic response to training (possibly closer to 40 g)
Study Limitations

1. Examining pre- and post-exercise supplementation simultaneously
2. Neglecting to match total protein intake between the conditions compared
3. Conservative dosing strategies (~10-20 g)
4. Using untrained individuals
5. Differing methods used to assess muscle hypertrophy (accuracy of measures inexact)
Practical Applications

- High-quality protein dosed at 0.4-0.5 g/kg of LBM at both pre- and post-exercise
  - Reflects current evidence showing maximal acute anabolic effect of 20-40 g

- Pre- and post-exercise meals should not be separated by more than ~3-4 h (given a typical training bout lasts ~45-90 min)
  - If protein delivered within a large mixed-meal, possibly lengthen the interval to 5-6 h
Introduction: Nutrient Distribution

- Recovery from exercise happens 24/7
  - Not just post-exercise

- How can we maximize recovery in areas outside of the “anabolic window?”
  - Protein intake?
Dietary Protein Distribution Positively Influences 24-h Muscle Protein Synthesis in Healthy Adults

Madonna M. Mamerow et al.

Background

- Current RDA sufficient to prevent deficiency
  - Optimal health?
  - Prevention of sarcopenia?
  - Enhancement of LBM and strength?
- Adult protein intake 3x greater at dinner than breakfast (NHANES, 2012)
  - 38g V 13g
Background

- 30g PRO per meal has been shown to increase MPS maximally

- Is this the complete picture?

- MPB may be more dynamic than originally thought
  - Populations...
Amino Acids:
- Potently stimulate MPS
- MPB mostly unchanged (Glynn et al, 2010)

The increase in MPS from exogenous amino acid delivery is transient, peaking at 1-2 hours and returns to baseline afterwards (Bohe et al, 2001)

Increases or decreases in muscle mass occur from changes in net protein balance over time.
Hypothesis

- Even spread (30-30-30) more beneficial than typical intake (10-15-65)
Participants and Methods

- **Participants**
  - 8 males and females 25-55 YO
  - No metabolic disease, obesity, recent weight loss/gain, anabolic steroid usage
  - Active, but not athletically trained

- **Design**
  - 7-d crossover with 30-d washout
  - Metabolic studies days 1 and 7
    - Account for habituation
Methods

- Hospital admittance
  - No exercise within 72h
  - Standardized meal the evening before
  - Overnight fast

- Metabolic Study
  - Primed, constant phenylalanine infusion
  - Biopsies at 900, 1230 and 900 (24h post)
  - Meals at 930, 1300 and 1700
  - Venous samples before meals and at 20 min intervals for 2-3 hour postprandial
Methods

- Are metabolic study conditions realistic for an athletic population?

- 7 days of constant PRO intake, then second metabolic study
  - Prepackaged meals

- Measuring MPS
  - Fractional Synthetic Rate (FSR)- measure incorporation of phenylalanine into mixed muscle protein
Results

Breakfast Meal (30g V 10g)
EVEN 30% higher

24-hour FSR
EVEN 25% higher
Discussion

- Daily quantity of protein not the sole determinant of increasing MPS
  - Recommendation/RDA?

- High protein containing evening meal (65g) not sufficient to make up for earlier deficits in MPS

- Muscle protein breakdown?
  - Inability to measure both MPS and MPB in same study
  - 40-40-40 better than 30-30-30?
Discussion

- Higher frequency?
  - 4x/day? 5x/day? 8x/day?

- Small contribution to increasing LBM and strength
Final Discussion

- Efficacy of post exercise supplementation when other protein is equally distributed?

- Class question- optimal protein dosing throughout the day?

- Meal-EAA-meal-EAA-meal for lower caloric intake but higher optimal MPS?

- Application- how to increase protein frequency?
  - What foods would be good for on-the-go? Refrigeration consideration?