What is a calorie?

Genie Moore, PhD
What is a calorie?

- The amount of energy needed to increase the temperature of 1 g of water from 14.5° to 15.5°C
How much energy is a kcal?

- Women usually use slightly <1 kcal/min and men use slightly more
  - One kcal/min corresponds approximately to the heat released by a burning candle or by a 75-watt light bulb (i.e., 1 kcal/min corresponds to 70 J/sec or 70 W)
How are kcal in food measured?

- **Bomb calorimetry** used historically and still widely used
Overview of food energy flow

Ingested energy (IE) = gross energy (GE)

- Faecal energy (FE)
- Combustible gas (GaE) (from microbial fermentation)

Digestible energy (DE)

- Urinary energy (UE)
- Surface energy (SE)

Metabolizable energy (ME)

- Heat of microbial fermentation
- Obligatory thermogenesis, i.e. excess heat relative to glucose during ATP synthesis

Net (metabolizable) energy (NME)

- Non-obligatory dietary thermogenesis
- Thermogenesis due to effects of cold, drugs, hormones, bioactive compounds or other stimulants

Net energy for maintenance (NE)

- Basal metabolism
- Physical activity

Metabolizable energy

- The difference between gross energy in consumed food (determined by bomb calorimetry) and energy in feces and urine (also measured by bomb calorimetry)
  - Rubner (Germany): heat of combustion of protein in a bomb calorimeter is higher than the energy value available to the host
    - The body oxidizes protein to urea, creatinine, uric acid, and other nitrogenous end products, and these products can be further oxidized in a bomb calorimeter
    - Estimated 23% of energy from protein lost in urine and feces
  - Atwater and Bryant extended Rubner’s observation to other nutrients
Atwater factors

<table>
<thead>
<tr>
<th>Macronutrient</th>
<th>Heat of combustion</th>
<th>Coefficient of availability</th>
<th>Available energy kcal/g total nutrients</th>
</tr>
</thead>
<tbody>
<tr>
<td>Protein</td>
<td>5.65</td>
<td>92</td>
<td>4.0&lt;sup&gt;1&lt;/sup&gt;</td>
</tr>
<tr>
<td>Fat</td>
<td>9.40</td>
<td>95</td>
<td>8.9</td>
</tr>
<tr>
<td>Carbohydrate</td>
<td>4.10</td>
<td>97</td>
<td>4.0</td>
</tr>
</tbody>
</table>

<sup>1</sup> Corrected for unoxidized material in the urine, i.e., \((5.65 \text{ kcal/g} \times 0.923) - 1.25 \text{ kcal/g}\).
Atwater factors

- Coefficient of availability
  - \( = (\text{intake} - \text{excretion}) \div \text{intake} \)
  - Derived from mixed diets typical of the time: beef, butter, ginger snaps, parched cereal, baked beans, canned pears
  - Meant to be applied to mixed diets, not individual foods

- Energy factors were average values
  - In error to some extent for every food in the diet due to
    - Differences in chemical structure
      - Heat of combustion for starch = 4.18 kcal/g; for glucose 3.72 kcal/g
    - Differences in availability

- Despite the caveats, subsequent investigations have tended to support the results
Atwater specific factors

- Originally proposed by Merrill and Watt in 1955
- Recognize different energy factors for carbohydrate, protein, and fat, depending upon the foods that they are derived from, e.g.,

<table>
<thead>
<tr>
<th></th>
<th>Protein (kcal/g)</th>
<th>Fat (kcal/g)</th>
<th>CHO (kcal/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs</td>
<td>4.36</td>
<td>9.02</td>
<td>3.68</td>
</tr>
<tr>
<td>Meat/fish</td>
<td>4.27</td>
<td>9.02</td>
<td>*</td>
</tr>
<tr>
<td>Milk/milk products</td>
<td>4.27</td>
<td>8.79</td>
<td>3.87</td>
</tr>
</tbody>
</table>

* Carbohydrate factor is 3.87 for brain, heart, kidney, liver; and 4.11 for tongue and shellfish

Nitrogen analysis

- **Kehladl technique - commonly used**
  - Digestion with concentrated $\text{H}_2\text{SO}_4$ in the presence of heat (370-400°C) and an inorganic catalyst; neutralization
  - Distillation and capture of $\text{NH}_3$ with weak acid
  - Quantification by titration with a strong acid ($\text{H}_2\text{SO}_4$)

- **Dumas technique**
  - Combustion nitrogen analysis (900-1000°C)

- **Near- and mid-infrared spectrometry**
  - Can detect protein, fat, starch, moisture, and fiber simultaneously

- **Liquid chromatography and triple-quadrupole tandem MS**
  - Contaminant testing

Hui Y. Handbook of food science. Marcel Dekker, 2005
Conversion factors: nitrogen to crude protein

- Quantitative amino acid analysis
  - Can be a method of protein analysis
    - $\Sigma$(Formula weight $\times$ [AA]) = [protein]
  - Usually used for conversion factors

- Nitrogen-to-protein factor allows estimation of crude protein content
  - Animal proteins assumed to be 16% nitrogen (6.25 g N/g protein)
  - Less consensus for plant proteins
    - 4.14 to 4.7 g N/g protein found in various investigations

Hui Y. Handbook of food science. Marcel Dekker, 2005
Fat analysis

- Extraction with a nonpolar solvent
  - Yields only “crude fat” unless samples or pretreated with mild acid or alkali or enzymatic digestion
    - Releases lipids from their bound forms – lipoproteins, glycolipids (“total lipid”)

- Instrumental methods
  - Infrared spectroscopy, NMR, densitometry
Carbohydrate analysis

- “By difference” vs analytic
  - Refractometry, colorimetric, HPLC, GC
- Fiber
  - Multi-step process: detergent, enzyme treatment, gravimetric
Resistant starch

- Indigestible or only slowly digested by body enzymes
- Three reasons for indigestibility
  - Physically inaccessible
    - Partially milled grains and seeds
  - Ungelatinized resistant granules, slowly degraded by amylase
    - Raw potato, green bananas, some legumes
  - Retrograded starch
    - Cooked and cooled potato, cornflakes
Much of the variation relates to fiber in the diet

Comparison of gross energy and measured and calculated metabolizable energy between 2 diets with different fiber content that were fed to 12 healthy, free-living men for 5 wk.

<table>
<thead>
<tr>
<th></th>
<th>High-fiber diet</th>
<th>Low-fiber diet</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Value</td>
<td>Difference from measured metabolizable energy</td>
</tr>
<tr>
<td>Gross energy</td>
<td>kcal/d</td>
<td>kcal (%)</td>
</tr>
<tr>
<td>Measured metabolizable energy</td>
<td>3069 ± 448⁴</td>
<td>360 (13.3)</td>
</tr>
<tr>
<td>Metabolizable energy (Atwater general factors)</td>
<td>2709 ± 402</td>
<td></td>
</tr>
<tr>
<td>Metabolizable energy (Atwater specific factors)</td>
<td>2925 ± 427</td>
<td>216 (8.0)</td>
</tr>
<tr>
<td></td>
<td>2892 ± 422</td>
<td>183 (6.8)</td>
</tr>
</tbody>
</table>

⁴ Adapted from reference 29.
⁵ Containing 37 g fiber and 14% of energy from protein, 33% from fat, and 53% from carbohydrate.
⁶ Containing 16 g fiber and 14% of energy from protein, 36% from fat, and 50% from carbohydrate.
⁷ x ± SD (all such values).
Correlation between fat intake and fecal fat

Regression equation, $y=0.0102x + 2.93$, $r = 0.314$ excludes the high fiber group

Does energy expenditure differ if calories come from fat vs CHO vs protein?

Zone: 40-30-30% for CHO-pro-fat

LEARN: 55-60%
CHO, <10% sat fat

Ornish: <10% fat

Atkins: CHO ≤ 20 g/d for induction (2-3 mo); ≤ 50 g/d for ongoing weight loss

Does the thermic effect of food vary with the diet?

- 10 studies of energy expenditure with diets of varying CHO:fat ratios
  - Low fat diets 3-20% energy from fat
  - High fat diets 40-60%
  - Protein held constant

Whole-room Indirect Calorimeter
Activity Measurement System

- EE & RQ measurement system
- physical activity & exercise measurement system
- behavior monitoring system
- environment control & monitoring
- measurement & calibration instruments
- noise filtering & alarm
- cooling & heating
- sensors for chair, mattress, commode, bike, food, scale, TV, standing
- event recordings for meal, exercise, sleep, BMR, standing, biking
- chair, mattress, commode, bike, food, scale, TV, standing
- meal, exercise, sleep, BMR, standing, biking
Does the thermic effect of food vary with the diet?

- Differences in total energy expenditure (24 h) between low and high fat diets were not different from zero.
- TEF only measured in 2 studies:
  - 58 kcal/d lower with high fat in one study.
  - Tended to be higher with high fat in the other study.
- In another review (Eisenstein et al. *Nutr Rev* 2002;60:189–200), the effect of high fat vs high CHO only accounted for 40 kcal/d.
<table>
<thead>
<tr>
<th></th>
<th>Atkins</th>
<th>Zone</th>
<th>LEARN</th>
<th>Omish</th>
<th>P Value†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, kcal/d</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>1888 (512)</td>
<td>1975 (567)</td>
<td>1925 (553)</td>
<td>1850 (541)</td>
<td>.52</td>
</tr>
<tr>
<td>2 mo</td>
<td>1381 (345)</td>
<td>1455 (464)</td>
<td>1476 (448)</td>
<td>1408 (453)</td>
<td>.52</td>
</tr>
<tr>
<td>6 mo</td>
<td>1538 (401)</td>
<td>1503 (348)</td>
<td>1598 (418)</td>
<td>1553 (530)</td>
<td>.64</td>
</tr>
<tr>
<td>12 mo</td>
<td>1599 (494)</td>
<td>1594 (523)</td>
<td>1654 (492)</td>
<td>1505 (437)</td>
<td>.43</td>
</tr>
<tr>
<td>Fat (% energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Baseline</td>
<td>36.2 (7.8)a</td>
<td>35.6 (6.5)b</td>
<td>33.2 (7.2)b</td>
<td>35.1 (7.0)a,b</td>
<td>.05</td>
</tr>
<tr>
<td>2 mo</td>
<td>54.7 (9.0)a</td>
<td>34.8 (6.6)b</td>
<td>30.2 (7.3)c</td>
<td>21.1 (8.0)d</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>6 mo</td>
<td>47.0 (11.9)a</td>
<td>35.7 (7.3)b</td>
<td>31.3 (7.8)c</td>
<td>28.3 (10.7)c</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>12 mo</td>
<td>44.3 (12.5)a</td>
<td>34.5 (7.8)b</td>
<td>32.9 (7.1)b</td>
<td>29.8 (10.5)b</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
Types of diets

- Cereal-based, unrefined, or nonpurified
  - Composed predominantly of unrefined plant and animal ingredients

- Purified
  - Composed primarily of purified commercially-available proteins, carbohydrates, and fats with vitamins and minerals added

- Chemically defined
  - Composed of chemically pure nitrogen, carbohydrate, fat, vitamin, and mineral sources

Nonpurified diet

- Inexpensive
- Some modifications are possible
  - Examples: ↑ or ↓ Na⁺, cholesterol, protein; vary the type of carbohydrate
- Composition can vary from batch to batch
  - Constant formula: Ingredients analyzed before manufacture of each batch to ensure that the diet nutrient composition does not vary substantially
  - Altering amount of ingredient (e.g., alfalfa) alters amount of nonnutritive components such as heavy metal contaminants, phytoestrogens
Nonpurified diet

- Natural phytoestrogens
  - Inhibit atherosclerosis, hypertension, obesity, diabetes and the formation of some cancers
- Natural fluorescence

Mouse fed nonpurified diet

Mouse fed purified diet
Certified diets

- Tested for a variety of potential contaminants before shipping
  - Aflatoxin
  - Heavy metals
  - Organophosphates
  - Chlorinated hydrocarbons

<table>
<thead>
<tr>
<th>Heavy Metals</th>
<th>Maximum Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arsenic</td>
<td>1.0 ppm</td>
</tr>
<tr>
<td>Cadmium</td>
<td>.5 ppm</td>
</tr>
<tr>
<td>Lead</td>
<td>1.5 ppm</td>
</tr>
<tr>
<td>Mercury</td>
<td>.2 ppm</td>
</tr>
<tr>
<td>Selenium</td>
<td>.5 ppm</td>
</tr>
<tr>
<td>Aflatoxin</td>
<td>5 ppb</td>
</tr>
</tbody>
</table>
Purified diets

- Made from individual ingredients rather than whole foodstuffs (e.g., casein, cornstarch)
- Potential for many nutrient variations
Chemically defined diets - example

Example: No lysine diet

<table>
<thead>
<tr>
<th>Formula</th>
<th>g/Kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>L-Alanine</td>
<td>3.6</td>
</tr>
<tr>
<td>L-Arginine HCl</td>
<td>12.1</td>
</tr>
<tr>
<td>L-Asparagine</td>
<td>6.0</td>
</tr>
<tr>
<td>L-Aspartic Acid</td>
<td>3.5</td>
</tr>
<tr>
<td>L-Cystine</td>
<td>3.5</td>
</tr>
<tr>
<td>L-Glutamic Acid</td>
<td>40.0</td>
</tr>
<tr>
<td>Glycine</td>
<td>23.3</td>
</tr>
<tr>
<td>L-Histidine HCl, monohydrate</td>
<td>4.5</td>
</tr>
<tr>
<td>L-Isoleucine</td>
<td>8.2</td>
</tr>
<tr>
<td>L-Leucine</td>
<td>11.1</td>
</tr>
<tr>
<td>L-Methionine</td>
<td>8.2</td>
</tr>
<tr>
<td>L-Phenylalanine</td>
<td>7.5</td>
</tr>
<tr>
<td>L-Proline</td>
<td>3.5</td>
</tr>
<tr>
<td>L-Serine</td>
<td>3.5</td>
</tr>
<tr>
<td>L-Threonine</td>
<td>8.2</td>
</tr>
<tr>
<td>L-Tryptophan</td>
<td>1.8</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>5.0</td>
</tr>
<tr>
<td>L-Valine</td>
<td>8.2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>508.88</td>
</tr>
<tr>
<td>Corn Starch</td>
<td>150.0</td>
</tr>
<tr>
<td>Corn Oil</td>
<td>100.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>30.0</td>
</tr>
<tr>
<td>Mineral Mix, AIN-76 (170915)</td>
<td>35.0</td>
</tr>
<tr>
<td>Calcium Phosphate, dibasic</td>
<td>4.6</td>
</tr>
<tr>
<td>Vitamin Mix, Teklad (40060)</td>
<td>10.0</td>
</tr>
<tr>
<td>Ethoxyquin, antioxidant</td>
<td>0.02</td>
</tr>
</tbody>
</table>
Chemically defined diets

- Expensive
- Palatability often reduced
Choice of diet affects gene expression in mouse liver

- Nonpurified diet (Lab Rodent Diet-5001) vs purified (AIN-76a)
- Studies were testing the effect of different levels of arsenic in food or drinking water
- Lung also showed differential expression (not shown)

Gene categories affected by arsenic exposure and/or type of diet

- 132 categories including
  - Fatty acid metabolism
  - Glycolysis/gluconeogenesis (including GCK, G6PC, GPI, PKLR)
  - Starch and sucrose metabolism
  - Synthesis and degradation of ketones
  - PPAR signaling
  - Amino acid metabolism (many)

Factors Affecting Feed Efficiency

- Age
- Sex
- Season
- Stage of reproduction
- Behavior and activity
- Temperature
- Humidity
- Heredity
  - Estimates in mice range from 0.1 to 0.56 heritability
Energy costs of depositing tissue

- Depositing 1 g of protein or fat: 12.6 kcal of metabolizable energy
- Energy costs to gain lean vs fat tissue
  - Differ because muscle is largely water
    - 1 g lean tissue = 2.6 kcal
    - 1 g fat = 12.6 kcal
Based on a database maintained at PMI®, the following example shows how widely protein, alone, can vary in a fixed formula:

<table>
<thead>
<tr>
<th>Fixed-Formula Diet</th>
<th>The ingredients could conceivably contain the following protein levels:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
</tr>
<tr>
<td>Corn</td>
<td>30.0</td>
</tr>
<tr>
<td>Wheat</td>
<td>17.0</td>
</tr>
<tr>
<td>Soybean Meal</td>
<td>15.0</td>
</tr>
<tr>
<td>Midds</td>
<td>6.0</td>
</tr>
<tr>
<td>Fish</td>
<td>4.0</td>
</tr>
<tr>
<td>Oats</td>
<td>4.0</td>
</tr>
<tr>
<td>Alfalfa</td>
<td>3.0</td>
</tr>
<tr>
<td>Misc</td>
<td>21.0</td>
</tr>
<tr>
<td></td>
<td>100.0</td>
</tr>
</tbody>
</table>

Calculated protein level in formula above:

<table>
<thead>
<tr>
<th>Low</th>
<th>High</th>
</tr>
</thead>
<tbody>
<tr>
<td>14.5</td>
<td>17.3</td>
</tr>
</tbody>
</table>
Metabolizable energy: why doesn’t what goes in always equal what goes out?

- Inaccurate assessment of metabolizable energy
  - Fecal losses
    - High fat intake
    - High fiber intake
    - Resistant starches
  - Unassessed losses
    - Exceeding the renal threshold
    - Environmental temperature – thermoneutral environment
  - Energy costs of depositing different types of tissues