Almonds: Food for Fitness

Sponsored by the Almond Board of California
Almonds: Food for Fitness

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Research Facility: Appalachian State University Human Performance Laboratory, North Carolina Research Campus

Sponsor: Almond Board of California  
OTR-19-NIEMAND-NR-01; ClinicalTrials.gov: NCT04958018

# Disclosures

<table>
<thead>
<tr>
<th>AFFILIATION/FINANCIAL INTERESTS (prior 12 months)</th>
<th>ENTITIES</th>
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<tr>
<td>Grants/Research Support</td>
<td>Almond Board of California, National Mango Board, Highbush Blueberry Council, Ocean Spray, LycoRed</td>
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<tr>
<td>Scientific Advisory Board/Consultant/Board of Directors</td>
<td>Vitapod</td>
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<td>Speakers Bureau</td>
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<tr>
<td>Stock Shareholder</td>
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<tr>
<td>Employee</td>
<td>Appalachian State University</td>
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<tr>
<td>Other</td>
<td>The Almond Board had no role in the study design, data collection, analysis and interpretation, the preparation of the manuscript, or the decision to submit the article for publication.</td>
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</table>
Physiological Stress from Prolonged and Intensive Exercise

Proteomics (LC-MS/MS)
Protein S100-A8

Nutrition interactions

Genomics, transcriptomics, epigenetics, gut microbiome (sequencers)

Metabolomics, lipidomics, oxylipins (UPLC-MRM-MS)

15-Hydroxyicosatetraenoic acid (15-HETE)

Palmitoleic Acid

Nutrition interactions

Systems biology approach

Immune Responses


Oxylipins are bioactive oxidation products derived from n-6 and n-3 PUFAs in the linoleic acid and α-linolenic desaturation pathways; sensitive to exercise and nutrition interventions. Signini, Nieman et al. Metabolites 2020 Jun 25;10(6):264

Oxylipins have vital regulatory roles in numerous physiological processes including upstream regulation of inflammation.

Dr. Qibin Zhang
(Associate Professor, Center for Translational Biomedical Research, UNC Greensboro)
Monocyte Neutrophil Oxylipins Upstream Regulation Function depends on the physiological context

Physical Exercise

Duration

PUFAs (ω-6 ω-3)

CYP

LOX

COX

PLA₂

Workload

Intensity

Post-exercise condition:

↑ level of most oxylipins (0-5h)
Plant foods support athletic endeavor and health (compared to sugar water).

Carbohydrate ingestion

↓ inflammation
(reduced neutrophilia, monocytosis, plasma IL-6, IL-1ra, IL-10, MCP-1; muscle IL-6, IL-8 mRNA)

↓ cortisol, epinephrine

↑ performance (2-6%)

↑ serum glucose, fructose, insulin

Metabolomics, Proteomics
Plant polyphenols: oxylipin and immune protein responses.

Dr. Camila Olson
Research Manager
Human Performance Lab, NCRC

AppState-NCRC Human Performance Lab

Mid-1990s

Exercise and Nutrition Immunology, Multiomics Approach

Carbohydrate ingestion

↑ performance (2-6%)

↑ serum glucose, fructose, insulin

↓ inflammation
(reduced neutrophilia, monocytosis, plasma IL-6, IL-1ra, IL-10, MCP-1; muscle IL-6, IL-8 mRNA)

↓ cortisol, epinephrine

2009-2023

NCRC

Plant foods support athletic endeavor and health (compared to sugar water).

Carbohydrate ingestion

↓ inflammation
(reduced neutrophilia, monocytosis, plasma IL-6, IL-1ra, IL-10, MCP-1; muscle IL-6, IL-8 mRNA)

↓ cortisol, epinephrine

↑ performance (2-6%)

↑ serum glucose, fructose, insulin

Metabolomics, Proteomics
Plant polyphenols: oxylipin and immune protein responses.
Anthocyanin

Punicalagin (ellagitannin, hydrolyzable tannin)

Hydroxybenzoic Acid

Hippuric Acid

anti-inflammatory, antioxidant, anti-pathogenic, immune regulatory effects

Earlier studies reported few discernable benefits of increased polyphenol intake for athletes: design shortfalls.
**HYPOTHESIS:** Almonds provide a unique and complex nutrient and polyphenol mixture that may support metabolic recovery from stressful levels of exercise. The total (poly)phenol content of almonds is **164 mg / 57-g serving.**

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>100 g raw almonds</th>
<th>57 g raw almonds</th>
<th>Male RDA</th>
<th>Female RDA</th>
<th>% RDA Males</th>
<th>% RDA Female</th>
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<tr>
<td>Kilocalories</td>
<td>579</td>
<td>324</td>
<td></td>
<td></td>
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<tr>
<td>Protein (g)</td>
<td>21.2</td>
<td>11.9</td>
<td></td>
<td></td>
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<tr>
<td>Arginine (g)</td>
<td>2.47</td>
<td>1.4</td>
<td></td>
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<tr>
<td>Total fat (g)</td>
<td>49.9</td>
<td>28.0</td>
<td></td>
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<tr>
<td>Monounsaturated fat (g)</td>
<td>31.6</td>
<td>17.7</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Carbohydrate (g)</td>
<td>21.6</td>
<td>12.1</td>
<td></td>
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<tr>
<td>Fiber (g)</td>
<td>12.5</td>
<td>7.0</td>
<td></td>
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<tr>
<td>Sugars (g)</td>
<td>4.35</td>
<td>2.44</td>
<td></td>
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<tr>
<td>Calcium (mg)</td>
<td>269</td>
<td>151</td>
<td>1000</td>
<td>1000</td>
<td>15.1</td>
<td>15.1</td>
</tr>
<tr>
<td>Iron (mg)</td>
<td>3.71</td>
<td>2.1</td>
<td>8</td>
<td>18</td>
<td>26.3</td>
<td>11.7</td>
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<tr>
<td>Magnesium (mg)</td>
<td>270</td>
<td>151</td>
<td>400</td>
<td>320</td>
<td>37.8</td>
<td>47.2</td>
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<tr>
<td>Zinc (mg)</td>
<td>3.12</td>
<td>1.76</td>
<td>11</td>
<td>8</td>
<td>16.0</td>
<td>22.0</td>
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<tr>
<td>Copper (mg)</td>
<td>1.031</td>
<td>0.58</td>
<td>0.9</td>
<td>0.9</td>
<td>64.0</td>
<td>64.0</td>
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<tr>
<td>Manganese (mg)</td>
<td>2.18</td>
<td>1.23</td>
<td>2.3</td>
<td>1.8</td>
<td>53.5</td>
<td>68.3</td>
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<tr>
<td>Vitamin E (ug alpha-TE)</td>
<td>25.6</td>
<td>14.4</td>
<td>15</td>
<td>15</td>
<td>96.0</td>
<td>96.0</td>
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<tr>
<td>Leucine (g)</td>
<td>1.47</td>
<td>0.834</td>
<td></td>
<td></td>
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</table>
• (Poly)phenols in almonds include
  • flavonols (kaempferol, isorhamnetin, and quercetin),
  • flavanols (several types of catechins),
  • flavanones (eriodictyol and naringenin),
  • simple phenolic acids such as protocatechuic acid and vanillic acid.
• Proanthocyanidins (PACs) are the most abundant class of (poly)phenols in almonds.
Previous Studies

Metabolic Signatures Induced by Heavy Exertion and Pistachios

Funding: American Pistachio Growers
Research Design

2-wks supplementation
3 oz/d pistachio nuts or NONE, randomized assignment.

75-km cycling trial
(with 3 oz pistachio nuts or water only)

2-wks washout, then crossover

2-wks supplementation
(opposite treatment)

75-km cycling trial
(with 3 oz pistachio nuts or water only)

= blood draw

75-km Performance
time (hours) 4.8% slower

75-km Performance Time (h)

Pistachio

Water
Pistachio nuts contain the trisaccharide raffinose, a soluble carbohydrate also found in onions and many types of legumes.
• 9,10-DiHOME is a leukotoxin derivative of linoleic acid and is produced by neutrophils and macrophages.
• Disrupts mitochondrial function by altering inner membrane integrity and increasing cytochrome C release.
Raffinose Blood Signature:
Enhanced by the “leaky gut” effect of exercise.

Raffinose Ingestion:
Passes through small intestine to colon.

9,10-DiHOME impairs muscle performance
Visit #1: Pre-Study Consent, Orientation
Visit #2: Body composition and Muscle Function: Vertical jump, bench press, leg-back strength, shuttle run, Wingate
Visit #3: Monday: Eccentric Exercise Bout
Visit #4: Tuesday: Recovery
Visit #5: Wednesday: Recovery
Visit #6: Thursday: Recovery
Visit #7: Friday: Recovery

Outcome Measures

- **Energy, vitality**: Profile of Mood States (POMS), performance
- **Soreness**: Delayed onset of muscle soreness (DOMS)

- **Inflammation**: 70 oxylipins, lipid mediators
- **Inflammation**: 6 cytokines and C-reactive protein (CRP)
- **Muscle damage**: Creatine kinase, myoglobin, LDH

- Urine metabolites from 4-weeks almond ingestion
- 3-day food record (flavonoids, macro- and micro-nutrients)

N=66 males & females (randomized treatment)

Almonds (57 g/d) (n=33)
Snack bar (324 calorie matched) n=33

Visit #3: Monday: Eccentric Exercise Bout

- **Muscle Function**: Vertical jump, bench press, leg-back strength, shuttle run, Wingate
- **Eccentric exercise**: 90-min
- **Half snack dose**: AM, PM
- **POMS; DOMS; Blood**: PM
- **Blood**: PM

Visit #4: Tuesday: Recovery
(7 am POMS, DOMS, blood sample, half snack dose before muscle function testing, and then afternoon)

Visit #5: Wednesday: Recovery
(7 am POMS, DOMS, blood sample, half snack dose before muscle function testing, and then afternoon)

Visit #6: Thursday: Recovery
(7 am POMS, DOMS, blood sample, half snack dose before muscle function testing, and then afternoon)

Visit #7: Friday: Recovery
(Repeat body composition; 7 am POMS, DOMS, blood sample, half snack dose, muscle function testing)
### Final Group Subject Statistics

[Almond n=33 (20 males, 13 females)]
[Snack Bar n=31 (18 males, 13 females)]

<table>
<thead>
<tr>
<th></th>
<th>Supplement Group</th>
<th>Mean</th>
<th>Std. Deviation</th>
<th>Std. Error Mean</th>
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</thead>
<tbody>
<tr>
<td><strong>Age (yr)</strong></td>
<td>Almond</td>
<td>46.06</td>
<td>8.39</td>
<td>1.46</td>
</tr>
<tr>
<td></td>
<td>Snack Bar</td>
<td>46.77</td>
<td>9.04</td>
<td>1.62</td>
</tr>
<tr>
<td><strong>Weight (kg)</strong></td>
<td>Almond</td>
<td>77.40</td>
<td>11.24</td>
<td>1.96</td>
</tr>
<tr>
<td></td>
<td>Snack Bar</td>
<td>79.74</td>
<td>10.11</td>
<td>1.82</td>
</tr>
<tr>
<td><strong>Height (cm)</strong></td>
<td>Almond</td>
<td>173.63</td>
<td>9.75</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Snack Bar</td>
<td>171.50</td>
<td>7.73</td>
<td>1.39</td>
</tr>
<tr>
<td><strong>BMI</strong></td>
<td>Almond</td>
<td>25.56</td>
<td>2.12</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td>Snack Bar</td>
<td>27.08</td>
<td>2.70</td>
<td>0.49</td>
</tr>
<tr>
<td><strong>Body Fat (%)</strong></td>
<td>Almond</td>
<td>27.43</td>
<td>7.75</td>
<td>1.35</td>
</tr>
<tr>
<td></td>
<td>Snack Bar</td>
<td>28.84</td>
<td>9.98</td>
<td>1.82</td>
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- Healthy, non-obese, non-athletes and not engaged in regular resistance training.
- **NOTE:** A limitation of the study is that it only included non-smoking participants without obesity who exercised occasionally; therefore, we cannot generalize the findings to other demographic and health status groups.
N=64 untrained subjects.
90 min eccentric exercise bout: 17 different exercises, multiple sets
Performance Testing

• 1. Vertical jump: Countermovement vertical jump, repeated three times, with the best score recorded as the difference between the jump and standing reach heights.

• 2. Leg-back strength: Grasp a bar attached via a chain to a force measuring device with straight arms, and then lift up with maximal effort for several seconds (best of 3 attempts).

• 3. Bench press to exhaustion: Bench press a weighted bar equal to 50% (female) 75% (male) body weight as many times as possible (to a metronome set at 60 beats/min or 30 lifts/min).

• 4. 30-second Wingate cycling test: Sprint cycle at maximal speed for 30 seconds on Lode cycle ergometer adjusted to weight (7 watts per kilogram) (peak power, mean power, adjusted to body mass).

• 5. 60 yard shuttle run test: Participants run to 5 yards, 10 yards, 15 yards, there and back, for a total of 60 yards.
7 Blood Samples and 2 urine samples: Outcomes
1. Creatine kinase, myoglobin, LDH (muscle damage)
2. C-reactive protein and six cytokines (IL-6, IL-8, IL-10, IL-1ra, MCP-1, GCSF) (inflammation)
3. Comprehensive diagnostic chemistry panel
4. Urine almond gut-derived metabolites
5. Plasma oxylipins (n=70) (inflammation)

Post-90-minutes exercise blood draw
Almonds

Snack bars

PreStudy

4 wks Supplementation

Composite Variable

Sum of 6 Urine Gut-Derived Phenolics

(6 metabolites related to proanthocyanidin catabolism)

Interaction effect $p=0.0013$; FDR $Q=0.1258$

$5\text{-}(3',4'\text{-Dihydroxyphenyl})\text{-γ-valerolactone}$; hydroxybenzoic acid-sulfate* (2 isomers of unknown structural configuration); 5-hydroxyphenylpropanoic acid-3-sulfate; benzoic acid-4-sulfate; 5-hydroxyphenylpropanoic acid-3-O-glucuronide; 3-(4-methoxyphenyl)propanoic acid-sulfate

Dr. Colin Kay
**Delayed Onset of Muscle Soreness (DOMS) (1-10 scale)**

Time effect, \( p<0.001 \); Time x treatment effect, \( p=0.187 \); Interpretation: The 90-minute eccentric exercise protocol had a significant effect in increasing muscle soreness, with no treatment effect (almonds vs snack bar).

---

**Estimated Marginal Means of MEASURE_1**

- **Supplement**
  - Almond
  - Snack Bar

**Delayed Onset of Muscle Soreness Scale:**

- 1: No soreness
- 1.5
- 2
- 2.5: Dull, vague ache
- 3
- 3.5
- 4: Slight soreness
- 4.5
- 5
- 5.5: More than slight soreness
- 6
- 6.5
- 7: Sore
- 7.5
- 8
- 8.5: Very sore
- 9
- 9.5
- 10: Unbearably sore

Error bars: +/- 1 SE
Serum Creatine Kinase

Time effect, p<0.001; Time x treatment effect, p=0.151; Interpretation: The 90-minute eccentric exercise protocol had a significant effect in increasing muscle damage (to a large and sustained level). The overall interaction effect was not significant, but change in CK differed between groups immediately post-exercise and after 1-day of recovery (i.e., lower muscle damage at those time points for the almond group).

Estimated Marginal Means of MEASURE_1

Error bars: +/- 1 SE
Serum C-Reactive Protein (CRP)

Time effect, $p<0.001$; Time x treatment effect, $p=0.145$; Interpretation: The 90-minute eccentric exercise protocol had a significant effect in increasing inflammation, with no treatment effect (almonds vs snack bar). (Weak trend for an almond anti-inflammatory effect).
RESULTS: Plasma Cytokine IL-6

Time effect, p<0.001; Time x treatment effect, p=0.323; Interpretation: The 90-minute eccentric exercise protocol had a significant effect in increasing IL-6 (a measure of inflammation), with no treatment effect (almonds vs snack bar).

Estimated Marginal Means of MEASURE_1

Supplementation

4-wks

Post-90 min eccentric exercise

4-day recovery period

Supplement
Almond
Snack Bar

Monday
Pre-Suppl Pre-Exerc Post-Exerc Tues am Wed am Thurs am Fri am

Error bars: +/- 1 SE
Body levels of EpOMEs and DiHOMEs are dependent on CYP450 and sEH, and dietary intake of linoleic acid. 


PMID: 31931080, PMID: 31030167

sEH is a target for pharma and nutrition interventions.

**Linoleic Acid**

- **9(10)-EpOME**
- **12(13)-EpOME**
- **9(10)-DiHOME**
- **12(13)-DiHOME**
9,10-diHOME can DISRUPT mitochondrial function (Sisemore et al. Arch Biochem Biophys. 2001 Aug 1;392(1):32-7).

Almond intake (4 weeks) countered the 9,10-diHOME increase immediately following the 90-min eccentric exercise protocol.
Almond intake (4 weeks) augmented the 12-13-diHOME increase immediately following the 90-min eccentric exercise protocol.
Prior research indicated that 12,13-diHOME is secreted from brown adipose tissue (BAT) during exercise and increases fatty acid uptake and mitochondrial respiration in skeletal muscle. This lipokine is positively correlated with aerobic fitness and negatively related to BMI and fat mass.

Human brown adipose tissue (1.5% of body mass)

RED = activated BAT.

Increased fatty acid uptake and mitochondrial respiration and improved recovery.

Rats treated with antibiotics to deplete gut bacteria: sEH activity was strongly altered.

Proanthocyanidin

5-(3',4'-Dihydroxyphenyl)-γ-valerolactone

Influence on sEH and diHOME production

↑12,13-diHOME, ↓9,10-diHOME
Profile of Mood States: Fatigue Domain

Time effect, \( p<0.001 \); Time \times\ treatment effect, \( p=0.051 \); Interpretation: The 90-minute eccentric exercise protocol had a significant effect in increasing fatigue. The pattern of change across all time points tended to be different between groups (interaction effect, \( p=0.051 \)) with lower fatigue levels in the almond group. Change contrast between groups on Friday morning, \( p=0.031 \).

Estimated Marginal Means of MEASURE_1

Supplement
- Almond
- Snack Bar

4-wks

4-day recovery period

Supplementation

Monday
- Pre-Suppl
- Pre-Exerc
- Post-Exerc
- Tues am
- Wed am
- Thurs am
- Fri am

Post-90 min eccentric exercise

\( p=0.094 \)

\( p=0.031 \)

Error bars: +/- 1 SE
Profile of Mood States: Tension Domain

Time effect, p=0.006; Time x treatment effect, p=0.033; Interpretation: The pattern of change across all time points was different between groups (interaction effect, p=0.033) with lower tension levels in the almond group. P-values represent change contrast between groups.

Estimated Marginal Means of MEASURE_1

Supplement
- Almond
- Snack Bar

4-wks
4-day recovery period

Monday
Pre-Suppl  Pre-Exerc  Post-Exerc  Tues am  Wed am  Thurs am  Fri am

Post-90 min eccentric exercise

Error bars: +/- 1 SE
**Bench Press (repetitions)**

Time effect, \( p<0.001 \); Time x treatment effect, \( p=0.338 \); Interpretation: Bench press capacity (reps to fatigue) was significantly decreased after the 90-min exercise bout, but without group differences.

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**Estimated Marginal Means of MEASURE_1**

Error bars: +/- 1 SE
**Leg-Back Strength (kg/kg body weight)**

Time effect, $p=0.004$; Time x treatment effect, $p=0.029$; Interpretation: Leg-back strength (weight adjusted) was negatively affected in the snack bar group, but not the almond group (significant treatment effect). $P$-value contrasts showed group differences after 1 and 4 days recovery.

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**Estimated Marginal Means of MEASURE_1**

- **Supplement**
  - Almond
  - Snack Bar

- **Error bars:** +/- 1 SE
Conclusions

• Elevated post-exercise plasma levels of 12,13-DiHOME and lower 9,10-DiHOME with almond intake support positive metabolic outcomes for adults engaging in unaccustomed eccentric exercise bouts.

• Other almond related benefits for exercisers include reduced feelings of fatigue and tension, better leg-back strength during recovery, and decreased muscle damage during the first day of recovery.
Implications for Health Professionals

• Almond intake can be encouraged for physically active people to improve recovery from stressful levels of exercise.

• Supporting recovery with an accessible, whole food intervention such as almonds could increase compliance with exercise recommendations.

• Almonds can be a valuable addition to athletes’ diets because of the high and varied amounts of nutrients provided, and novel findings that almond intake augmented release of 12,13-DiHOME.

Implications for Consumers

• Almonds can be added to sports nutrition strategies to help with recovery from exercise.

• Regular almond intake may improve fatigue, tension and mood state after exercise, which could help encourage future exercise sessions.

• Almonds are food for fitness offering a nutrition package of healthy fats, the antioxidant vitamin E, and polyphenols that can help explain these beneficial outcomes. One serving of almonds (28g) has 13 g of good unsaturated fat and only 1 g of saturated fat and 50% of the Daily Value of Vitamin E.

Q&A