Therapeutic Diets for Cardiometabolic Disorders: What is the Evidence?
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Needs Assessment

The science of Nutrition is rapidly growing with respect to scientific discovery. There exists in the field of Endocrinology multiple disease states which are directly affected by diet and nutrition. This course will address the need for expertise in diet, and nutritional support to improve patient care for those with cardiometabolic disorders through understanding of nutritional principles and disease process. To better standardize the fund of knowledge, a curriculum dedicated to exposure of nutritional demands in various cardiometabolic disorders is very much needed.

Target Audience

The target audience for this meeting is the Physicians and interested Allied Health Professionals in attendance at the AACE Annual Meeting – Boston MA, April 21-25, 2010.

Overall Learning Objective

Improve care of patients with cardiometabolic disorders through better understanding of nutritional principles and the effect on disease process.

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Therapeutic Diets for Cardiometabolic Disorders: What is the Evidence?

7:30-8:00 a.m.  Registration/Continental Breakfast

8:00-8:05 a.m.  Introduction by co-chairs (Drs. Roubenoff and Mechanick)

Session 1.  Moderator – Ronenn Roubenoff, MD, MHS

8:05-8:30 a.m.  Low Carb-High Protein Diets for Obesity
Jeffrey Mechanick, MD, FACP, FACE, FACN
  • Be able to discuss these diets with your patients based on the scientific evidence
  • Understand the clear and not-so-clear differences among the various high carb - low protein diets in the management of obesity
  • Appreciate the knowledge gaps in research in the field of low carb – high protein diets for obesity

8:30-8:35 a.m.  Q&A

8:35-9:00 a.m.  Antioxidants and Vascular Inflammation
Mohsen Meydani, DVM, PhD, FAHA, FACN
  • Define vascular inflammation and its contribution to the development of cardiovascular diseases including arteriosclerosis.
  • Define and present the rate of oxidative stress associated with inflammation of the vascular system.
  • Address current concepts and evidence about the role of dietary antioxidants on vascular inflammation and their potential health benefits.

9:00-9:05 a.m.  Q&A

9:05-9:30 a.m.  Diets for Heart Disease Prevention and Treatment
Jun Dai, MD, PhD
  • Review the role of dietary fat in the prevention and treatment of heart disease
  • Describe the use of fish oil supplements in the prevention and treatment of heart disease
  • Discuss potential mechanisms underlying the role of dietary fat in heart disease

9:30-9:35 a.m.  Q&A

9:35-9:50 a.m.  Panel Discussion
9:50-10:05 a.m.  Break

**Session 2. Moderator – Dr. Jeff Mechanick**

10:05-10:30 a.m.  **Diet and Exercise for Sarcopenia and Cachexia**  
*Ronn Roubenoff, MD, MHS*

- Participants should be able to identify the difference between cachexia, sarcopenia, and wasting in terms of populations at risk, pathophysiology, and goals of therapy.
- Participants should be able to prescribe specific levels of dietary intake, micronutrient needs, and exercise prescription for sarcopenia and for cachexia.
- Participants should be able to develop a differential diagnosis for reduced physical performance in the setting of sarcopenia and cachexia.

10:30-10:35 a.m.  Q&A

10:35-11:00 a.m.  **Probiotics and Obesity**  
*Caroline Apovian, MD, FACP, FACN*

- Understand the interest in the gut microbiome and possible association with obesity.
- Understand what probiotics are and how they protect immune function
- Broaden understanding of the various possible causes of obesity.

11:00-11:05 a.m.  Q&A

11:05-11:30 a.m.  **Dietary Restrictions and Aging**  
*Susan Roberts, PhD*

- Understand the benefits of calorie restriction in animal species including rodents and non human primates
- Understand the state of human research on calorie restriction
- Consideration of whether calories restriction may be a feasible lifestyle intervention for humans

11:30-11:35 a.m.  Q&A

11:35-11:50 a.m.  Panel Discussion

11:50 a.m. – 12 Noon  Closing Remarks
AACE/ ASN Joint Meeting on Nutrition
April 21, 2010
Boston, MA

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AACE/ASN Meeting on Nutrition: Therapeutic Diets for Cardiometabolic Disorders: What is the Evidence?
April 21, 2010
Boston, MA

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Director, AACE
Chair, AACE Nutrition and Publications Committees

Program Co-Chair: Therapeutic Diets for Cardiometabolic Disorder: What is the Evidence?

LOW CARBOHYDRATE HIGH PROTEIN DIETS FOR OBESITY
Disclosures

- Honoraria for lectures from
  - Sanofi-Aventis
  - Abbott Nutrition
Outline

- **Perspective**
- What are high fat, High Protein, Low Carbohydrate diets (HPLC)?
- What is the theoretical advantage of HPLC?
- What is the clinical evidence supporting the use of HPLC?
- What is the potential impact of this evidence on the incorporation of HPLC in clinical practice guidelines for the treatment of obesity?
Perspective

- Clinical problem of obesity, type-2 diabetes (T2DM), and cardiovascular disease (CVD) risk
- Historically, low fat (<30%) with protein only 10-20% should manage fat accumulation with obesity and CVD (despite this, increased T2DM and obesity rates)

- Competing hypotheses
  - That it is total calories that matter to correct overweight/obesity and that this correction leads to CVD risk reduction
  - That there is a significant impact of macronutrient composition of our diets not only on weight loss but on CVD development

- Therefore, that HPLC diets may be therapeutic in reducing cardiometabolic disease
More controversy

- ADA does not recommend HPLC diets
- Institute of Medicine, Dietary Guidelines for Americans 2005 – calorie amount more important than macronutrient distribution
- Joslin Diabetes Center recommends a diet with 40% carbohydrate, 30-35% fat, and 20-30% protein (for overweight/obese patients with or at risk for T2DM)
- Conscious restriction of carbohydrate results in net reduction of total calories (also high fat decreases appetite); so weight loss is not an effect of diet composition *per se*
What are high fat, high protein, low carbohydrate diets (HPLC)?

- HPLC have protein and carbohydrate distributions deviating from “standard” recommendations
- Minimal amount of daily carbohydrate recommended is 130 g/day (ADA; IOM)
- Institute of Medicine healthy eating
  - Carbohydrate 45-65% (ADA 60%; DASH 57%; USDA 55%)
  - Fat 20-35% (ADA 25%; DASH 22%; USDA 29%)
  - Protein 10-35% (ADA 15%; DASH 21%; USDA 18%)
Summary of the HPLC “Diets”

- Atkins: induction phase (ketosis; < 20g/d)
- Bernstein: < 30g/d + supplements for nl BG
- Caveman (paleolithic): avoid processed food
- Mediterranean: red wine, lo-GI, MUFA, nuts
- Protein powder: 30-40 g/d + supplements
- Sonoma: Mediterranean-like + lo GI
- Sugar-Busters: avoid refined carbs
- South Beach: phased lo-GI diet
- The Zone: 40c-30F-30P highly structured
What about the Paleolithic diet?

- Based on the genomic programming (Cordain et al AJCN 2005;81:341–54)
  - Carbohydrate 22-40%
  - Fat 28-58% (high PUFA+MUFA; low ω-6/ω-3 ratio)
  - Protein 19-35%
  - Unresolved: genetic adaptation to lactose tolerance and consuming dairy products

- Benefit demonstrated but data limited
  - Frassetto et al Eur J Clin Nutr 2009; 63: 947-955
  - O’Dea Diabetes 1984 33:596-603
Mediterranean DIETS

- Not just one diet, but many different types
- Large amounts of fruits, vegetables, nuts, breads, cereals, potatoes, and legumes (wild plants; high in folate)
  - Plant-based with little meat
  - Moderate fish and alcohol (as red wine)
  - High in olive oil
  - High in polyphenols (protects plants from hot sun)
    - Extra virgin olive oil high in polyphenols
  - Cardiovascular benefits related to fatty acid changes and not LDL or HDL levels
Professional Society Recommendations for Diabetes

ADA: protein 15-20% with saturated fat < 7%, minimal trans fat, cholesterol < 200 mg/day, 2 servings of fish/week for ω-3 PUFA

- EASD: protein 10-20%, fat < 30%, carb 45-60%; sat and trans fat < 8-10%, MUFA 10-20%, PUFA up to 10%, 2-3 servings fish/wk with other plant sources ω-3 FA

- CDA: protein 15-20%, fat < 35%, carb 45-60%, low-GI foods, sat fat < 7%, MUFAs nonspec and limit trans fat, PUFA < 10%, incr ω-3 FA foods

- BDA/ Diab UK: protein up to 1 g/kg/day, fat up to 35%, carb 45-60%, sucrose up to 10%, sat and trans fat < 10%, ω-6 FA < 10%, fish 1-2 serv/wk

- Commonalities: grains, legumes, fiber and healthy wt
What is the theoretical advantage of HPLC?

- Restricts refined carbohydrates and lowers glycemic excursions, insulin levels, total calorie intake, and weight
- High protein may increase satiety – short term (Weigle et al AJCN 2005; 8241-48)
- Very low carbohydrate ketogenic diet may have anti-inflammatory effects – short term (decreased leptin, insulin resistance, weight, and fat mass) (Volek et al Lipids; 2009: 44: 297-309; Forsythe et al Lipids; 2008; 43: 65-77)
Concerns about HPLC diets

- Evidence lacking for long-term (1 year) benefit
- Increased risk for kidney stones
- Ketosis and bad breath
- Can be low in fiber, calcium, magnesium, potassium, iron, folate, thiamine
- (if) high in saturated fat then increase CVD risk
- (if) low in plant proteins, fiber, and fresh fruit, then increased constipation and increased cancer risk
Compromise

- Tailor an HPLC where
  - carbohydrate is replaced
    - With healthy fats, such as MUFA, PUFA and ω-3 FA (and not easily accessible saturated fats)
    - High biological value protein, especially plant proteins (which can be found in low-saturated fat foods)
  - Remaining carbohydrate is derived from high fiber-containing whole foods (low glycemic index/load)
<table>
<thead>
<tr>
<th>Dietary factor</th>
<th>Level of evidence for an association with</th>
<th>Methodological considerations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Risk of overweight(^b)</td>
<td>Risk of type 2 diabetes</td>
</tr>
<tr>
<td>Total carbohydrate intake</td>
<td>$\leftrightarrow [17, 20]$</td>
<td>$\leftrightarrow [21-24]$</td>
</tr>
<tr>
<td>Dietary fibre</td>
<td>$\downarrow \downarrow [15-17]$</td>
<td>$\downarrow \downarrow \downarrow [18]$</td>
</tr>
<tr>
<td></td>
<td>- May be particularly prone to residual confounding by lifestyle or socioeconomic factors, which epidemiological studies cannot fully control for</td>
<td></td>
</tr>
<tr>
<td>Whole grain intake</td>
<td>$\downarrow \downarrow [15, 16]$</td>
<td>$\downarrow \downarrow [19]$</td>
</tr>
<tr>
<td></td>
<td>- May be particularly prone to residual confounding by lifestyle or socioeconomic factors, which epidemiological studies cannot fully control for</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Definition of whole grain product varies between studies</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Ascertainment of whole grain content is difficult</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Whole grain-specific over-reporting cannot be precluded</td>
<td></td>
</tr>
<tr>
<td>High GI/GL</td>
<td>$\uparrow [20, 29]$</td>
<td>$\uparrow \uparrow [22, 23, 26]$</td>
</tr>
<tr>
<td></td>
<td>- Dietary assessment methods generally not designed to determine GI/GL. In some studies the validity for GI/GL may hence be very low</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Residual confounding by lifestyle related factors less likely</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- GI/GL specific under-reporting unlikely</td>
<td></td>
</tr>
<tr>
<td>Western food pattern(^c)</td>
<td>$\uparrow \uparrow [36, 37]$</td>
<td>$\uparrow \uparrow [31-35]$</td>
</tr>
<tr>
<td></td>
<td>- Food pattern depend on the population they were derived from</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Specific under-reporting of these socially less desirable foods cannot be precluded</td>
<td></td>
</tr>
<tr>
<td></td>
<td>- Identifiable food groups are determined by the dietary assessment tool (mostly FFQ) used</td>
<td></td>
</tr>
</tbody>
</table>

\(^a\) Level of evidence: $\uparrow$ strong, $\downarrow$ weak, $\leftrightarrow$ mixed, $\uparrow\uparrow$ strong, $\downarrow\downarrow$ weak, $\leftrightarrow\leftrightarrow$ balanced.

\(^b\) Risk of overweight and type 2 diabetes.

\(^c\) Based on Western food pattern.
Buyken et al. Diabetologia 2010; 53: 406-418
Molecular mediators of HPLC therapeutics effect – recent studies

- TaqIB polymorphism of the cholesterol ester transfer protein (CETP) gene (Du et al J Nutr Biochem 2010)
- C358A missense polymorphism of the degrading enzyme fatty acid amide hydrolase (FAAH) gene (Deluis et al Metab Clin Exp 2010)
- TCF7L2 rs7903146 T-risk allele macronutrient interactions (Grau et al AJCR 2010; 91: 472-479)
Evidence

No difference among diets: all had decreased adherence

Modified DASH diets associated with decreased BP and LDL

Replacing 10% DASH carb with protein (48c-27L-25P) OR with MUFA (48C-37L-15P)

Hi MUFA or Pro improved GTT and lipids and decreased appetite

meta-analysis of PRCTs low carb vs. low fat – unfavorable ↑LDL

<table>
<thead>
<tr>
<th></th>
<th>60% Carbohydrate diet (n = 30)</th>
<th>40% Carbohydrate diet (n = 27)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbohydrate</td>
<td>57 ± 4&lt;sup&gt;2&lt;/sup&gt;</td>
<td>41 ± 4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Protein</td>
<td>18 ± 2</td>
<td>18 ± 2</td>
<td>0.70</td>
</tr>
<tr>
<td>Total fat</td>
<td>25 ± 4</td>
<td>41 ± 5</td>
<td>&lt; 0.0001</td>
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<tr>
<td>Saturated fat</td>
<td>9 ± 2</td>
<td>8 ± 2</td>
<td>0.14</td>
</tr>
<tr>
<td>Polyunsaturated or</td>
<td>16 ± 3</td>
<td>33 ± 4</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>monounsaturated fat</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

No difference in weight loss but Mediterranean-type diet improved CVD markers (insulin and TG)

Carbohydrate replaced by MUFA/PUFA

Figure 2. Weight Changes during 2 Years According to Diet Group. Vertical bars indicate standard errors. To statistically evaluate the changes in weight measurements over time, generalized estimating equations were used, with the low-fat group as the reference group. The explanatory variables were age, sex, time point, and diet group.

Only improvements in surrogate markers – no outcome studies (Wylie-Rosett and Davis Curr Diab Rep 2009; 9: 396-404)

811 randomized patients x 2 years on calorie restricted diets – no difference based on nutrient composition (adherence was poor)
### A High Protein Diet With Resistance Exercise Training Improves Weight Loss And Body Composition In Overweight And Obese Patients With Type 2 Diabetes

Thomas P Wycherley¹,² (BSc (Hons)), Manny Noakes¹ (PhD), Peter M Clifton¹ (PhD), Xenia Caneuţou³ (MND), Jennifer B Keogh¹ (PhD), Grant D Brinkworth¹ (PhD)

<table>
<thead>
<tr>
<th></th>
<th>CON (n=16)</th>
<th>HP (N=12)</th>
<th>CON + RT (n=17)</th>
<th>HP + RT (n=14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kJ)</td>
<td>6278 ± 648</td>
<td>6321 ± 763</td>
<td>6199 ± 696</td>
<td>6339 ± 649</td>
</tr>
<tr>
<td>Carbohydrate (g)</td>
<td>197.4 ± 16.3</td>
<td>176.3 ± 23.7</td>
<td>195.0 ± 21.5</td>
<td>170.0 ± 23.1</td>
</tr>
<tr>
<td>Carbohydrate (% of energy)</td>
<td>53.6 ± 2.6</td>
<td>47.4 ± 1.6</td>
<td>53.6 ± 3.9</td>
<td>45.5 ± 2.4</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>68.4 ± 5.9</td>
<td>119.0 ± 7.8</td>
<td>68.0 ± 8.3</td>
<td>117.1 ± 6.7</td>
</tr>
<tr>
<td>Protein (% of energy)</td>
<td>18.6 ± 0.9</td>
<td>32.3 ± 2.8</td>
<td>18.7 ± 1.3</td>
<td>31.6 ± 2.2</td>
</tr>
<tr>
<td>Fat (g)</td>
<td>38.5 ± 7.7</td>
<td>30.5 ± 8.2</td>
<td>37.5 ± 9.6</td>
<td>33.7 ± 5.5</td>
</tr>
<tr>
<td>Fat (% of energy)</td>
<td>22.6 ± 3.0</td>
<td>17.7 ± 3.0</td>
<td>22.3 ± 4.5</td>
<td>19.6 ± 1.9</td>
</tr>
<tr>
<td>Saturated fat (% of total fat)</td>
<td>34.1 ± 5.5</td>
<td>33.9 ± 5.0</td>
<td>33.2 ± 2.8</td>
<td>34.3 ± 4.3</td>
</tr>
<tr>
<td>Polyunsaturated fat (% of total fat)</td>
<td>19.8 ± 4.5</td>
<td>22.3 ± 3.6</td>
<td>21.4 ± 4.5</td>
<td>21.0 ± 4.2</td>
</tr>
<tr>
<td>Monounsaturated fat (% of total fat)</td>
<td>46.1 ± 6.6</td>
<td>43.9 ± 4.1</td>
<td>45.5 ± 5.4</td>
<td>44.8 ± 5.1</td>
</tr>
<tr>
<td>Diet Fibre (g)</td>
<td>31.1 ± 2.9</td>
<td>24.7 ± 4.0</td>
<td>30.5 ± 4.4</td>
<td>22.6 ± 4.1</td>
</tr>
</tbody>
</table>

Data are means ± SD. The treatment groups were a standard carbohydrate, low protein, low fat diet alone (CON) or with resistance exercise training (HP); or an isocaloric higher protein, low fat diet alone (HP) or with resistance exercise training (HP+RT). Differences between groups (one way ANOVA)

¹ Comparison of the difference between the diets (CON and CON+RT vs. HP and HP+RT) (planned contrast)
<table>
<thead>
<tr>
<th></th>
<th>Group</th>
<th>CON</th>
<th>HP</th>
<th>CON + RT</th>
<th>HP + RT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n=16)</td>
<td>(n=12)</td>
<td>(n=17)</td>
<td>(n=14)</td>
<td><strong>P</strong></td>
</tr>
<tr>
<td><strong>Body Weight (kg)</strong></td>
<td>Week 0</td>
<td>97.0 ± 10.6</td>
<td>102.7 ± 15.4</td>
<td>105.0 ± 15.3</td>
<td>107.6 ± 15.5</td>
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<tr>
<td></td>
<td>Week 16</td>
<td>88.4 ± 11.2</td>
<td>93.7 ± 13.8</td>
<td>94.5 ± 15.4</td>
<td>93.8 ± 13.5</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>-8.6 ± 4.6*</td>
<td>-9.0 ± 4.8*</td>
<td>-10.5 ± 5.1</td>
<td>-13.8 ± 6.0</td>
</tr>
<tr>
<td><strong>Body Mass Index (kg/m²)</strong></td>
<td>Week 0</td>
<td>34.8 ± 4.9</td>
<td>35.6 ± 3.8</td>
<td>34.9 ± 4.9</td>
<td>36.6 ± 5.0</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>31.7 ± 5.1</td>
<td>32.5 ± 3.1</td>
<td>31.4 ± 4.3</td>
<td>31.9 ± 4.3</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>-3.1 ± 1.6</td>
<td>-3.2 ± 1.7</td>
<td>-3.5 ± 1.7</td>
<td>-4.7 ± 2.1</td>
</tr>
<tr>
<td><strong>Total Body Fat Mass (kg)</strong></td>
<td>Week 0</td>
<td>38.5 ± 8.0</td>
<td>40.4 ± 8.4</td>
<td>40.4 ± 10.0</td>
<td>42.9 ± 11.6</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>32.1 ± 9.5</td>
<td>33.2 ± 6.9</td>
<td>32.3 ± 10.7</td>
<td>31.5 ± 11.6</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>-6.5 ± 3.7*</td>
<td>-7.1 ± 4.0*</td>
<td>-8.1 ± 3.8</td>
<td>-11.4 ± 3.9</td>
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<tr>
<td><strong>Waist Circumference (cm)</strong></td>
<td>Week 0</td>
<td>111.3 ± 10.7</td>
<td>114.3 ± 6.8</td>
<td>113.7 ± 8.5</td>
<td>116.2 ± 12.7</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>103.2 ± 12.8</td>
<td>105.4 ± 6.7</td>
<td>102.4 ± 9.6</td>
<td>102.5 ± 11.8</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>-8.2 ± 4.6*</td>
<td>-8.9 ± 3.9*</td>
<td>-11.3 ± 4.6</td>
<td>-13.7 ± 4.6</td>
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<tr>
<td><strong>Total Fat Free Mass (kg)</strong></td>
<td>Week 0</td>
<td>58.5 ± 10.7</td>
<td>62.3 ± 13.0</td>
<td>64.6 ± 12.4</td>
<td>64.7 ± 11.5</td>
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<tr>
<td></td>
<td>Week 16</td>
<td>56.3 ± 10.6</td>
<td>60.4 ± 13.2</td>
<td>62.2 ± 12.0</td>
<td>62.3 ± 10.7</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>-2.2 ± 1.9</td>
<td>-1.9 ± 1.5</td>
<td>-2.4 ± 2.5</td>
<td>-2.4 ± 3.1</td>
</tr>
<tr>
<td><strong>Single Repetition Bench Press (kg)</strong></td>
<td>Week 0</td>
<td>60.0 ± 18.1</td>
<td>68.5 ± 27.4</td>
<td>67.1 ± 22.4</td>
<td>64.6 ± 25.5</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>58.1 ± 17.7</td>
<td>66.0 ± 25.1</td>
<td>76.2 ± 23.6</td>
<td>75.5 ± 28.9</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>-1.9 ± 4.8*</td>
<td>-2.5 ± 8.0*</td>
<td>9.1 ± 8.5</td>
<td>10.9 ± 8.2</td>
</tr>
<tr>
<td><strong>Single Repetition Lat Pull-down (kg)</strong></td>
<td>Week 0</td>
<td>49.8 ± 15.1</td>
<td>56.7 ± 15.4</td>
<td>55.0 ± 14.8</td>
<td>55.7 ± 18.7</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>50.4 ± 14.9</td>
<td>57.2 ± 15.9</td>
<td>66.2 ± 17.4</td>
<td>65.0 ± 20.7</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>0.6 ± 4.9*</td>
<td>0.6 ± 3.7*</td>
<td>11.2 ± 6.0</td>
<td>9.3 ± 4.9</td>
</tr>
<tr>
<td><strong>Systolic Blood Pressure (mmHg)</strong></td>
<td>Week 0</td>
<td>137 ± 12</td>
<td>141 ± 11</td>
<td>137 ± 10</td>
<td>138 ± 9</td>
</tr>
<tr>
<td></td>
<td>Week 16</td>
<td>124 ± 11</td>
<td>125 ± 11</td>
<td>122 ± 9</td>
<td>124 ± 9</td>
</tr>
<tr>
<td></td>
<td><strong>Change</strong></td>
<td>-13 ± 11</td>
<td>-16 ± 13</td>
<td>-16 ± 7</td>
<td>-14 ± 9</td>
</tr>
<tr>
<td><strong>Diastolic Blood Pressure (mmHg)</strong></td>
<td>Week 0</td>
<td>79 ± 9</td>
<td>83 ± 9</td>
<td>81 ± 8</td>
<td>79 ± 8</td>
</tr>
</tbody>
</table>
**Conclusion:** An energy restricted HP diet combined with RT achieved greater weight loss and more favourable changes in body composition. All treatments had similar improvements in glycemic control and CVD risk markers.
Hernandez et al. Lack of suppression of circulating free fatty acids and hypercholesterolemia during weight loss on a high fat, low-carbohydrate diet. AJCN 2010

20 g carb/day vs. 55% carb; both groups lost wt and suppressed insulin levels but low carb diet failed to suppress FFA and this may be reason for persistently elevated LDL-c

The two groups were similar re weight loss, lipids, and glycemic control (results content with Wadden et al Obesity 2009 – no effect lo carb diet on weight)

Low carb diet better for BP reduction
Analyzing the evidence

- HPLC diets found to improve certain surrogate markers for CVD risk (LDL-c, CRP, BP, insulin)

- However, HPLC diets found to accelerate atherosclerosis via other physiological pathways (non-esterified fatty acids [NEFA] and endothelial progenitor cells [EPC])

- Methodological pitfalls: adherence, incorporating physical activity and other lifestyle change

Smith SR A look at the Low-Carbohydrate Diet NEJM 2009; 361:23
What about Carbohydrate Blockers?

- Soluble fiber
- Resistant starches
- Pulses (dietary non-oil seeds: chickpeas, beans, peas, lentils)
- Low glycemic index/load foods
- Amylase-inhibitor (white kidney bean, wheat, hibiscus extracts)
- Alpha-glucosidase inhibitors (L-arabinose)
- Glucose transport inhibitors (Gymnema sylvestre, apple extract, flavonoids, eucalyptus leaf)
What is the potential impact of this evidence on the incorporation of HPLC in clinical practice guidelines for the treatment of obesity?
Conclusions

- Inconclusive results of HPLC diets based on well-conducted PRCTs
- Inconsistencies due to problems with surrogate markers and extrapolating experimental conditions to real-life scenarios
- Need a systems approach to better understand complexities of cardiometabolic disorders and fashion practical solutions incorporating healthy eating, physical activity, pharmacology, novel molecular targeted therapies, and even surgery
- For now, best to compute protein needs based on weight, compute nonprotein kcal based on energy for a healthy body composition, and choose healthy foods
- Future CPG will retain many conservative features while incorporating emerging data and innovative ideas
AACE Nutrition Initiatives

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POWER OF PREVENTION®

AMERICAN COLLEGE OF ENDOCRINOLOGY

Co-edited by Jeffrey I. Mechanick, MD, FACP, FACE, FACN and Elise M. Bierer, MD, FaCE, CNSP
Genetic polymorphisms as determinants for disease-preventive effects of vitamin E

Jean-Marc Zingg, Angelo Azzi, and Mohsen Meydani

Polymorphisms in genes involved in vitamin E uptake, distribution, metabolism, and molecular action may be important determinants for the protective effects of vitamin E supplementation. The haptoglobin 2-2 polymorphism is associated with increased production of oxygen free radicals and reduces levels of vitamin E and C; the consequent elevated risk for cardiovascular disease can be prevented by vitamin E supplementation.

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INTRODUCTION

Atherosclerosis underlies important adverse vascular events, such as coronary artery disease, stroke, and peripheral artery disease, which are responsible for most of the cardiovascular morbidity and mortality. In the last two decades, numerous clinical studies have addressed the possible benefits of supplementation with vitamin E (α-tocopherol) and other micronutrients against atherosclerosis and other diseases such as cancer and neurodegeneration (reviewed previously1–6). Randomized clinical trials and epidemiologic studies with vitamin E supplementation intended to protect against cardiovascular disease (CVD) reported both positive and negative effects (reviewed previously5). Recent meta-analyses of the clinical studies even suggested there is increased all-cause mortality with high doses of vitamin E supplementation.7,8 However, there is little evidence for adverse effects of vitamin E in adults when taken below the tolerable upper limit intake (1000 mg/day vitamin E according to the Food and Nutrition Board of the Institute of Medicine).5 Vitamin E supplementation studies were generally aimed at reducing the amount of free radicals generated by inflammatory processes during disease development, and they were based on the findings that vitamin E levels in plasma and tissues can be increased by dietary supplementation where it chemically can act as an antioxidant.

Several factors have been proposed to explain the often null outcome of vitamin E supplementation in human studies, in particular, the relatively short duration of supplementation and the presence of high baseline levels of vitamin E in the normal diet sufficient to prevent disease symptoms.9,10 Moreover, the dosage of vitamin E supplementation may have been too low, since a detectable reduction in the biomarkers of oxidative damage has only been achieved with much higher doses of vitamin E (>1600 IU) than those used in most primary and secondary prevention studies.11,12 The potential health effects of vitamin E supplementation may become evident only under specific environmental and pathophysiological circumstances, such as local depletion of vitamin E by free radicals associated with inflammation, infection, smoking, or UV irradiation. However, patients with severe atherosclerosis display an imbalance of oxidant/antioxidant status in plasma that can be corrected by vitamin E supplementation, but with no effect on atherosclerotic plaques.13 A recent study by Milman et al.14 suggests that polymorphisms in specific genes, such as the haptoglobin gene, may increase the level of free radicals in diabetic patients and contribute to individual differences in response to vitamin E supplementation.

ROLE OF OXIDATIVE DAMAGE AND HAPTOGLOBIN GENOTYPE IN THE PATHOGENESIS OF Atherosclerosis

Oxidation of LDL (oxLDL) as a result of excess production of free radicals or their insufficient scavenging by antioxidant enzymes and redox-reactive micronutrients...
is recognized to play a central role in atherogenesis; however, a variety of other factors contribute to its development and progression (reviewed previously). The oxidized lipids in oxLDL stimulate proliferation of vascular smooth muscle cells (VSMC) and accumulate in macrophages and VSMC, converting them to foam cells forming fatty streaks and atherosclerotic plaques. LDL oxidation is also catalyzed by hemoglobin through heminitiated globin radical formation that can be prevented by irreversible binding of hemoglobin to haptoglobin, a plasma acute-phase α2-glycoprotein induced during inflammatory and neoplastic disease. A higher plasma oxLDL/LDL ratio has been observed in individuals with the haptoglobin 2-2 polymorphism, a genotype that occurs with a frequency of about 36% in Western societies. 

Hemoglobin (Hb), which is released by intravascular hemolysis of red blood cells, is a strong lipid oxidant through oxidation of its bound Fe to Fe. Haptoglobin (Hp) is a plasma protein that binds with high affinity to hemoglobin forming a Hb-Hp complex, which prevents hemoglobin’s oxidative damage (reviewed previously). This complex is then recognized and degraded by the reticuloendothelial system via endocytosis through the hemoglobin scavenger receptor CD163, as well as by other receptors. In the context of cardiovascular disease, the CD163 receptor is expressed in monocytes and upregulated during their differentiation to macrophages; moreover, several cytokines such as interleukin (IL)-6 and IL-10 trigger induction of CD163 and of Hp (Figure 1).

In humans, the Hp gene exists mainly as two alleles Hp 1 and Hp 2, leading to haptoglobin 1-1, 1-2, and 2-2 genotypes. The magnitude of antioxidant activity of the Hp protein is measured to be in the order of Hp 1-1 > Hp 1-2 > Hp 2-2 > probucol > vitamin E. The Hp-1-1 genotype is associated with resistance to the development of several diseases such as diabetic retinopathy, diabetic nephropathy, and CVD. In addition to that, the expression level of the CD163 receptor on macrophages at sites of vascular injury may play an important role in clearing the Hb released during plaque progression.

Since the Hb-Hp 1-1 complex is readily recognized by CD163 and more rapidly cleared, oxidative damage caused by hemoglobin in this complex may be low. In contrast, the Hp 2-2 protein binds with 10-fold higher affinity to hemoglobin, but this complex is less efficiently cleared, which may lead to oxidative damage to the vascular wall. In this situation, supplementation with antioxidants such as vitamin E or C may show potent preventive effects. The formation of glycated hemoglobin and advanced glycation endproducts in diabetic patients may further accelerate the damage induced by non-degraded hemoglobin in the vascular wall.

**INFLUENCE OF GENETIC POLYMORPHISMS ON VITAMIN E-MEDIATED HEALTH BENEFITS**

A recent, prospective, randomized, double-blind, placebo-controlled clinical trial by Milman et al. found that middle-aged patients (1424 patients, ≥55 years) with type-2 diabetes and the haptoglobin 2-2 genotype show reduced cardiovascular events when supplemented with vitamin E (400 IU/d) for 18 months. These results confirm a previous association of the haptoglobin 2-2 phenotype and the preventive effects of vitamin E supplementation against myocardial infarction and cardiovascular death, which was obtained from reanalyzing data from the HOPE (Heart Outcomes Prevention Evaluation) study. Interestingly, the recent findings of Milman et al. are in contrast with the main conclusion of the original HOPE study, which failed to demonstrate the clinical benefit of vitamin E in patients with advanced CVD, as well as with a follow-up study (HOPE-TOO) that reported no detectable preventive effect of vitamin E against cancer or major cardiovascular events in patients with vascular disease or diabetes mellitus. Thus, as exemplified by the study of Milman et al., the preventive effects of vitamin E supplementation may depend on the presence of polymorphisms of specific genes involved in the production and scavenging of reactive oxygen and reactive nitrogen species and maintaining the balance within the antioxidant network.

In addition, polymorphisms in genes involved in the uptake, distribution, metabolism, and secretion of antioxidant micronutrients may play an important role in micronutrient bioavailability. For example, a number of genetic and epigenic polymorphisms (that can occur in the homozygote or heterozygote state) may lower the bioavailability and cellular activity of vitamin E, which could be circumvented by vitamin E supplementation (Table 1). As reviewed by Rigotti, several proteins participate in the uptake, distribution, and metabolism of vitamin E, and polymorphisms in these proteins and their cellular expression levels may explain individual differences in vitamin E uptake and response, thereby influencing a differential susceptibility to disorders such as atherosclerosis, certain cancers, and neurodegenerative diseases. For reasons as yet unknown, patients respond differently to supplemented vitamin E, and they achieve different levels of plasma vitamin E after supplementation. These differences could be the consequence of gene defects or polymorphisms resulting in changes in vitamin E transport efficiency, the rate of metabolism, the structure and plasma levels of lipoproteins, the status of other micronutrients involved in α-tocopherol protection, as well as some environmental factors. Furthermore, it appears possible that polymorphisms in the postulated kinases, which are required for the synthesis of the more active
**Figure 1** Preventive action of vitamin E against cardiovascular disease in subjects with the haptoglobin 2-2 genotype.

**A. Depletion of vitamin E and C in subjects with the Hp 2-2 genotype.** Hemoglobin (Hb) oxidation leads to depletion of vitamin E (αT) and C (AA) that can be prevented by haptoglobin (Hp). Hp 2-2 is less efficient than Hp 2-1 or 1-1 in preventing the oxidation of Hb; supplementation of subjects with the Hp 2-2 genotype prevents vitamin E and C depletion, restores the cellular functions of these vitamins, and reduces the formation of oxidized lipoproteins (oxLDL). Oxidation of vitamin E and C may be increased in subjects with diabetes or inflammation (stippled arrows). DHA, dehydroascorbic acid; αTQ, α-tocopheryl quinone.

**B. Increased oxidation of LDL in subjects with the Hp 2-2 genotype.** Hemoglobin released during vascular injury and hemolysis increases the formation of oxLDL. Uptake of oxLDL by macrophages by the scavenger receptors (SR) can convert them to foam cells. Alternatively, released Hb is bound by Hp and the complex removed and inactivated via the CD163 receptor by macrophages or the reticuloendothelial system. Hp 2-2 is less efficient than Hp 2-1 or 1-1 in removing Hb; increased formation of oxLDL in subjects with the Hp 2-2 genotype can be prevented by supplementation with vitamin E and C. Oxidation of LDL may be increased in subjects with diabetes or inflammation (stippled arrows).

**C. Increased uptake of oxLDL in subjects with the Hp 2-2 genotype.** When the formation of oxLDL is a strong determinant for the development of atherosclerosis, such as in subjects with the Hp 2-2 genotype, overexpression of scavenger receptors on monocytes/macrophages and vascular smooth muscle cells (VSMC) may increase the formation of atherosclerotic foam cells. Vitamin E reduces the uptake of oxLDL by antagonizing the induction of the CD36 scavenger receptor by oxLDL on monocytes/macrophages and VSMC. Oxidation of LDL and CD36 expression may be increased in subjects with diabetes or inflammation (stippled arrows).
<table>
<thead>
<tr>
<th>Candidate gene</th>
<th>Function in relation to vitamin E</th>
<th>Possible effects on vitamin E action when mutated or polymorph</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apolipoprotein E (ApoE)</td>
<td>Increased levels of free radicals and depletion of vitamin E and C, plasma lipoprotein turnover, vitamin levels in plasma, and tissue</td>
<td>Polymorphisms in ApoE, such as ApoE4, ApoE3, or ApoE2 are known to generate increased oxidative stress (apoE4 &gt; apoE3 &gt; apoE2). ApoE determines the uptake of HDL vitamin E into peripheral tissues via binding to SR-BI, ApoE4 genotype is associated with a lower tissue level, but an increased plasma level of vitamin E</td>
<td>Peroutka et al. (2000) [33] Gomez-Coronado et al. (2002) [34] Mardones et al. (2002) [35] Lodge et al. (2004) [36] Borel et al. (2007) [37] Jofre-Monseny et al. (2007) [38] Jofre-Monseny et al. (2008) [39]</td>
</tr>
<tr>
<td>Apolipoprotein A (ApoA)</td>
<td>Plasma lipoprotein turnover, vitamin E levels in plasma and tissues</td>
<td>Polymorphisms in ApoA, such as ApoA-IV may affect the level of γ-tocopherol in plasma and tissue</td>
<td>Borel et al. (2007) [37]</td>
</tr>
<tr>
<td>SR-BI scavenger receptor</td>
<td>Vitamin E uptake and transport to peripheral tissues</td>
<td>Polymorphisms in SR-BI may influence vitamin E levels in peripheral cells and tissues</td>
<td>Borel et al. (2007) [37]</td>
</tr>
<tr>
<td>CD36 scavenger receptor</td>
<td>Vitamin E downregulates the expression of CD36 with consequent reduced foam cell formation</td>
<td>Polymorphisms in the CD36 gene (promoter polymorphisms, alternative splicing) may influence responsiveness to vitamin E</td>
<td>Ma et al. (2004) [40] Zingg et al. (2002) [41] Andersen et al. (2006) [42] Cheung et al. (2007) [43]</td>
</tr>
<tr>
<td>LDL-receptor</td>
<td>Removal of LDL form plasma, vitamin E uptake</td>
<td>Polymorphisms may influence plasma lipid profile and possibly vitamin E levels in plasma and tissues</td>
<td>Doring et al. (2004) [44] Davis et al. (2005) [45]</td>
</tr>
<tr>
<td>Phospholipid transfer protein (PLTP)</td>
<td>Exchange of vitamin E between lipoproteins</td>
<td>Polymorphism in PLTP may influence the level of vitamin E in different lipoproteins (VLDL; LDL, HDL, chylomicrons)</td>
<td>Kostner et al. (1995) [46] Jiang et al. (2002) [47]</td>
</tr>
<tr>
<td>Microsomal triglyceride transfer protein (MTTP)</td>
<td>Incorporation of vitamin E into chylomicron</td>
<td>Polymorphisms in MTTP may influence the uptake efficiency of vitamin E</td>
<td>Anwar et al. (2007) [48]</td>
</tr>
<tr>
<td>Afamin</td>
<td>Vitamin E transport in cerebrospinal liquid and brain</td>
<td>Polymorphisms in afamin may influence levels of vitamin E in the nervous system</td>
<td>Voegele et al. (2002) [55]</td>
</tr>
</tbody>
</table>
Potentially, phosphatases, which are required for hydrolyzing it, may play a role in determining the cellular activity of vitamin E.

Potential Benefits of Combined Antioxidant Therapy

Vitamin E supplements may have to be coadministered with vitamin C (L-ascorbic acid) to effectively lower the amount of free radicals. In fact, several prevention studies indicate that the combination of vitamins E and C is more potent in preventing atherosclerosis, and it remains to be determined whether subjects with the Hp 2-2 genotype benefit even more when both vitamins are supplemented together. Vitamin C present in plasma and in the arterial wall efficiently prevents vitamin E oxidation and improves the myocardial and endothelial functions, indicating that an intact cellular antioxidant network is important for maintaining cellular homeostasis.

On the other hand, vitamin C may have a pro-oxidant activity by facilitating redox-cycling of free iron and increasing hemoglobin-iron-driven peroxidation. Thus, polymorphisms of L-ascorbic acid binding proteins involved in uptake, tissue distribution and metabolism, and their cellular expression levels could contribute to the individual vitamin E response (Table 1). In line with this, serum vitamin C concentrations were lowest in serum from subjects with Hp 2-2, whereas other endogenous antioxidants (uric acid, bilirubin, albumin, ceruloplasmin, and total antioxidant status) were not different.

<table>
<thead>
<tr>
<th>Candidate gene</th>
<th>Function in relation to vitamin E</th>
<th>Possible effects on vitamin E action when mutated or polymorph</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lipoprotein lipase (LPL)</td>
<td>Vitamin E transfer from lipoproteins into peripheral tissues</td>
<td>Polymorphisms of LPL may influence plasma and tissue levels of vitamin E</td>
<td>Traber et al. (1985)</td>
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<td>Traber et al. (1992)</td>
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<td></td>
<td></td>
<td>Doring et al. (2004)</td>
</tr>
<tr>
<td>ATP binding cassette transporter A1 (ABCA1)</td>
<td>Vitamin E export from cells</td>
<td>Polymorphisms in ABCA1 may influence cellular and tissue levels of vitamin E</td>
<td>Oram et al. (2001)</td>
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<td></td>
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<td>Mustacich et al. (2006)</td>
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<td>Frikke-Schmidt et al. (2008)</td>
</tr>
<tr>
<td>Pregnane X receptor (PXR)</td>
<td>Vitamin E-mediated gene expression</td>
<td>Polymorphisms may influence vitamin E-dependent gene expression of PXR target genes</td>
<td>Landes et al. (2003)</td>
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<td></td>
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<td>Doring et al. (2004)</td>
</tr>
<tr>
<td>Multidrug resistance protein (MDR2)</td>
<td>Involved in biliary secretion of vitamin E</td>
<td>Polymorphisms in MDR2 may influence levels of vitamin E and enterohepatic circulation</td>
<td>Mustacich et al. (1998)</td>
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<td></td>
<td></td>
<td></td>
<td>Doring et al. (2004)</td>
</tr>
<tr>
<td>P450-cytochromes (Cyp3A and Cyp4F2)</td>
<td>Vitamin E metabolism</td>
<td>Polymorphisms in metabolic genes may influence plasma and tissue levels of α-, β-, γ-, and δ-tocopherols, as well as the level of their metabolites</td>
<td>Birringer et al. (2001)</td>
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<td>Sontag et al. (2002)</td>
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<td>Doring et al. (2004)</td>
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<td></td>
<td></td>
<td></td>
<td>Mustacich et al. (2006)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Abe et al. (2007)</td>
</tr>
<tr>
<td>Dehydroascorbate reductase, e.g., omega class glutathione transferases (GSTO1-1 or GSTO2-2)</td>
<td>Regeneration of vitamin C and thus regeneration of vitamin E</td>
<td>Polymorphisms in GSTO1-1 or GSTO2-2 may influence plasma and tissue levels of vitamin C, and thus of vitamin E</td>
<td>Withbread et al. (2005)</td>
</tr>
<tr>
<td>Sodium-coupled vitamin C transporters 1 and 2 (SVCT1/SLC23A1, SVCT2/SLC23A2) or dehydroascorbate transporters (GLUT1, GLUT3)</td>
<td>Regeneration of vitamin E by vitamin C</td>
<td>Polymorphisms in SVCT1 or SVCT2 may influence the plasma and tissue level of vitamin C, and thus that of vitamin E</td>
<td>Hediger (2002)</td>
</tr>
<tr>
<td></td>
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<td>Na et al. (2006)</td>
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<td>Seno et al. (2004)</td>
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<td>Erichsen et al. (2006)</td>
</tr>
</tbody>
</table>
most likely resulting in a lower efficiency of regeneration of vitamin E by vitamin C in these subjects.\textsuperscript{32,69}

At present, the effects of vitamin E supplementation in diabetic patients with the haptoglobin 2-2 genotype are mainly explained by its antioxidant action, but other effects of vitamin E supplementation cannot be excluded. Decreased levels of vitamins E and C as a result of oxidative utilization in subjects with the Hp 2-2 genotype most likely also affect alternative non-antioxidant mechanisms resulting from the modulation of cellular signaling and gene expression by interacting with and regulating specific enzymes and transcription factors or influencing cellular structures, such as membranes and lipid domains (reviewed in \textsuperscript{73,89–91}). Hp 2-2 in the Hb-Hp-complex may induce a different cellular response when compared to Hp 1-2 or Hp 1-1, and it can be speculated that the higher preventive effects of vitamin E may also result from the modulation of Hb-Hp 2-2-induced and CD163-mediated signal transduction in antioxidant or non-antioxidant manners.

**CONCLUSION**

According to the study of Milman et al.,\textsuperscript{14} clear preventive effects of vitamin E are only seen after selection of a patient group with the Hp 2-2 genotype and diabetes mellitus, which is characterized by increased production of oxygen free radicals. Compared to previous clinical trials with vitamin E supplementation, these findings suggest that confounding factors, such as polymorphisms in the Hp 2-2 genotype, are insufficient to increase the statistical power of studies to demonstrate a positive outcome effect of vitamin E supplementation, even though the prevalence of the Hp 2-2 genotype is 36% in the general population. Moreover, as shown with the HOPE-TOO study,\textsuperscript{31} even when focusing on diabetic patients, the Hp 2-2 allele, which occurs together with diabetes in only 2–3% of the general population, did not shift the overall outcome of this large clinical trial.

In view of these findings, it is likely that additional mutations and polymorphisms in those genes known to modulate vitamin E absorption, distribution, transport, activity, metabolisms, and excretion, will be found that could shift the balance in the redox-active cellular network and generate low vitamin E bioavailability, resulting in similar deficiency syndromes with possible influences on atherosclerotic and associated cardiovascular events that can be prevented by additional vitamin E supplementation. Moreover, species-specific polymorphisms and gene variants may help explain why animal studies of vitamin E supplementation generally show more positive outcomes against atherosclerosis in many experimental settings, such as the clearer beneficial effects of vitamin E supplementation seen in vitamin E-deficient animals\textsuperscript{92} or in animals having specific mutations, such as in knockout mice for the apolipoprotein E gene (ApoE\textsuperscript{−−}), the low-density lipoprotein receptor (LDL-R\textsuperscript{−−}) or the α-tocopherol transfer protein (α-TTP\textsuperscript{−−}).

Whereas the concept of individualized medicine with drugs designed specifically against disease-causing molecular targets is currently well accepted, the data from the haptoglobin polymorphism and vitamin E study by Milman et al.\textsuperscript{14} provide a stronger scientific basis to help establish guidelines for recommending individualized supplementation with micronutrients based on polymorphisms in specific genes.

**Acknowledgment**

_Funding:_ This work is supported by grant USDA contract #58-1950-7-707. JMZ is supported by a Phosphagenics Ltd. Research fellowship.

**REFERENCES**


Potential health benefits of avenanthramides of oats
Mohsen Meydani

Oats are known to be a healthy food for the heart due mainly to their high β-glucan content. In addition, they contain more than 20 unique polyphenols, avenanthramides, which have shown strong antioxidant activity in vitro and in vivo. The polyphenols of oats have also recently been shown to exhibit anti-inflammatory, antiproliferative, and anti-itching activity, which may provide additional protection against coronary heart disease, colon cancer, and skin irritation.

INTRODUCTION

Epidemiological evidence has indicated that a high intake of whole-grain foods is associated with a lower risk for coronary heart disease (CHD) and diabetes.\(^1-4\) Whole-grain foods contain a significant amount of fiber, which is believed to be the major factor contributing to their beneficial effects on CHD and diabetes. Thus, several epidemiological studies have focused on the association of cereal fibers (representing whole-grain fiber) with the risks of CHD. An inverse relationship has been reported between the high intake of cereal fiber and the risk of myocardial infarction. Early meta-analysis of multiple, controlled studies has suggested that consumption of whole grains including wheat, rice, maize, and oats reduces the risk of CHD slightly better than even fruit or vegetables.\(^5\) In addition to having dietary fiber, whole grain foods are a rich source of many nutrients, including complex carbohydrates, starch, oligosaccharides, minerals, vitamins, and phytochemicals.\(^1,6\) The contribution of components of whole grains other than fiber and the mechanism by which whole-grain foods provide health benefits have not been clearly identified.

OAT CONSUMPTION AND HEART HEALTH

A recent comprehensive review of the literature by Kelly et al.\(^7\) provided evidence that the beneficial effect of consuming whole grains on CHD in clinical intervention trials is mainly limited to whole-grain oats. The beneficial effect of consuming other whole grains remains to be elucidated by long-term, controlled clinical intervention trials.

Oats are unique among the whole grains. The consumption of oatmeal and oat bran, even for a short period of time, has been shown in most studies to reduce total plasma cholesterol and LDL-cholesterol levels, the main risk factors for CHD.\(^8\) This is mainly attributed to β-glucan, the soluble fiber content of oats. β-glucan, the active component of the soluble fiber in oats, interferes with the reabsorption of bile acid in the gut and reduces cholesterol levels.\(^5\) Due to this well-established effect of oats on the risk of CHD, the United States Food and Drug Administration in 1997 approved the heart-health-benefit claim on food labels of food containing soluble fiber from oats. In addition to its cholesterol-lowering effect, oats have been shown to improve endothelial function when consumed with supplements of vitamins C and E\(^9\) and to reduce blood pressure.\(^10\) Although the mechanisms of these effects are not known, it is plausible that the effects are mediated through increasing vessel wall endothelium production of nitric oxide (NO), which mediates relaxation of smooth muscle cells in the vascular wall.

Oats (\textit{Avena sativa} L.), like all monocot cereal grains, contain relatively high levels of soluble fibers compared to other cereals; they also contain one-third more protein, nearly four times more fat, and have less starch.
Importantly, oats contain a number of phytochemicals possessing a phenolic moiety with free-radical scavenging capability and thus exhibit antioxidant properties in vitro. Based on their chemical structure and biosynthetic pathways, these phytochemicals can be roughly divided into low-molecular-weight, readily soluble “free phenols” (such as tocopherols, tocotrienols, flavonoids, hydroxycinnamates, etc.), and bound phenols, or those covalently linked to complex high-molecular-weight and insoluble cell components (such as lignin, cell wall polysaccharides, structural and/or storage protein, etc.). The “free phenols” appear to represent readily absorbed sources of antioxidants in the human diet; however, insoluble “bound phenols” present different challenges for researchers attempting to evaluate their long-term efficacy, since they require further metabolism before absorption from the gastrointestinal tract.

**AVENANTHRAMIDES**

Oats contain unique, low-molecular-weight, soluble phenolic compounds called avenanthramides (Avns), which are not present in other cereal grains. These compounds are antipathogens (phytoalexins), which are produced by the plant in response to exposure to pathogens such as fungi. Avns are conjugates of a phenylpropanoid with anthranilic acid or 5-hydroxy anthranilic acid. More than 20 different forms of Avns are present when extracted from oats, and the three major forms are A, B, and C (Figure 1). Investigation of commercial processing of oats including steaming, autoclaving, and drum drying indicates that not all Avns are affected equally by processing. Steaming and flaking moderately reduce the Avn-A content of dehulled oat groats, whereas Avn-C and -B are not affected by steaming. Autoclaving of oat grains and drum drying of steamed rolled oats significantly decrease the Avn content. However, the loss of Avns from drum drying of the whole meal made from autoclaved grains is less.

**Antioxidant properties**

Avns extracted from oats and those synthetically prepared exhibit potent antioxidant properties in vitro and in vivo. The antioxidant activity of Avns is 10–30 times greater than that of oats' other phenolic antioxidants such as vanillin and caffeic acid. Avn-C, one of the three major Avns of oats, often comprises about one-third of the total concentration of Avns in oat grain (although the relative proportion of Avns is highly variable), and it has the highest antioxidant activity in vitro. By far, these Avns constitute the major phenolic antioxidants present in the oat kernel. They occur in relatively high concentrations in the whole grain (up to 300 ppm or 0.03%) and in the oat kernel’s outer regions (e.g., bran and subaleurone layers), although they are not restricted to these plant tissues. The antioxidant activity of Avn-enriched extract of oats has been investigated in laboratory animals. Supplementing the diet of rats with Avn-enriched extract of oats at 100 mg/kg diet (providing about 20 mg Avns/kg bw) has been reported to increase superoxide dismutase (SOD) activity in skeletal muscle, liver, and kidneys, and to enhance glutathione peroxidase activity in heart and skeletal muscles. Supplementation at 200 mg/kg diet, which provides about 40 mg Avns/kg bw in rats, attenuated the exercise-induced production of reactive oxygen species (ROS).
Bioavailability

The bioavailability of Avns has been demonstrated in Golden Syrian hamsters. Following oral administration of Avn-enriched extract of oats, the peak plasma concentration of Avns in hamster blood appeared after 40 min. Since most polyphenols exhibit antioxidant activity, their protective effect on vascular function and the prevention of atherosclerosis has been attributed to their protection of LDL oxidation. In this regard, Avn-enriched extract of oats combined with vitamin C, synergistically inhibited LDL oxidation in vitro.

The bioavailability of Avns has also been reported in humans. In a randomized, placebo-controlled, crossover study, Chen et al. reported that the peak plasma concentration of Avns appeared at about 2 h following intakes of 0.5 and 1 g of Avn-enriched extract of oats. The plasma concentrations of different forms of Avns were 40–110 nmol/L after intakes of 0.5 g and 90–370 nmol/L after doubling the dose to 1 g. Interestingly, consumption of Avn-enriched extract of oats significantly increased the plasma concentration of a reduced form of glutathione. These observations suggest that consumption of oat-derived Avns may increase the total antioxidant capacity in laboratory animals and humans.

Anti-inflammatory effects

In addition to demonstrating antioxidant activity, Avn compounds may interact with cellular components, not only through their antioxidant activity, but also through their interactions with the molecular and signaling pathways that govern cellular responses during inflammation. Using the human aortic endothelial cell (HAEC) culture system, the potentially beneficial health effects of oat Avns was found to be mediated via modulation of the cellular and molecular processes that are known to play an important role in the inflammation of arteries and the development of atherosclerosis. These unique oat polyphenols have been shown to inhibit vascular endothelial cell expression of adhesion molecules, including ICAM-1, VCAM-1, and E-selectin. Suppression of these adhesion molecules by Avns resulted in inhibition of monocyte adhesion to HAEC monolayers and reduced production of several inflammatory cytokines and chemokines, including IL-6, IL-8, and MCP-1, the inflammatory components involved in fatty streak formation in arteries. The production of proinflammatory cytokines, chemokines, and adhesion molecules by endothelial cells has been shown to be regulated by redox-sensitive signal transduction involving nuclear transcription factor NF-κB. The above-observed effects of Avns on HAEC and other cells are reported to be mediated through inhibition of NF-κB. More recently, dihydroavenanthramide (DHAv), a synthetic analog of Avn, has been shown to protect pancreatic β-cells from damage via inhibition of NF-κB. In a series of experiments, Guo et al. determined that suppression of the expression of NF-κB activity by Avns is mediated via inhibition of the phosphorylation of IKK and IKB, and by suppression of proteasome activity in endothelial cells.

Antiproliferative effects

It is important to note that Tranilast [N-(3′,4′-dimethoxyaminomethyl)-anthranilic acid], a synthetic drug (Rizaban, Kissei Pharmaceutical Co, Japan) with structural similarity to the Avns (Figure 1), was originally developed and is currently used in Japan as an antihistamine. Later, it was discovered to have an antiproliferative effect on vascular smooth muscle cells (VSMCs), and in clinical trials it prevented restenosis after percutaneous transluminal coronary angioplasty. Cell culture studies have also revealed that, like Tranilast, Avns inhibits proliferation of VSMCs, a process that is known to be a major contributing factor to the development of atherosclerosis and restenosis after angioplasty. Subsequently, Nie et al. studied the molecular mechanism of Avns’ inhibition of VSMC proliferation and showed that, through modulation of cell cycle regulatory proteins such as p53, p21cip1, p27kip1, cyclin-D1, and pRb, Avns inhibits cell cycle signaling at the G1 to S phase transition. It was reported that Avns and Avn-C (synthetically prepared) suppressed phosphorylation of pRb, whose hyperphosphorylation is a hallmark of the G1 to S transition in the cell cycle. This was accompanied by a decrease in cyclin D1 expression and an increase in cyclin-dependent kinase inhibitor p21cip1 expression, without significant changes in p27kip1 expression. Furthermore, Avn-C treatment increased the expression level and stability of p53 protein, which could account for the increase in p21cip1 expression. In a recent set of studies, our research group examined the antiproliferative effects of Avns on several cancerous cell lines and found that Avn-enriched extracts of oats, Avn-C, and the methyl-ester derivative of Avn-C are more effective on colonic cancer cell lines, including Caco-2, HT29, LS174T, and HCT116, than on prostate or breast cancer cell lines. While this is a preliminary observation, it provides additional insight into the mechanism by which consumption of oats, with their high fiber content and Avns, may reduce the risk of colon cancer.

Vasodilation effects

Another potentially interesting biological effect of Avns on the cardiovascular system would be their effect on nitric oxide (NO)-dependent vasodilation. Nie et al.
reported that Avns increase NO production and endothelial NO synthase expression by both endothelial cells and VSMC. This effect of oat Avns might have contributed to the previously observed increase in flow-mediated vessel dilation and reduction of blood pressure in humans following consumption of oats and oatmeal in earlier studies.\(^9\),\(^10\) The inhibitory effects of oat Avns on VSMC proliferation and on the increase of NO production are additional characteristics that potentially lend another CHD-related health benefit to oats, beyond their known effect of lowering blood cholesterol.

**Anti-itch effects**

For some time, oatmeal has been recognized as a remedy for the treatment of poison ivy, sunburn, eczema, and psoriasis. Oat colloidal extract containing Avns has also proved to have antihistamine and anti-irritation activity.\(^43\) While the anti-itching property of oats and oatmeal has been known for centuries, a recent report provided molecular evidence for the mechanism by which oat Avns may exert their soothing effect on irritated skin. Sur et al.\(^44\) reported that at concentrations as low as 1 ppb Avns inhibited NF-κB activation in keratinocytes and reduced release of IL-8, a proinflammatory cytokine. In addition, topical application of Avns at 1–3 ppm mitigated inflammation in murine models of contact hypersensitivity and neurogenic inflammation and reduced puritrogen-induced scratching in a mouse model of itching. These observations indicate that Avns of oats appear to mediate oats’ anti-inflammatory and anti-irritant effects, and that they probably work through inhibition of histamine signaling. DHAvn, a synthetic derivative of Avn, which has been developed as a drug, reduces histamine-related skin disorders like itching, redness, and wheals.\(^45\)

**Cytoprotection effects**

It was also reported that DHAvn increased resistance of RINm5F cells and pancreatic islets to cytokine-induced toxicity and decreased β-cell destruction and maintained normal insulin secretion capacity. In vivo, pretreatment of mice with DHAvn blocked the development of Type 1 diabetes induced by streptozotocin-treatment, probably by preserving functional β-cells in the pancreas.\(^32\)

**CONCLUSION**

Taken together, current evidence suggests that consumption of foods containing oats is beneficial. Oats keep the heart healthy by lowering total and LDL cholesterol through β-glucan content and by suppressing inflamma-

**REFERENCES**


Diet for Heart Disease
Prevention and Treatment

Jun Dai, MD, MSc, PhD.
Assistant Professor
Department of Applied Health Science
Indiana University - Bloomington
Abbreviations

- American Heart Association (AHA)
- Body mass index (BMI)
- Mediterranean Diet (MedD)
- Mediterranean Diet Score (MedDS)
- Saturated Fatty Acids /Saturated Fats (SFA)
- Trans Fatty Acids/Trans-unsaturated Fat/Trans Fats
- Monounsaturated Fatty Acids /Monounsaturated Fats (MUFA)
- Polyunsaturated Fatty Acids /Polyunsaturated Fats (PUFA)
- Eicosapentaenoic acid (EPA)
- Docosahexaenoic acid (DHA)
- Total Cholesterol (TC)
- Low-density lipoprotein cholesterol (LDL-C)
- High-density lipoprotein cholesterol (HDL-C)
- Triglyceride s (TG)
- Myocardial infarction (MI)
- Coronary Heart Disease (CHD)
- Cardiovascular Disease (CVD)
- Ischemic Heart Disease (IHD)
Abbreviations

- Heart Rate Variability (HRV)
- Standard deviation of all normal to normal (NN) R-R intervals (SDNN)
- Standard deviation of 5-minute average NN intervals (SDANN)
- Mean of the standard deviations of all NN intervals for all 5-minute segments of the entire recording (SDNNI)
- Square root of the mean of squares of successive NN interval differences (rMSSD)
- Percentage of intervals above 50 ms different from preceding interval (pNN50)
- Total power (TotPow, < 0.40 ms²),
- Ultra-low frequency (ULF, 0 ~ 0.0033 ms²),
- Very-low frequency (VLF, 0.0033 to < 0.04 ms²)
- Low frequency (LF, 0.04 to < 0.15 ms²)
- High frequency power (HF, 0.15 to < 0.40 ms²).
AHA Dietary Guidelines on the Cardiovascular Disease (CVD) Prevention Among Children and Adults

Dietary Quality (whole diet)
The Mediterranean Diet (MedD)

**Daily**
- Olive Oil
- Vegetables
- Beans, Legumes & Nuts
- Fruits
- Fish
- Poultry
- Eggs
- Cheese & Yogurt
- Sweets
- Wine in Moderation

**Weekly**
- Pasta, Rice, Couscous, Polenta, Other whole grains & potatoes

**Monthly**
- Meat
Following the Mediterranean Diet (MedD) and Heart Disease

**Greece 8-Year Study**
- 22,043 aged 20~86 years
- Endpoint: CHD death

**The US 10-Year Study**
- 214,284 men; 166,012 women
- Median age: 62 years
- Endpoint: CVD death

**Nurses’Health Study 20-Year Study**
- 74,886 aged 38~83 years
- Endpoint: CVD death

- Men & Women*
  - Adjusted hazard ratio
    - Men†: 0.67
    - Women‡: 0.76
    - Women$: 0.80

- 2-unit increment in the MedDS
  - Greece 8-Year Study
    - Men & Women*
      - Adjusted for calories intake, intake of eggs and potatoes, gender and age education, smoking, physical activity, BMI, and WHR
    - Adjusted for calories intake, age, gender, race, education, marital status, smoking, physical activity, and BMI
    - Further adjusted for menopausal hormone therapy

- The US 10-Year Study
  - [6,9] vs. [0,3] MedDS
    - 4th vs. 1st quintile MedDS
      - Adjusted for age, smoking, BMI, menopausal status and postmenopausal hormone use, energy intake, multivitamin intake, alcohol intake, family history, physical activity, and aspirin use.

- Nurses’Health Study 20-Year Study
  - Adjusted for age, smoking, BMI, menopausal status and postmenopausal hormone use, energy intake, multivitamin intake, alcohol intake, family history, physical activity, and aspirin use.

---

Randomized Controlled Trials on Alpha-Linolenic Acid-Rich Mediterranean Diet and CVD

Outcomes

Adjusted Risk Ratio (95% CI)

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Lyon Heart Study</th>
<th>Indo-MedD Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total MI</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A+unstable angina etc¹ + CVD death¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A+B+stable angina etc. requiring hospital admission¹</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sudden cardiac death²</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Fatal MI³</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fatal MI²</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Adjusted Risk Ratio**

- **Lyon Heart Study, Intervention:** mean, 44 month MedD
  - ALA: 1.8 g/d
  - Prudent diet: 0.67 g/d
  - Start, n: 303
  - End, n: 144

- **Indo-MedD Study, Intervention:** 2-year MedD NCEP-I diet
  - ALA: 1.8 g/d
  - Prudent diet: 0.8 g/d
  - Start, n: 499
  - End, n: 485

---

Marine ω-3 Polyunsaturated Fatty Acids (PUFA) and Heart Disease Primary Prevention

% Decrement in Death

Coronary death: 36% (P<0.001)
Total death: 17% (P=0.046)

Fish 1-2 servings/week (250 mg/day of EPA and DHA)

Mozaffarin and Rimm. JAMA. 2006
Randomized Controlled Trials on Fish Oil Supplements and CVD

Outcomes

<table>
<thead>
<tr>
<th>Total Cardiac death¹</th>
<th>Coronary death¹</th>
<th>Sudden cardiac death¹</th>
<th>Total cardiac events²</th>
<th>Coronary death² + MI²</th>
<th>Sudden Cardiac death²</th>
<th>Nonfatal Cardiac event²</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.70</td>
<td>0.28</td>
<td>0.65</td>
<td>0.55</td>
<td>0.81</td>
<td>0.77</td>
<td>0.94</td>
</tr>
</tbody>
</table>

GISSI: 2-way analysis (Italy)
RCT: 3.5 years
Fish oil: EPA+DHA 850-882 mg/d
Control: 0
n = 2836 2828

JELIS (Japan)
Intervention: 4.6 years (mean)
Fish oil+Statin: EPA 1.8 g/d
Statin: 0
n = 9326 9319

1 - GISSI. Lancet. 1999;
2 - Yokoyama, Lancet. 2007
ω-3 PUFA and Heart Disease

Include plant and marine ω-3 PUFA from diet and supplement
Meta-Analysis for 11 randomized controlled trials
Follow-up: ≥ 6 months;
7951 patients in the intervention; 7855 patients in the control

Risk Ratio (95% CI)

Nonfatal
Myocardial
Infarction (MI)

Fatal
MI

Sudden
Cardiac
Death

Overall
Mortality

0.80
0.70
0.70
0.80

1.5

0.0

Bucher HC, et al. 2002
Other Fatty Acids and Coronary Heart Disease Meta-Analysis on Epidemiologic Studies

Prospective Studies

Fatal or nonfatal CHD

Relative Risk (95% CI)

1.00

1.07

Prospective Studies

Fatal or nonfatal CVD

1.00

1.23

Pro- & Retropective Studies

Fatal and nonfatal CHD

1.29

Saturated Fatty Acids*  Trans-unsaturated Fatty Acids#

Mixed Lipids and Coronary Heart Disease (CHD)

Adjusted Relative Risk (95% CI)

- \( \uparrow \) MUFA & \( \downarrow \) SFA*
- \( \uparrow \) PUFA & \( \downarrow \) SFA*

Coronary Event Coronary Death
Coronary Event Coronary Death

CHD Death
- CHD Death
  - Keys Score #
    - Keys score/modified Hegsted score, \( \uparrow \) SFA, \( \downarrow \) PUFA, \( \uparrow \) cholesterol in diet
  - Modified Hegsted Score#

5% Energy change in fat

3\textsuperscript{rd} vs. 1\textsuperscript{st} tertile

## Dietary Fat and CVD

### Increase CVD risk
- Saturated Fatty Acids (SFA)
- Trans-Fatty Acids (trans-FA)

### Reduce CVD risk
- Polyunsaturated Fatty Acids (PUFA)
  - N-6/ω-6 PUFA
    - Linoleic acid, essential fatty acid (LA)
  - N-3/ω-3 PUFA
    - Alpha-linolenic acid, essential fatty acid (ALA)
    - Eicosapentaenoic acid (EPA)
    - Docosahexaenoic acid (DHA)

### Uncertain
- Monounsaturated Fatty Acids (MUFA)

---

<table>
<thead>
<tr>
<th>Individuals</th>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Without documented CHD</td>
<td>Fish (mainly oily fish) 2 times/week Foods and oils rich in alpha-linolenic acid (flaxseed, canola, and soybean oils)</td>
</tr>
</tbody>
</table>
| With documented CHD*                | 1 g EPA+DHA/day  
1. From oily fish  
2. If from the supplements, consultation with physician is needed. |
| Needing triglyceride-lowering       | 2-4 g EPA+DHA/day from capsules under care of a physician                     |

<table>
<thead>
<tr>
<th>Oily Fish for 1 gram EPA+DHA</th>
<th>Foods for 1 gram Alpha-Linolenic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mackerel, fresh (1.5 oz)</td>
<td>Flaxseed oil (1.5 teaspoons)</td>
</tr>
<tr>
<td>Herring, fresh (2.5 oz)</td>
<td>Canola oil (2.5 teaspoons)</td>
</tr>
<tr>
<td>Salmon, atlantic (3 oz)</td>
<td>Soybean oil (1 tablespoons or 3 teaspoons)</td>
</tr>
<tr>
<td>Tuna, fresh (3 oz)</td>
<td></td>
</tr>
<tr>
<td>Salmon, pink (3.5 oz)</td>
<td></td>
</tr>
<tr>
<td>Trout, fresh (5-6 oz)</td>
<td></td>
</tr>
</tbody>
</table>
Randomized Controlled Trials on Alpha-Linolenic Acid-Rich Diet and CVD

44 months vs. Baseline\(^1\)

- **TC**: Experiment, -5%; Control, -8%
- **LDL-C**: Experiment, 11%; Control, -10%
- **HDL-C**: Experiment, 3%; Control, -12%
- **TG**: Experiment, -18%; Control, -20%

2 years vs. Baseline\(^2\)

- **TC**: Experiment, -12%; Control, -18%
- **LDL-C**: Experiment, 3%; Control, -20%
- **HDL-C**: Experiment, P<0.001
- **TG**: Experiment, P<0.001

**Lyon Heart Study**


**Indo-MedD Study**

2 – Singh RB et al, Lancet. 2002
Randomized Controlled Trials on Fish Oil Supplements and CVD

6th month vs. Baseline
- Fish oil:
  - TC: 7.9%
  - LDL-C: 9.1%
  - HDL-C: 8.8%
  - TG: -3.4%
  - Statin: P<0.05

5 years vs. Baseline
- EPA+Statin:
  - TC: 3%
  - LDL-C: -20%
  - HDL-C: -24%
  - TG: -9%
  - Statin: P<0.001

GISSI Study
1 - GISSI. Lancet. 1999;

JELIS Study
2 - Yokoyama, Lancet. 2007
Fish Oil Supplement and Triglyceride-Lowering Effects

3.25 g/day (1.9 g EPA + 1.35 g DHA) versus placebo
47 Randomized trials (placebo/control)
Meta-analysis

Associations Between Following the Mediterranean Diet (MedD) and Inflammation Independent of Common Factors

% Difference in Geometric Means of Levels of Interleukin-6 (IL-6) per 1 unit increment in MedD Score

Associations Between Following the Mediterranean Diet (MedD) and Oxidative Stress Independent of Common Factors

% Difference in Geometric Means of Reduced to Oxidized Glutathione Ratio (GSH/GSSG Ratio)
Per Unit Within-Pair MedD Score Difference

↑ ratio
↓ Oxidative stress

Pooled data for zygosity


GSH/GSSG difference (%) | Model 1: + Nutritional factors | Model 2: + Socio-demographic + Lifestyle factors | Model 3: + CHD risk factors | Model 4: + Medications
---|---|---|---|---
9.5% | P=0.01 | 10.7% | P=0.01 | 10.2% | P=0.007 | 10.1%
Associations Between Following the Mediterranean Diet (MedD) and Heart Rate Variability (HRV)
Independent of Common Factors

% Difference in Geometric Means of Time-Domain HRV Per Unit Within-Pair MedD Score Difference

- SDNN: 2%
- SDANN: 2%
- SDNNI: 3%
- rMSSD: 4%
- pNN50: 11%

Pooled data by zygosity due to interaction term $P>0.05$
Additionally control for education, marital status, physical activity, WHR, Framingham risk score; plasma fasting triglyceride; and use of fish oil supplements, β-blockers, aspirin, statins, antihypertensives, & antihyperglycemics.
Associations Between Following the Mediterranean Diet (MedD) and Heart Rate Variability (HRV) Independent of Common Factors

% Difference in Geometric Means of Frequency-Domain HRV Per Unit Within-Pair MedD Score Difference

<table>
<thead>
<tr>
<th>Frequency-domain</th>
<th>TotPow</th>
<th>ULF</th>
<th>VLF</th>
<th>LF</th>
<th>HF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Difference (%)</td>
<td>4%</td>
<td>3%</td>
<td>5%</td>
<td>8%</td>
<td>5%</td>
</tr>
</tbody>
</table>

- Pooled data by zygosity due to interaction term $P > 0.05$ except LF
- Additionally control for education, marital status, physical activity, WHR, Framingham risk score; plasma fasting triglyceride; and use of fish oil supplements, β-blockers, aspirin, statins, antihypertensives, & antihyperglycemics.

Dai J, et al. Submitted to Circulation Outcomes
<table>
<thead>
<tr>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy body weight: Balance energy intake with its expenditure</td>
</tr>
<tr>
<td>Choose whole-grain, high-fiber foods</td>
</tr>
<tr>
<td>Consume a diet rich in vegetables and fruits</td>
</tr>
<tr>
<td>Minimize intake of beverages and foods with added sugars</td>
</tr>
<tr>
<td>Choose and prepare foods with little or no salt</td>
</tr>
<tr>
<td>If you consume alcohol, do so in moderation</td>
</tr>
</tbody>
</table>

## AHA Dietary Recommendation for CVD Prevention — Emphasis on a Whole Diet

<table>
<thead>
<tr>
<th>Recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Limit Lipid intake</strong></td>
</tr>
<tr>
<td><strong>Saturated Fatty Acids (SFA)</strong>                                             ≤ 7% energy</td>
</tr>
<tr>
<td><strong>Trans-unsaturated Fatty Acids</strong>                                           ≤ 1% energy</td>
</tr>
<tr>
<td><strong>Cholesterol</strong>                                                              ≤ 300 mg</td>
</tr>
<tr>
<td><strong>Consume fish, especially oily fish, ≥ twice/week</strong></td>
</tr>
<tr>
<td><strong>Choosing lean meats and vegetable alternatives</strong></td>
</tr>
<tr>
<td><strong>Selecting fat-free (skim), 1% fat, and low-fat dairy products</strong></td>
</tr>
<tr>
<td><strong>Minimizing intake of partially hydrogenated fats</strong></td>
</tr>
<tr>
<td><strong>When you eat food prepared outside of home, follow the AHA diet and lifestyle recommendations</strong></td>
</tr>
</tbody>
</table>

Diet and Exercise for Sarcopenia, Cachexia & Wasting

Ronenn Roubenoff, MD, MHS
Global TM Head, Musculoskeletal Diseases
Novartis Institutes for Biomedical Research, Cambridge, MA
Adjunct Professor of Nutrition & Associate Professor of Medicine
Tufts University & Tufts Medical Center, Boston, MA
Body Compartments of Standard Man

ICRP 1975
Why Lean Loss Matters

- **Mortality** – fatal at 40% loss
- **Morbidity** – demonstrable at 5% loss
- **Function**
  - Muscle → strength, independence
  - Viscera → resting energy expenditure
  - Immune system → infections (*P. carinii*)
- **Reversible** – with intervention
**Body Composition: Definitions**

- **Wasting**
  - Loss of weight and cell mass
  - Inadequate intake

- **Cachexia**
  - Loss of cell mass without loss of weight
  - Hypermetabolism -- cytokines

- **Sarcopenia**
  - Loss of muscle mass/function in normal aging
  - Withdrawal of anabolic stimuli
  - ± increased catabolic stimuli

Sarcopenia

Young, active

Old, sedentary
Sarcopenia: Type II Fiber Loss

Lexell, *J Gerontol* 1995
Sarcopenia Affects Muscle Strength More than Mass

Figure 1. Annualized rates for declines in leg lean mass (hatched bar) and muscle strength (black bar) by gender and race. Gender difference within race, $p < .01$. Racial difference within gender, $p < .05$.

Figure 3. Declines in leg lean mass and muscle strength over 3 years by weight change groups, stratified by gender. Values of $p$, analysis of variance between groups within the same gender.

Prevalence of Sarcopenia, Cachexia and Related Indications

<table>
<thead>
<tr>
<th>Condition</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sarcopenia</td>
<td>13.5 million</td>
</tr>
<tr>
<td>Catabolic illness</td>
<td>5.5 million</td>
</tr>
<tr>
<td>COPD cachexia</td>
<td>3.5 million</td>
</tr>
<tr>
<td>Cancer cachexia</td>
<td>0.5 million</td>
</tr>
<tr>
<td>Cardiac cachexia</td>
<td>0.2 million</td>
</tr>
<tr>
<td>Anorexia nervosa</td>
<td>0.3 million</td>
</tr>
<tr>
<td>HIV wasting</td>
<td>0.1 million</td>
</tr>
</tbody>
</table>

## Costs of Sarcopenia: NHANES

$18.5$ Billion (Osteoporosis $16.8$ B)

<table>
<thead>
<tr>
<th>Degree of Sarcopenia</th>
<th>Muscle Mass (kg/m²)</th>
<th>Prevalence (%)</th>
<th>RR Disability</th>
<th>PAR (%)</th>
<th>Cost (billion $)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MEN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>$\geq 10.76$</td>
<td>35.7</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moderate</td>
<td>8.51-10.75</td>
<td>53.1</td>
<td>3.48</td>
<td>56.8</td>
<td>7.18</td>
</tr>
<tr>
<td>Severe</td>
<td>$\leq 8.51$</td>
<td>11.2</td>
<td>4.60</td>
<td>28.7</td>
<td>3.63</td>
</tr>
<tr>
<td>WOMEN</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>$\geq 6.76$</td>
<td>68.7</td>
<td>1.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Moderate</td>
<td>5.76-6.75</td>
<td>21.9</td>
<td>1.46</td>
<td>9.2</td>
<td>2.70</td>
</tr>
<tr>
<td>Severe</td>
<td>$\leq 5.75$</td>
<td>9.4</td>
<td>3.15</td>
<td>16.8</td>
<td>4.96</td>
</tr>
</tbody>
</table>

Janssen et al. *JAGS*, 2003
Pathophysiology of Sarcopenia: What’s Lost (and Gained) with Age (not Disease)?

- **Anabolic signals decline**
  - Spinal cord α-motor units
  - Testosterone
  - Growth hormone
  - Estrogen
  - Physical activity
  - Dietary protein intake
  - Appetite
  - Sleep
  - Thirst

- **Catabolic signals increase**
  - IL-6
  - Subclinical inflammation (ESR, CRP, IL-1Ra)
  - Insulin resistance
  - Obesity (TNF, IL-6, etc.)

- **Unchanged**
  - Short-term energy balance
  - TNF and IL-1
IL-6 Production in the Elderly: The Framingham Heart Study

Roubenoff et al., *J Gerontol* 1998
IL-6 Predicts All-Cause Mortality: Framingham Heart Study

- 4–year all cause mortality, ages 72-92
  - High serum IL-6
  - High PBMC TNF-alpha
  - Low serum IGF-1
  - Greater loss of fat-free mass

(Adjusted for smoking, diabetes, CVD, arthritis, high CRP, being bedridden)

### Percentage of Elderly Falling Below 1989 RDA for Selected Nutrients

<table>
<thead>
<tr>
<th>Nutrient</th>
<th>Women</th>
<th>Men</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy (kcal.)</td>
<td>89</td>
<td>83</td>
</tr>
<tr>
<td>Protein (gm.)</td>
<td>38</td>
<td>37</td>
</tr>
<tr>
<td>Vitamin B-6 (mg.)</td>
<td>68</td>
<td>72</td>
</tr>
<tr>
<td>Calcium (mg.)</td>
<td>82</td>
<td>68</td>
</tr>
<tr>
<td>Iron (mg.)</td>
<td>51</td>
<td>35</td>
</tr>
<tr>
<td>Zinc (mg.)</td>
<td>87</td>
<td>88</td>
</tr>
<tr>
<td>Vitamin E (mg.)</td>
<td>79</td>
<td>80</td>
</tr>
<tr>
<td>Vitamin C (mg.)</td>
<td>40</td>
<td>48</td>
</tr>
</tbody>
</table>

Effect of 9 Weeks of 1/2-RDA Protein Intake in Elderly Women

Castaneda et al., AJCN 1995; 62:30

* p < 0.02

- BCM, kg
- Muscle Mass, kg
- LBM, kg
- N bal, g
Assessing Weakness in the Chronically Ill Elderly

- **Age-related**
  - Sarcopenia (exclusion)
  - Men: testosterone deficiency

- **Nutritional**
  - Vitamin Deficiency: B12, D, A
  - Dehydration

- **Disease related**
  - Shortness of breath
  - Cachexia
  - Adrenal insufficiency
  - Fluid overload
  - Infection (chronic, acute)
  - Myositis
Muscle strength and mass changes: effects of aging and training

Sarcopenia: A Simplified Causal Framework

α-motor neuron loss → SARCOPENIA
Anabolic factor loss → SARCOPENIA
Catabolic factor gain → SARCOPENIA

Testosterone, GH/IGF-1, IL-6

Dietary Intake → Protein

Wasting

Reduced physical activity → SARCOPENIA

Obesity

Cachexia
Cachexia

Age 51

Age 58
Diseases Commonly Associated with Cachexia

- Cancer (pancreas, NSCLC, colon, stomach, esophagus)
- CHF
- COPD
- HIV Infection
- Rheumatoid arthritis
- Tuberculosis (“Consumption”)
Fiber Type Preponderance in Various Forms of Cachexia

- **Type II Fiber Loss**
  - Steroid myopathy
  - Sarcopenia
  - Disuse atrophy
  - Cancer cachexia
  - Rheumatoid cachexia

- **Type I Fiber Loss**
  - Cardiac cachexia
  - COPD cachexia

Green et al., *J Mol Histol* 2009

Pu et al., *J Appl Phys* 2001
Rheumatoid Arthritis

- Most common inflammatory arthritis
  - 1-2% of population in developed countries
  - Increases with age (3-5% >60 y)

- No one dies OF RA, but people WITH RA have a 2-5-fold higher mortality rate
  - Even in the pre-steroid era
  - Same causes of death as general population, except for increased risk of infections

- Model of chronic hypercytokinemic state:
  - TNF, IL-6

- Normal renal and hepatic function
Body Composition in RA

Roubenoff et al. JCI 1994; 93: 2379

Fat Mass
BMC
ECW
BCM
PBMC Production of IL-1β and TNF-α in RA and Matched Controls

Roubenoff et al., J Clin Invest 1994
Hypermetabolism: REE per g TBK in RA vs. Matched Controls

Roubenoff et al., *J Clin Invest* 1994
Energy and Protein Intake in RA does not Differ from Matched Controls

<table>
<thead>
<tr>
<th>Outcome</th>
<th>RA</th>
<th>Control</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Energy Intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>kcal/d</td>
<td>1780 ± 697</td>
<td>1997 ± 623</td>
<td>NS</td>
</tr>
<tr>
<td>MJ/d</td>
<td>7654 ± 2997</td>
<td>8587 ± 2697</td>
<td></td>
</tr>
<tr>
<td>kcal/kg/d</td>
<td>25.5 ± 10.0</td>
<td>27.9 ± 8.7</td>
<td>NS</td>
</tr>
<tr>
<td>MJ/kg/d</td>
<td>110 ± 43</td>
<td>120 ± 37</td>
<td></td>
</tr>
<tr>
<td><strong>Protein Intake</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>g/d</td>
<td>71.9 ± 23.7</td>
<td>79.0 ± 28.7</td>
<td>NS</td>
</tr>
<tr>
<td>g/kg/d</td>
<td>1.0 ± 0.3</td>
<td>1.1 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

Roubenoff et al., *J Clin Invest* 1994
Physical Activity in RA and Matched Controls

![Bar chart showing physical activity levels in RA and Controls. The chart indicates a statistically significant difference (* P < 0.02).]
Body Cell Mass And Spontaneous TNF-α & IL-1β Production

Walsmith et al. 2003

Note: The relationship remained significant in RA after adjusting for age. Physical activity, duration of RA, protein intake, prednisone dose, % body fat, and smoking status were not significant explanatory variables.
Cachexia: A Simplified Causal Framework

- **α-motor neuron loss**
- **Anabolic factor loss**
- **Catabolic factor gain**

**Dietary Intake**
- Protein
- Energy

**CACHEXIA**
- **Fiber Dominance**
  - Type I
  - Type II

**Tissue Hypoxia (CHF, COPD)**

**Reduced physical activity**

**Fever, hypermetabolism**

**Testosterone**
- GH/IGF-1

**IL-1, IL-6, TNF**

**Glucocorticoids**
Wasting: Starvation, Organ Failure, AIDS, Cancer
Effect of Weight Loss on Cancer Survival

- Pancreas
- Gastric
- NSCLC
- SCLC
- Prostate
- Colon
- Sarcoma
- Breast
- Hodgkin

Cachexia, Survival, and IL-1β in COPD

**FIGURE 1.** Survival in patients with chronic obstructive pulmonary disease according to the presence of cachexia. Median survival is almost twice as short in cachectic patients (●) as in noncachectic patients (■). Cachexia was considered to be a fat-free mass index <16 kg·m⁻² in males and <15 kg·m⁻² in females. Reproduced from [7], with permission from the publisher.

**FIGURE 2.** Association of the -511 CC polymorphism with cachexia in patients with chronic obstructive pulmonary disease (COPD). While the CC variant is not necessary for cachexia to develop, the TT genotype appears protective. ■: cachectic patients with COPD; □: noncachectic patients with COPD; □: normal subjects. *, p<0.05.

Cardiac “cachexia”: effect on survival

Anker S et al. Lancet 1997;349: 1050-53
Anker S et al. Lancet 2003; 361:1077-83
AIDS Wasting: Cachexia Component
AIDS Wasting: Poor Intake Drives Weight Loss
AIDS Wasting: Poor Intake Drives Weight Loss
Wasting: A Simplified Causal Framework

α-motor neuron loss

Anabolic factor loss

Catabolic factor gain

Testosterone
GH/IGF-1

IL-1, IL-6, TNF

Glucocorticoids

Reduced physical activity

Fever, hypermetabolism

Dietary Intake

Protein
Energy

Tissue Hypoxia (CHF, COPD)
Conclusions

- Sarcopenia is a withdrawal of anabolic stimuli with age, probably with an increased catabolic component. However, REE/FFM is generally normal.
  - Rx: Resistance exercise

- Cachexia is due to catabolic cytokine activation in chronic diseases, with altered protein and energy metabolism. REE/FFM is increased.
  - Rx: Treat underlying condition.

- Wasting is caused by inadequate intake, and REE/FFM is reduced.
  - Rx: Nutritional support, exercise as tolerated.
Back-Up Slides
### Determinants of 2-yr changes in fat-free mass

<table>
<thead>
<tr>
<th></th>
<th>$\beta$</th>
<th>SE</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>-.02</td>
<td>.02</td>
<td>0.40</td>
</tr>
<tr>
<td>FFM (kg) at baseline</td>
<td>-.04</td>
<td>.01</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cell. IL-6 (ng/ml)</td>
<td>-.21</td>
<td>.10</td>
<td>0.04</td>
</tr>
</tbody>
</table>

Framingham Study n=539, 72-94 yrs

Payette et al., JAGS, 2003
IL-1Ra Production in the Elderly: The Framingham Heart Study

Roubenoff et al., *J Gerontol* 1998
Effect of 9 Weeks of 1/2-RDA Protein Intake in Elderly Women: Muscle Function

Castaneda et al., AJCN 1995; 62:30

* p < 0.02
Lewis Rat Adjuvant Arthritis as a Cachexia Model

AA Weight Loss: 28% intake, 72% catabolism

Roubenoff et al., Arthr Rheum 1997

Loss of Body Cell Mass in AA Cachexia
Effect of TNF & IL-1 Blockade on Soleus Atrophy in Lewis Rat Adjuvant Arthritis

Hamada et al., FASEB J, 2000
Sarcopenia: Decline in Muscle Protein Synthesis with Age

Figure 7. Fractional synthesis rate of myofibrillar proteins, whole body myofibrillar protein synthesis rate, and whole body protein synthesis in young and old (taken from Welle et al., 1993). Whole body protein synthesis rate is not different between young and old, whereas myofibrillar protein synthesis rate (fractional rate and absolute rate) are lower in the old.

J Gerontol 1995, Supplement
Basal AA Kinetics with Age: No Decline in Synthesis

Volpi et al. JAMA 2001
Association of PBMC TNF-α Production with Whole Body Protein Breakdown Rate

Rall et al., *Arthr Rheum* 1996

Flux

\[ \text{Flux (µmoles/gm TBK/hour)} \]

\[ r = 0.47, p = 0.01 \]
Roubenoff et al., *J Clin Invest* 1994
Whole-Body Protein Metabolism in RA and Aging

Rall et al., *Arthr Rheum* 1996

* p < 0.05
Figure 8. Fractional total muscle protein synthesis rate (Yarasheski et al., 1992, 1995) increased in the young and the old by 3 month of resistance training, but a similar increase was not observed for fractional myofibrillar protein synthesis rate by Welle et al. (1995). Yarasheski (1993) observed a more substantial increase in total muscle protein synthesis after 2 weeks resistance exercise in young and old.
Cachexia: Summary

- TNF
- Inflammation
- Oxidative Stress
- Insulin Resistance
- Fat Mass
- Physical Inactivity
- Muscle Mass
- Muscle Quality
- IL-1
- IL-6
- Proteasome Activity
- IL-6
- Protein Intake
- Antioxidants?
- Weakness
- Metabolic Reserve
- Disability, Morbidity, Mortality
- Protein Intake
- Cachexia
PRT in Renal Insufficiency: IL-6 and Muscle Hypertrophy

Castaneda et al., 2003
Gene Expression in Skeletal Muscle of RA Patients vs. Controls

Healthy Controls

RA Patients

Rate of Skeletal Muscle Protein Synthesis

Catabolic

Anabolic

Index of leukocyte infiltration

TNF-α  TGF-β  IL-1β  IL-15  IGF-1  MyoD  CD18

* 3.0x  *  4.0x  1.4x  1.5x  1.8x  *  4.2x  *  3.0x

*P < 0.05

25%, P < 0.04

Walsmith et al. J Rheum 2003
Myostatin control of Muscle Growth
Pathways involved in muscle hypertrophy/atrophy

**Hypertrophy signaling**
- Ca\(^{2+}\) channels
- GPCRs: e.g. β-agonists
- IGF1
- PI3K
- AKT
- AC
- cAMP
- FoxO
- Proteinsynthesis pathways: AKT/GSK3/eIF2B, AKT/mTOR/p70S6
- Mitochondriogenesis
- Fibre type switch
- Muscle hypertrophy

**Atrophy signaling**
- ActRII Ab
- Cachexia Factors
- Myostatin
- TNF-α/Tweak
- SMAD2/3
- p38
- IKK
- TAK-1
- SMAD
- Mafbx / Atrogin-1
- MuRF1
- Protein breakdown pathways
- Muscle atrophy

**Sarcopenia**
- TGFβ2/3
- Klotho/FGF23
- FGFR1/3
- Tweak Ab
- Muscle regeneration
- Aging

**Cachexia Factors**
- AktRIB Ab
- TGFα/2/3
- TAK-1
- SMAD
- SIK
- Mnk2
- Activin A

**Muscle regeneration**
Objectives

- To understand the interest in the gut microbiome and possible association with obesity
- To understand what probiotics are and how they protect immune function
- To broaden understanding of the various possible causes of obesity
Prevalence of Overweight and Obesity - U.S. Adults

<table>
<thead>
<tr>
<th>Study</th>
<th>Overweight (%)</th>
<th>Obese (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NHANES II</td>
<td>32</td>
<td>15</td>
</tr>
<tr>
<td>NHANES III</td>
<td>32</td>
<td>23</td>
</tr>
<tr>
<td>NHANES</td>
<td>34</td>
<td>31</td>
</tr>
<tr>
<td>NHANES 2001-2002</td>
<td>35</td>
<td>31</td>
</tr>
<tr>
<td>NHANES 2002-2004</td>
<td>32</td>
<td>34</td>
</tr>
<tr>
<td>MASS 2007</td>
<td>37</td>
<td>22</td>
</tr>
</tbody>
</table>

US: 66%
Massachusetts: 59%
Etiology of Obesity

• Causes of obesity are multifactorial – Complex environmental and genetic factors

• A person’s weight and body composition are likely comprised of genetic makeup, social, cultural, behavioral and environmental factors

Etiology of Obesity

• Complex systems that regulate energy balance are now being considered as contributors/causes of obesity

• Gut has highly integrated, complex system of anorexic (e.g. CCK, PYY, GLP-1, etc) and orexigenic (ghrelin) hormones that contribute to satiety, appetite and energy balance

Interactions Among Hormonal and Neural Pathways That Regulate Food Intake and Body-Fat Mass

Thyrotropin-releasing, corticotropin-releasing, and melanin-concentrating hormones produced in different neurons of the paraventricular nucleus and lateral hypothalamic area

Paraventricular nucleus

Y1R

MC4R

NPY

AgRP

POMC

(α-MSH)

Arcuate nucleus

INSR

LEPR

Insulin

Leptin

To forebrain and pituitary and adrenal glands

Ingestive and autonomic responses

Ghrelin

Large intestine

Stomach

Adipose tissue

Small intestine

Pancreas

SOLID LINE = Hormonal stimulatory effects

DASHED LINE = Hormonal inhibitory effects
Etiology of Obesity – New Focus

• Recent focus on the contribution of individual bacterial species and microbial communities
  – Precipitated by change from culture-based techniques to genetic analysis (metagenomics)
    • Majority of intestinal organisms were not able to be cultured

New Obesity Treatments?

• Recent evidence suggests:
  – Gut microbiota is critical for maintaining normal gastrointestinal and immune function, and normal digestion of nutrients
  – Obese and lean people have different gut microbiota

• Gut microbiota may play important role in regulating weight and be partly responsible for development of obesity in some people

• New studies are investigating manipulation of gut microbiota using probiotics, antibiotics, and /or prebiotics as a treatment for obesity

Metagenomics

- Term used for cloning and analysis of microbial DNA using culture-independent techniques
  - DNA elucidates organism profiles and is a tool to explore function of heterogeneous communities
- Samples obtained from feces and mucosal biopsies are used to study luminal and/or mucosal communities

Metagenomics

• Molecular identification involves SSU rRNA* present in all cellular organisms
• Sequencing of 16S rRNA genes from bacterial nucleic acid extracted from fecal material or mucosal samples has expedited identification and classification of bacteria
• Human Gut Microbiome Initiative
  – Effort to sequence gut genomes
  – http://genome.wustl.edu

* Small sub-unit, ribosomal ribonucleic acid
Steps for Building a Clone Library to Fingerprint a Complex Microbial Community

1. Extract genomic DNA from stool

2. Amplify 16S rRNA genes by PCR with primers 8F and 1525R targeting the domain bacteria

3. Combine PCR reactions starting with different amounts of template and gel; purify products of correct size

4. Clone PCR products of 16S rRNA genes into the pCR4-TOPO plasmid vector (Invitrogen, Carlsbad, CA) by the topoisomerase reaction (TOPO TA [Invitrogen] for sequencing)

5. Transform chemically competent Escherichia coli cells with plasmids

6. Extract plasmids from transformed E. coli cells

7. Sequence plasmid inserts

8. Align library sequences with references from 16S rRNA databases to determine phylogenetic affiliations for each sequence

9. Generate phylogenetic trees using neighbor-joining and maximum-likelihood algorithms

10. Make clone libraries

---

PCR – polymerase chain reaction
rRNA – ribosomal RNA
Metagenomics

- Adult human gut contains up to 100 trillion microbial organisms (mostly anaerobic)
  - These microbiota contain 500-1000 bacterial species – and ~100x the number of genes found in the human genome
  - More recent research indicates 18,000 genera and 15,000–36,000 bacterial species

Mayo Clinic Proceedings April 2008 vol. 83 no. 4 460-469.
Key Physiologic and Microbiological Features of the Gut

Relative concentrations of bacteria and pH at various locations

500-1000 Microbial species

Stomach
<10^2 cfu/mL
pH, 1-2

Duodenum
10^1-3 cfu/mL
pH, 6-7

Jejunum
10^3-4 cfu/mL
pH, 6-7

Ileum
10^7-9 cfu/mL
pH, 6-7

Colon
10^{10-12} cfu/mL
pH, 5-7

Aerobes

Digestion and acid secretion

Small intestine

Digestion and absorption of carbohydrates, proteins, and fats

Absorption of bile acids and vitamin B_{12}

Large intestine

Absorption of water, electrolytes, and short-chain fatty acids

Less bacteria count
More aerobic

Higher bacteria count
More anaerobic

cfu = colony-forming unit
http://mayoclinproc.highwire.org/content/83/4/460.figures-only
# Major Bacteria and Archaea Phyla and Genera Found in Human Gut Microbiota

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Representative genera</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
</tr>
<tr>
<td>Firmicutes</td>
<td>Ruminococcus</td>
</tr>
<tr>
<td></td>
<td>Clostridium</td>
</tr>
<tr>
<td></td>
<td>Peptostreptococcus</td>
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<tr>
<td></td>
<td>Lactobacillus</td>
</tr>
<tr>
<td></td>
<td>Enterococcus</td>
</tr>
<tr>
<td>Bacteroidetes</td>
<td>Bacteroides</td>
</tr>
<tr>
<td>Proteobacteria</td>
<td>Desulfovibrio</td>
</tr>
<tr>
<td></td>
<td>Escherichia</td>
</tr>
<tr>
<td></td>
<td>Helicobacter</td>
</tr>
<tr>
<td>Verrucomicrobia</td>
<td></td>
</tr>
<tr>
<td>Actinobacteria</td>
<td></td>
</tr>
<tr>
<td>Cyanobacteria</td>
<td></td>
</tr>
<tr>
<td>Synergistes</td>
<td>Bifidobacterium</td>
</tr>
<tr>
<td>Archaea</td>
<td></td>
</tr>
<tr>
<td>Euryarchaeota</td>
<td>Methanobrevibacter</td>
</tr>
</tbody>
</table>

Account for more than 90% of all phylotypes of Bacteria

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a Prokaryotic phyla were identified by using an alignment of the 18,348-sequence dataset from reference 18.

b Not related to any known genera.
Microbiota and Inflammation

• LPS* is triggering factor linking inflammation to high fat diets and metabolic syndrome

• Type II diabetics have higher LPS levels than those without DM

*LPS = Lipopolysaccharide
Mechanisms by Which Gut Microbiota May Contribute to Obesity

Intestinal microbiota

- Fermentation of indigestible dietary polysaccharides
  - Increased intestinal absorption of monosaccharides and short-chain fatty acids
  - Increased hepatic lipogenesis via ChREBP and SREBP-1

- Increased LPL activity via suppression of Fiaf and induction of PGC-1α and AMPK activity
  - Increased fatty acid metabolism and storage of calories in fat

- Increased circulation of LPS modulated by dietary fat content
  - Increased inflammatory cytokines via CD14-dependent mechanism

AMPK = adenosine monophosphate-activated protein kinase
ChREBP = carbohydrate response element-binding protein
Fiaf = fasting-induced adipocyte factor
LPL = lipoprotein lipase
LPS = lipopolysaccharide
PGC-1α = peroxisome proliferator-activated receptor γ coactivator 1α
SREBP-1 = sterol response element-binding protein type 1

http://mayoclinproc.highwire.org/content/83/4/460.figures-only
Gut Microbiota in Infants

• Type of birth delivery has significant impact on development of gut microbiota¹
  – Based on vaginal exposure, neonatal care, breast feeding and conversion adult microbiota following weaning

• Differences in infant fecal microbiota composition may predispose to obesity later in childhood²

• Children who were overweight at 7 years had lower bifidobacterial concentrations and higher staphylococcus aureus during infancy

Gut Microbiota Composition in Obese Humans

• Study monitored fecal gut microbiota in 12 obese participants in weight-loss program for 1 year; randomly assigned to fat-restricted or carbohydrate-restricted low-calorie diet

• Before diet, obese participants had fewer Bacteroidetes (3%) and more Firmicutes than lean controls; post weight loss, Bacteroidetes increased to 15% and Firmicutes decreased

Unanswered Questions

• Why do obese people have more Firmicutes?
• Do Firmicutes contribute to more efficient energy extraction?
• How and why is microbiota shifted by differences in body weight?
• Would differences in microbiota contribute to significant differences in weight?
Future Directions

Attempt to Modulate Gut Microbiota Through
Use of Antibiotics, Probiotics and/or Prebiotics
Antibiotics and Obesity Treatment

• Antibiotics improved glucose intolerance in mice\(^1\)

• Antibiotic treatment decreased incidence and delayed the onset of diabetes in a diabetes-prone rat model\(^2\)
  – Rats that did not develop diabetes displayed a lower number of Bacteroides species
  – Speculation that antibiotic-induced alteration in gut microbiota led to reduction in antigenic load and subsequent inflammation that usually leads to pancreatic \(\beta\)-cell destruction
  – Does not directly address obesity; demonstrates potential of modulating intestinal microbiota as a therapeutic strategy

Prebiotics and Obesity Treatment

• Dietary components that stimulate growth and metabolism of beneficial organisms; “fertilizers” of colonic microbiota

• Examples: fructo-oligosaccharides, insulin, bran, and psyllium
Probiotics and Obesity Treatment

- Contain various bacterial species and concentrations
- Available as dietary supplements
- Minimal FDA regulation
- Possible uses in IBD, IBS, NFLD

Sartor RB Gastroenterology 2004; 126:162—1633.
Summary

• Human gut contains up to 100 trillion microbial organisms (microbiota)

• Proportions of communities important (Firmicutes and Bacteriodetes) in energy homeostasis

• Microbiota also affect immune and inflammatory human responses

• Probiotics, antibiotics and/or prebiotics are under investigation as obesity treatments
Resources


- Backhed F, et al. Proc Natl Acad Sci USA. 2007; 104:979-984. Examines how germ-free mice were protected from obesity on a Western-style diet.


Dietary Restriction and Aging: Evidence and Clinical Ramifications
Susan B. Roberts, PhD
Professor of Nutrition and Professor of Psychiatry at Tufts University

This talk describes current information on the longevity and health effects of caloric restriction, based on longitudinal primate studies. The talk also discusses how, potentially, CR, can be achieved in free living humans. The talk will discuss specifics of how to restrict energy intake, and also the biology underlying intrinsic factors influencing human eating behavior, and their implications for successful CR.

Learning Objectives
1. To understand key points of agreement in research studies on CR, longevity and health.
2. To understand factors influencing an individual’s ability to stay in CR, and implications for designing sustainable approaches to free living CR in human populations.
3. To understand the different roles of eating behavior, dietary intake and exercise in the initial and later stages of CR.