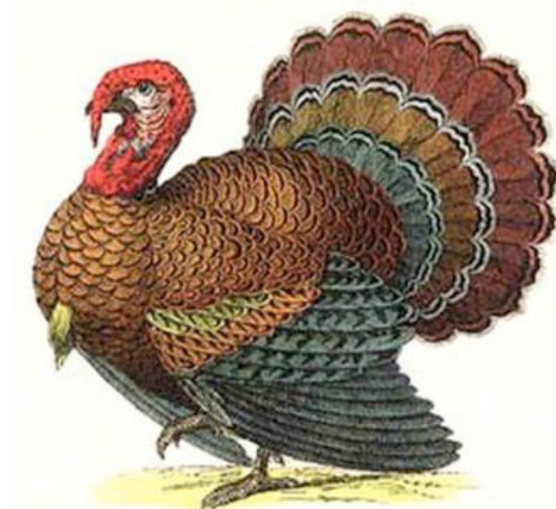
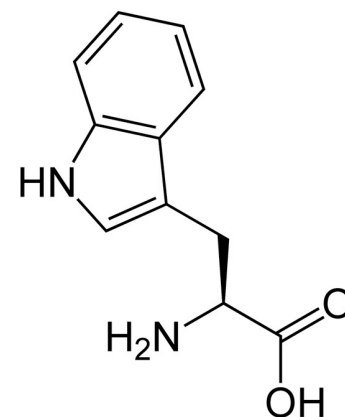
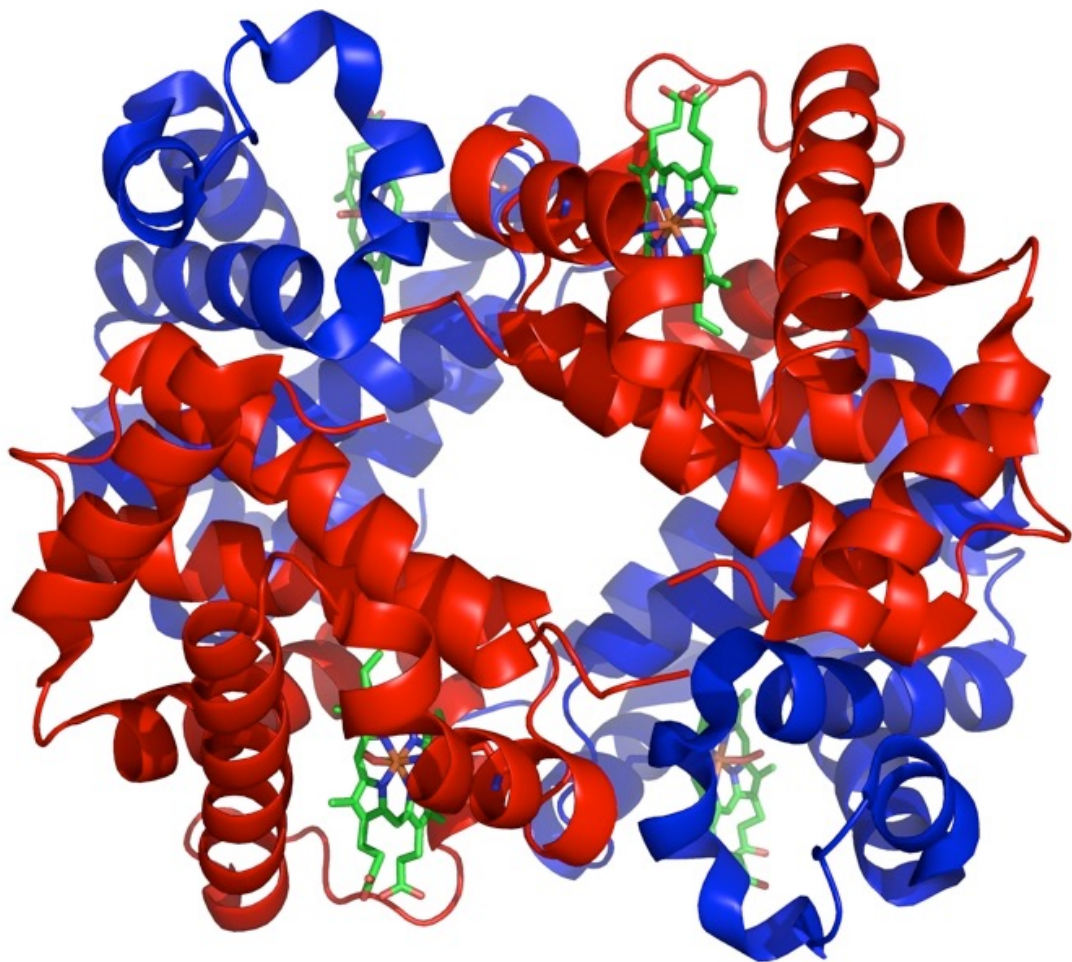


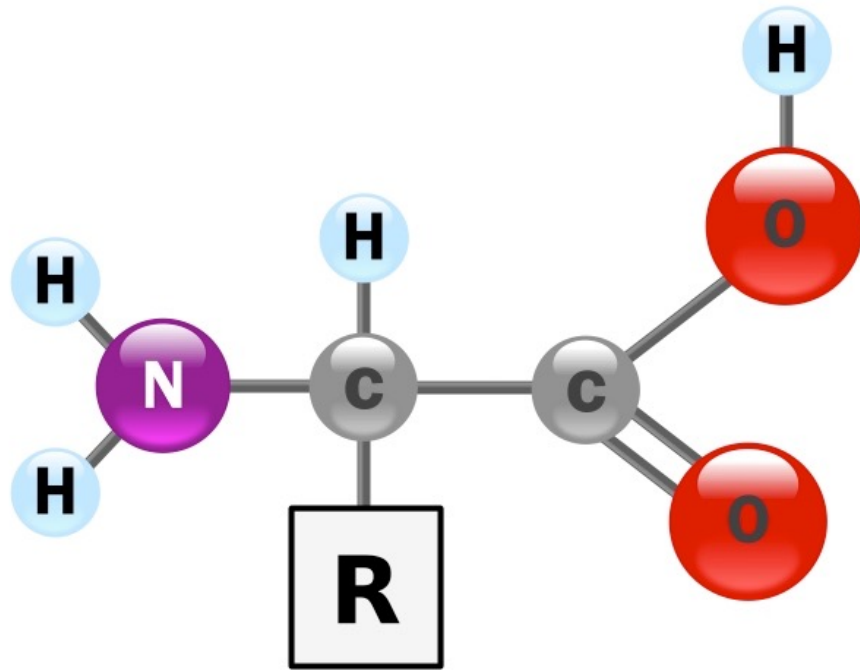
# Protein and Amino Acid Analysis



Chemistry M3LC

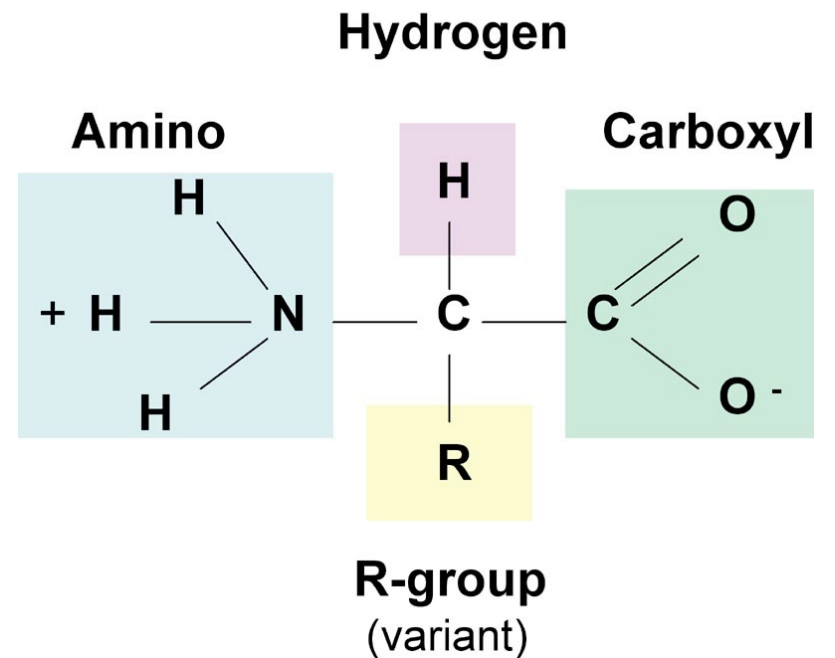
# Proteins

Proteins are made up of **amino acids**:



neutral form

## Amino Acid Structure

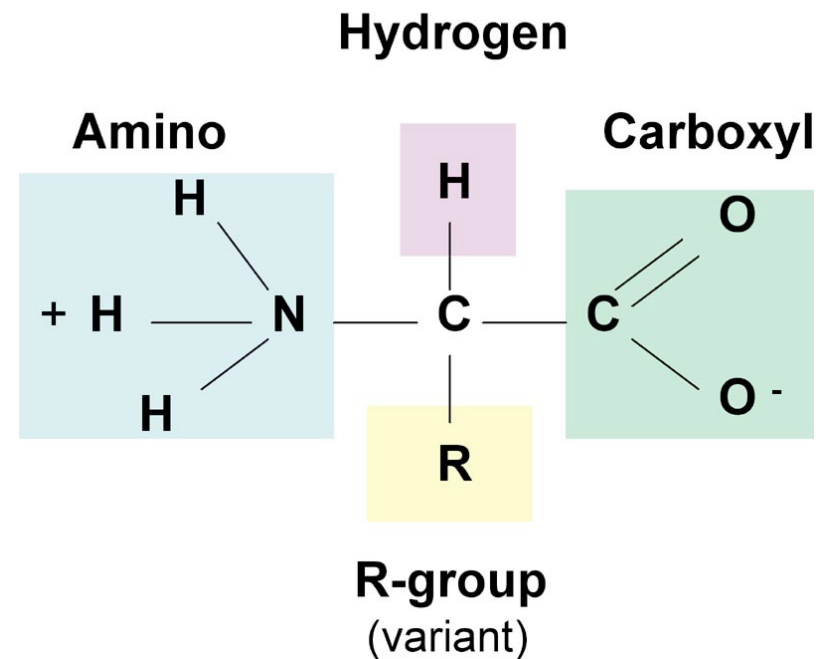


zwitterionic form

There are twenty standard amino acids:

A ala alanine  
R arg arginine  
N asn asparagine  
D asp aspartic acid  
C cys cysteine  
Q gln glutamine  
E glu glutamic acid  
G gly glycine  
H his histidine  
I ile isoleucine  
L leu leucine  
K lys lysine  
M met methionine  
F phe phenylalanine  
P pro proline  
S ser serine  
T thr threonine  
W trp tryptophan  
Y tyr tyrosine  
V val valine

## Amino Acid Structure



# There are twenty standard amino acids:

**A** ala alanine

**R** arg arginine

**N** asn asparagine

**D** asp aspartic acid

**C** cys cysteine

**Q** gln glutamine

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**G** gly glycine

**H** his histidine

**I** ile isoleucine

**L** leu leucine

**K** lys lysine

**M** met methionine

**F** phe phenylalanine

**P** pro proline

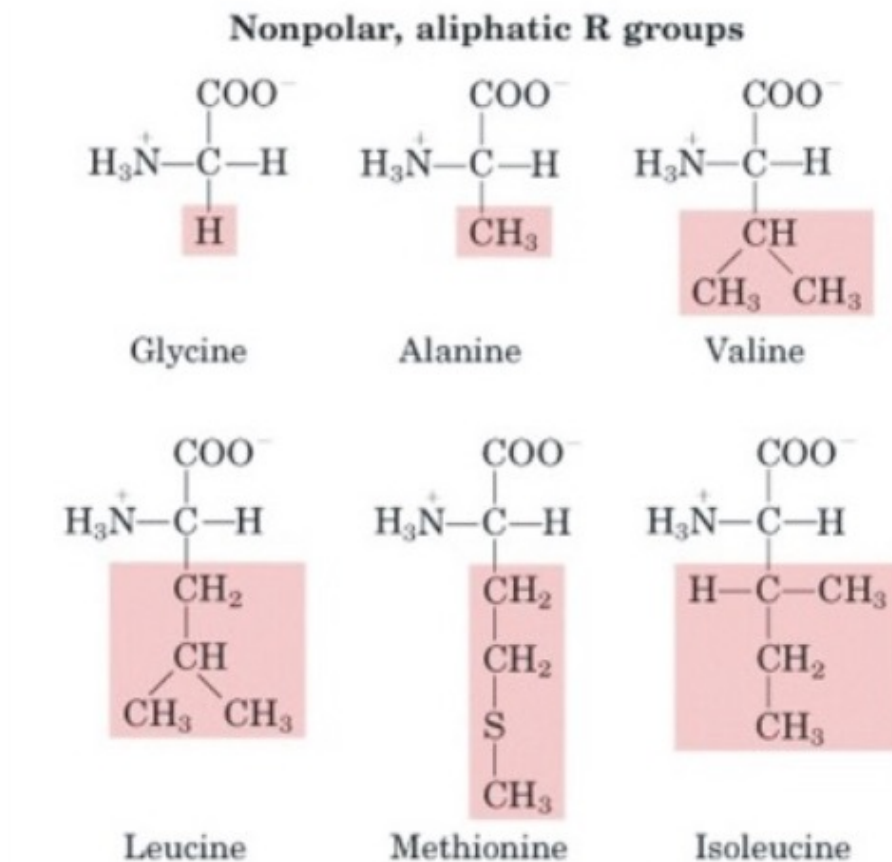
**S** ser serine

**T** thr threonine

**W** trp tryptophan

**Y** tyr tyrosine

**V** val valine



# There are twenty standard amino acids:

A ala alanine

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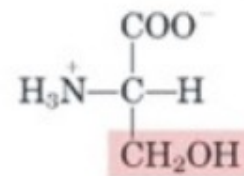
T thr threonine

W trp tryptophan

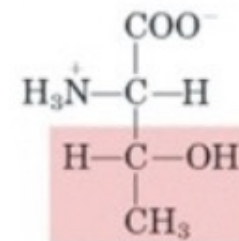
Y tyr tyrosine

V val valine

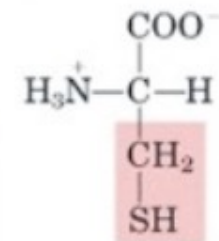
## Polar, uncharged R groups



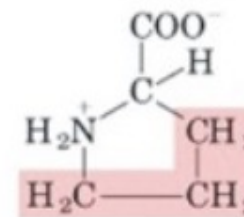
Serine



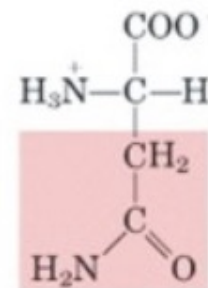
Threonine



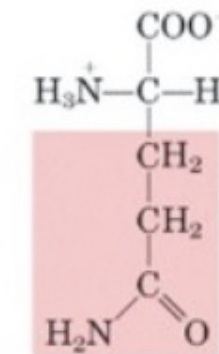
Cysteine



Proline



Asparagine



Glutamine

V val valine

The diagram shows the side chains of three amino acids, each highlighted in a pink box. Lysine's side chain is a straight chain of four methylene groups ending in a protonated amine. Arginine's side chain is a guanidinoethyl group. Histidine's side chain is an imidazole ring attached to a methylene group.

**Lysine**

$$\begin{array}{c} \text{COO}^- \\ | \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{NH}_3^+ \end{array}$$

**Arginine**

$$\begin{array}{c} \text{COO}^- \\ | \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{CH}_2 \\ | \\ \text{NH} \\ | \\ \text{C}=\text{NH}_2^+ \\ | \\ \text{NH}_2 \end{array}$$

**Histidine**

$$\begin{array}{c} \text{COO}^- \\ | \\ \text{H}_3\text{N}^+-\text{C}-\text{H} \\ | \\ \text{CH}_2 \\ | \\ \text{C}-\text{NH} \\ || \quad \diagup \quad \diagdown \\ \text{C}-\text{N} \quad \text{CH} \\ | \quad \quad \quad // \\ \text{H} \end{array}$$

Histidine

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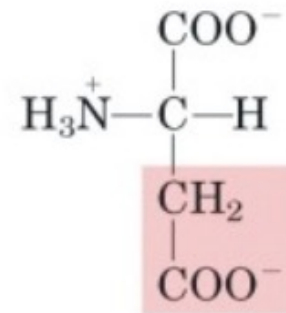
T thr threonine

W trp tryptophan

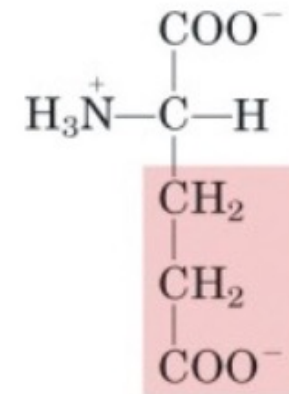
Y tyr tyrosine

V val valine

## Negatively charged R groups



Aspartate

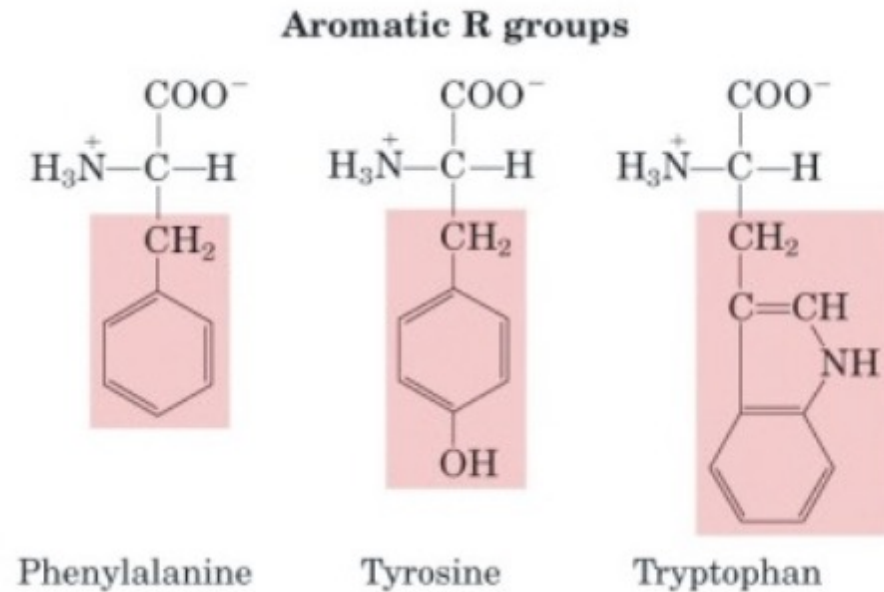


Glutamate



# There are twenty standard amino acids:

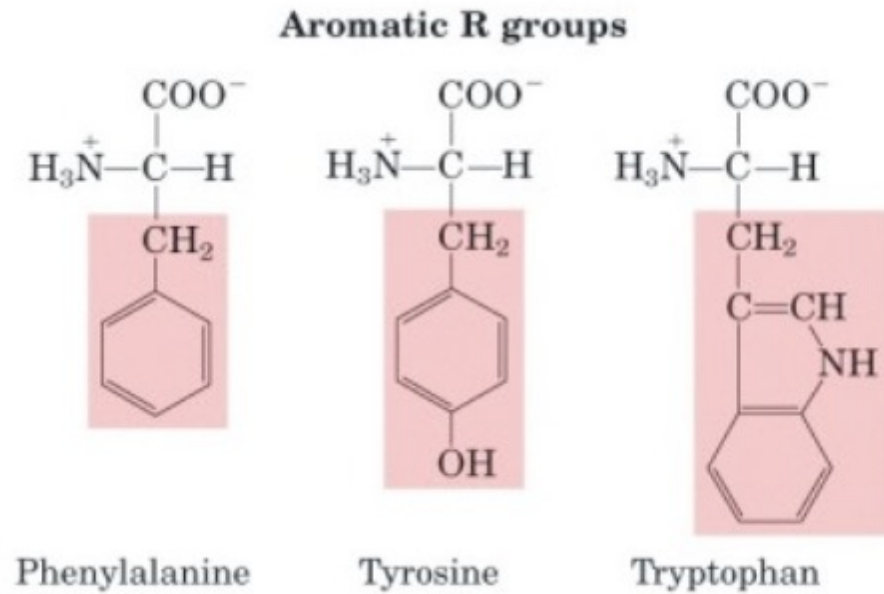
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M met methionine  
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# There are twenty standard amino acids:

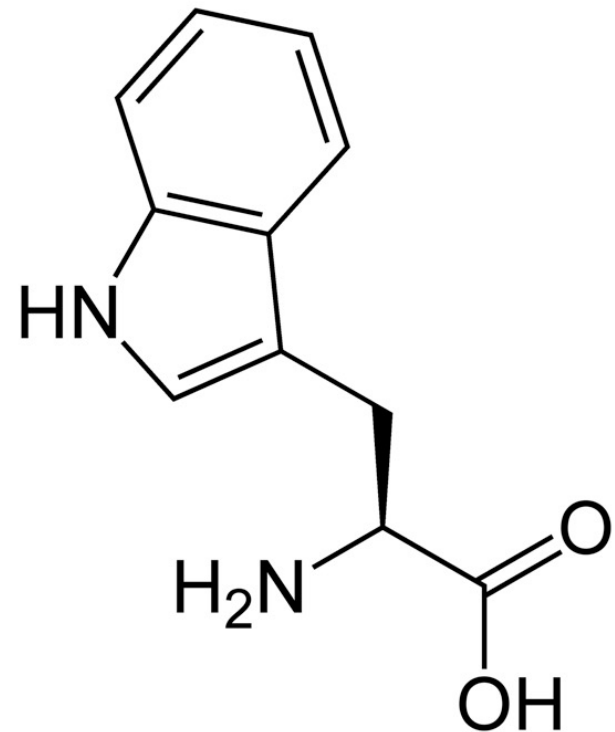
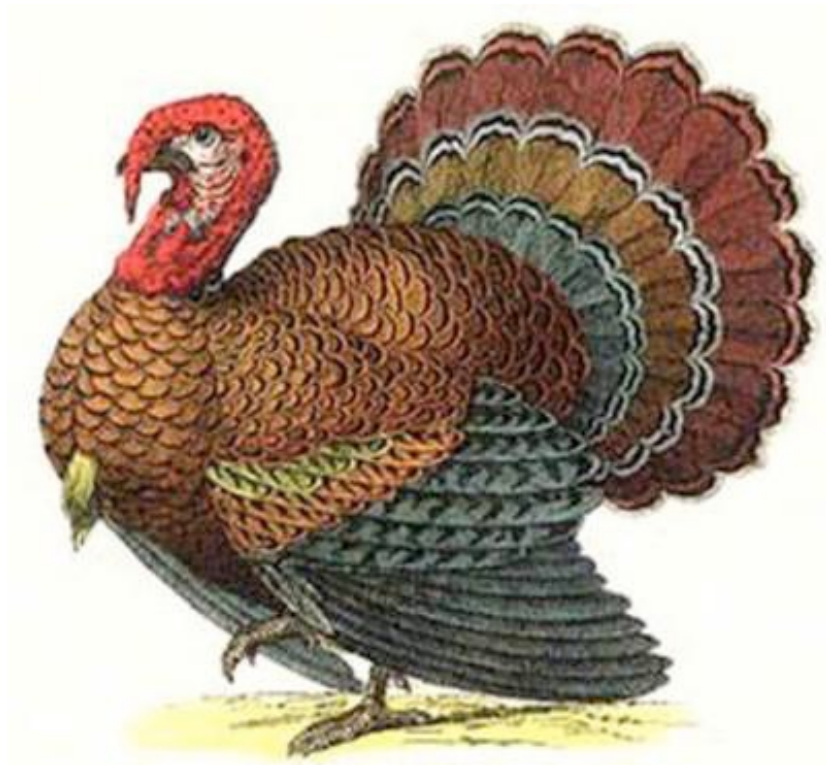
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L leu leucine  
K lys lysine  
M met methionine  
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V val valine



**Tryptophan**

# Tryptophan

A common belief is that heavy consumption of turkey meat (as for example in a Thanksgiving or Christmas feast) results in drowsiness, which has been attributed to high levels of the amino acid tryptophan contained in turkey. While turkey does contain tryptophan, the amount is comparable to that contained in most other meats. Furthermore, postprandial Thanksgiving sedation may have more to do with what else is consumed along with the turkey, in particular carbohydrates and alcohol.

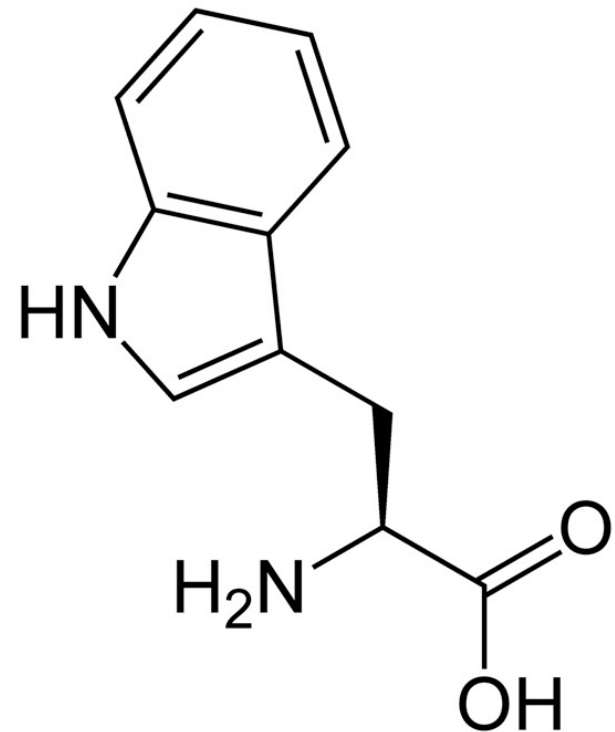


Tryptophan

# Tryptophan

Chicken and ground beef contain almost the same amount of tryptophan as turkey — about 350 milligrams per 4-ounce serving. Swiss cheese and pork actually contain more tryptophan per gram than turkey, and yet the American classic, a ham and cheese sandwich, somehow escapes blame.

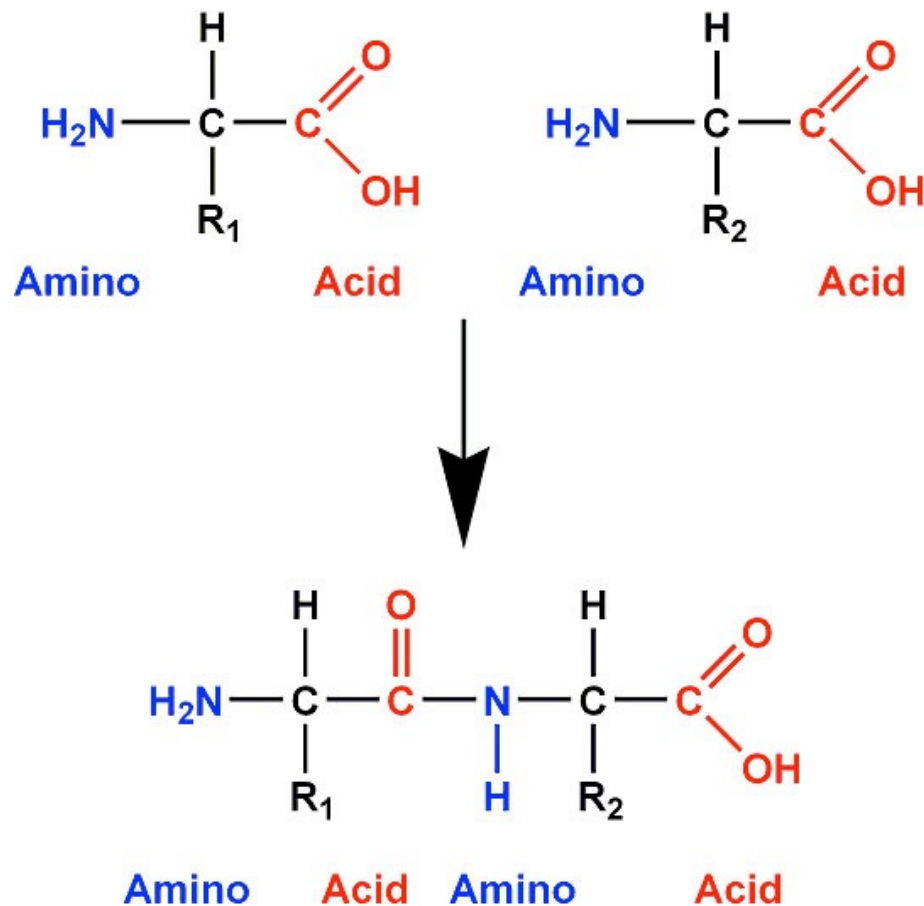
The amount of tryptophan in a single 4-ounce serving of turkey (350 milligrams) is also lower than the amount typically used to induce sleep. The recommendations for tryptophan supplements to help you sleep are 500 to 1,000 milligrams.



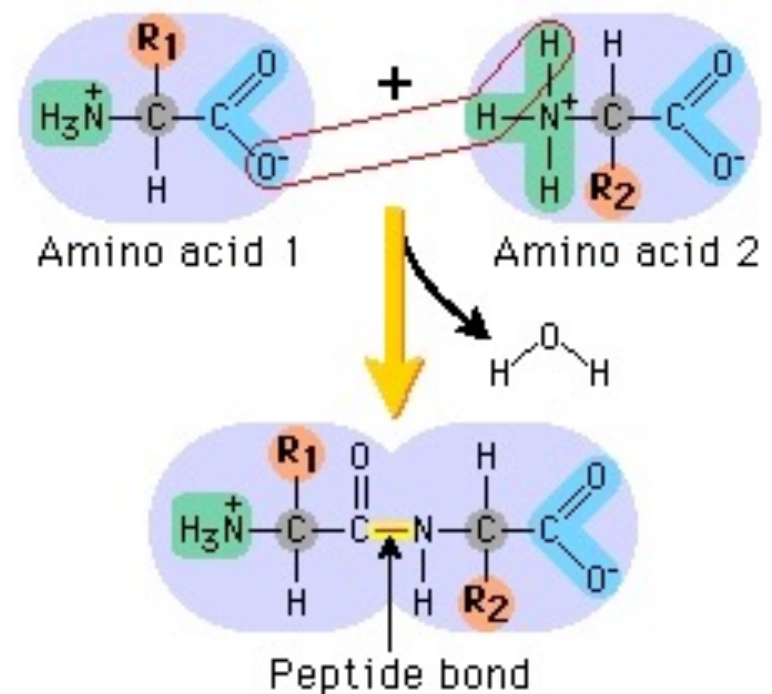
Tryptophan

# Peptides

The amino acids can be linked together with peptide bonds:

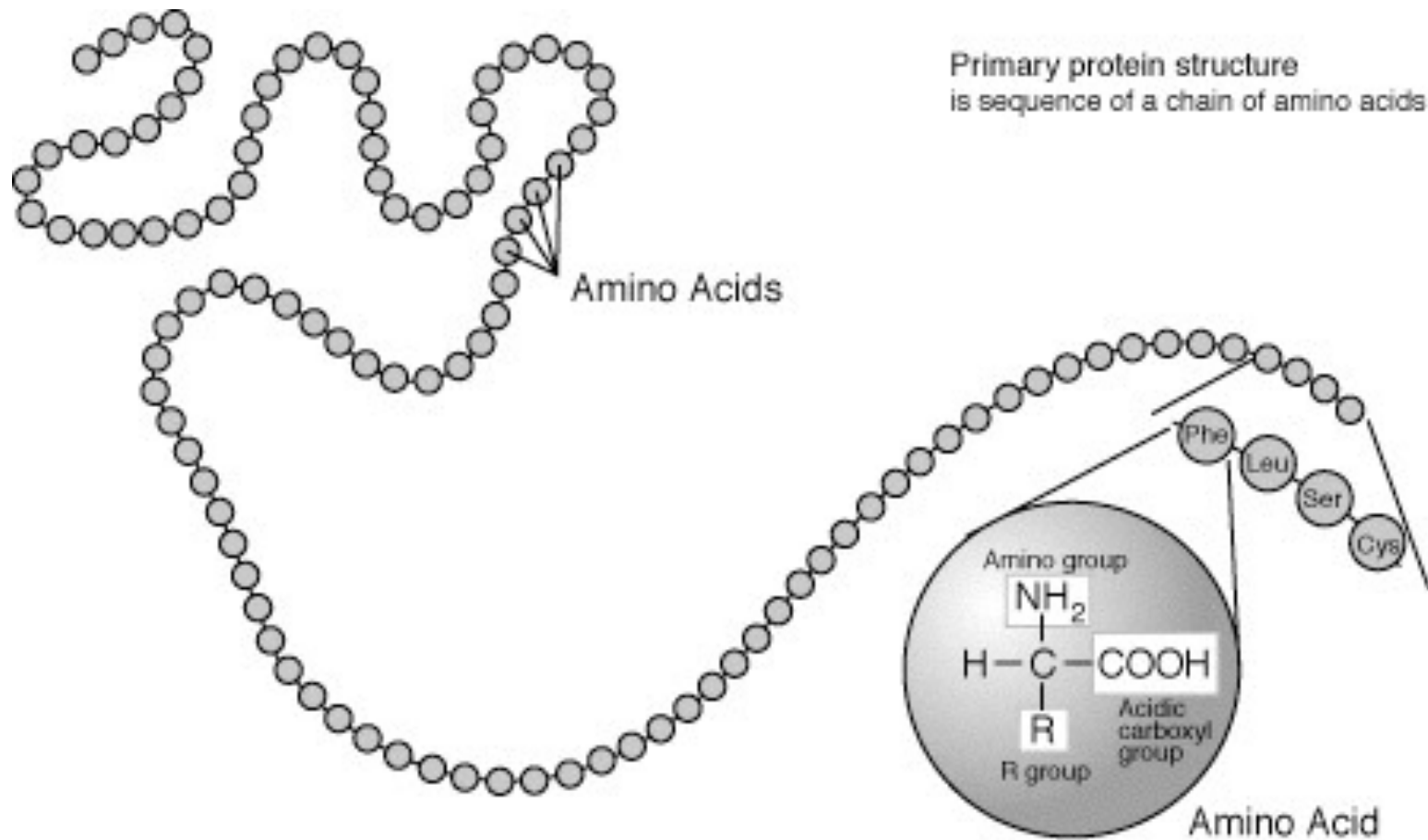


amide linkage



# Proteins

Proteins are made up of many amino acids linked together with many peptide bonds.





# Protein Analysis

Proteins fold into useful structures.

An example: Cytochrome C



x-ray crystal  
structure

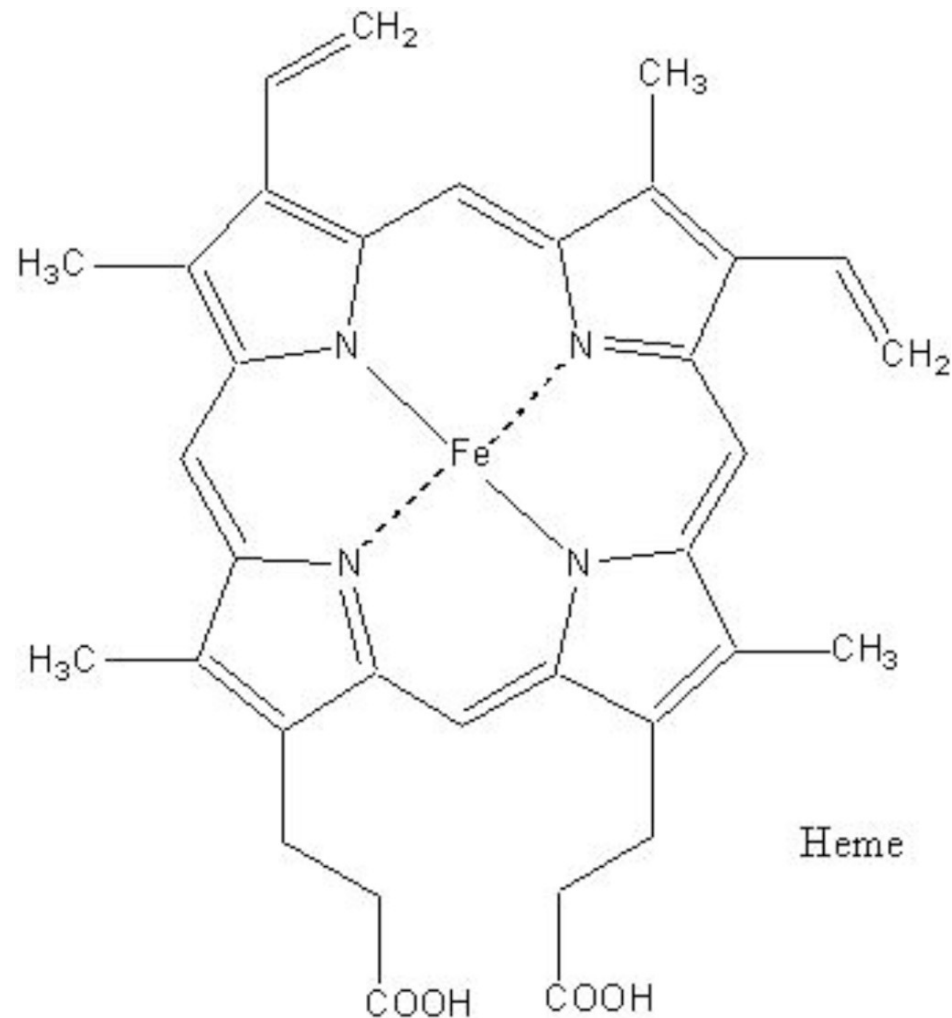
Cytochrome c is an electron-carrying mitochondrial protein. The transition of cytochrome c between the ferrous and ferric states within the cell makes it an efficient biological electron-transporter. It plays a vital role in cellular oxidation in both plants and animals, and is generally regarded as a universal catalyst of respiration.

# Protein Analysis

Cytochrome C transports oxygen and electrons with Fe-containing heme groups:

heme group

$\text{Fe}^{2+}$  or  $\text{Fe}^{3+}$





# Protein Analysis

Proteins fold into useful structures.

An example: Cytochrome C



x-ray crystal  
structure

Molecular mass:  
12,233 Da (Human Cyt c)

Residue Sequences:

Human:

mgdvekgkki fimkcsqcht vekggkhktg  
pnlhglfgrk tgqapgysyt aanknkgiw  
gedtlmeyle npkkyipgtk mifvgikkke  
eradliaylk katne

Fruit Fly:

mgvpagdvek gkklfvqrca qchtveaggk  
hkvgnlhgl igrktgqaag faytdankak  
gitwnedtlf eylenpkkyi pgtkmifagl  
kkpnergdli aylksatk

Why do we need people who know about Analytical Chemistry?

# Protein Analysis

## Kjeldahl method

The classic assay for protein concentration in food is the Kjeldahl method. This test determines the total nitrogen in a sample. The only major component of most food which contains nitrogen is protein (fat, carbohydrate and dietary fibre do not contain nitrogen). If the amount of nitrogen is multiplied by a factor depending on the kinds of protein expected in the food the total protein can be determined. On food labels the protein is given by the nitrogen multiplied by 6.25, because the average nitrogen content of proteins is about 16%. The Kjeldahl test is used because it is the method the AOAC International has adopted and is therefore used by many food standards agencies around the world.

### Supplement Facts

Serving Size: Two (2) scoops (60 g)

|                     | Amount<br>per serving | %Daily<br>Value* |
|---------------------|-----------------------|------------------|
| Calories            | 213                   |                  |
| Calories from Fat   | 27                    |                  |
| Total Fat           | 3 g                   | 5%               |
| Saturated Fat       | 1 g                   | 5%               |
| Polyunsaturated Fat | 1 g                   |                  |
| Monounsaturated Fat | 0.5 g                 |                  |
| Trans Fat           | 0 g                   |                  |
| Cholesterol         | 75 mg                 | 25%              |
| Sodium              | 90 mg                 | 3%               |
| Total Carbohydrate  | 7 g                   | 3%               |
| Dietary Fiber       | 1 g                   | 3%               |
| Sugars              | 3 g                   |                  |
| Protein             | 40 g                  | 70%              |

\* Percent Daily Values are based on a 2,000 calorie diet.

Other ingredients: Whey protein concentrate (containing Beta-Lactoglobulin (MW 18300), Alpha-Lactalbumin (MW 14000), Immunoglobulin G (MW 150000), Lactoferrin (MW 77000), Bovine Serum Albumin (MW 69000)), fiber blend (carragenan, xanthan, guar), natural vanilla flavor blend, sucralose, sodium chloride.)

# Protein Analysis

## 2008 Chinese Melamine Scandal

Hereunder are the companies affected with Melamine.  
Remember: Foods with creamer or milk should be avoided.



# Protein Analysis

## 2008 Chinese Melamine Scandal

In 2008, thousands of babies in China became ill, having suffered acute kidney failure, with several fatalities, having been fed formula milk contaminated with the industrial chemical melamine.

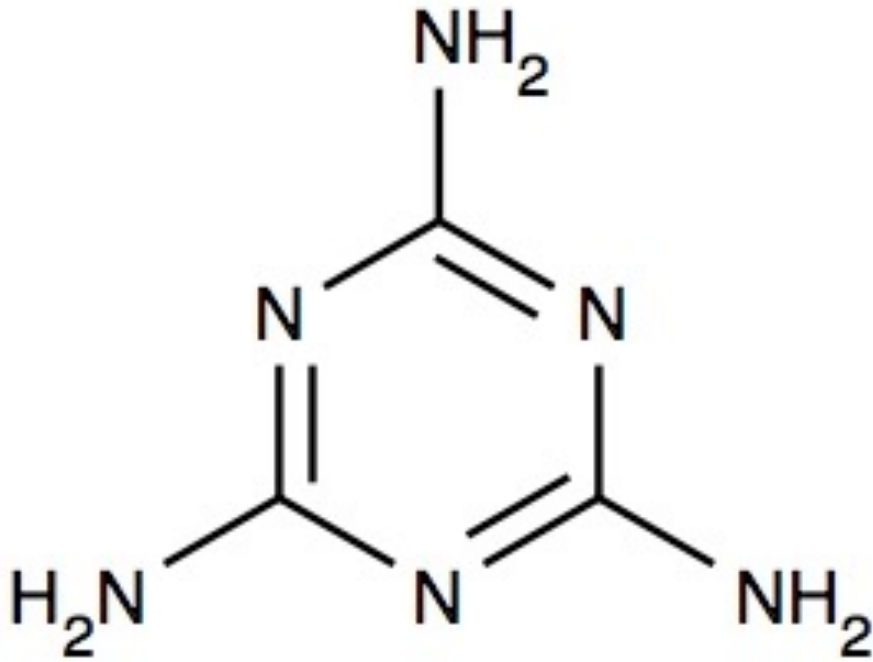
On 17 September 2008, Health Minister Chen Zhu stated that tainted milk formula had "sickened more than 6,200 children, and that more than 1,300 others, mostly newborns, remain hospitalised with 158 suffering from acute kidney failure". By 23 September, about 54,000 children were reported to be sick and 4 had died. An additional 10,000 cases were reported from the provinces by 26 September. A WHO official said 82 percent of the children made ill were 2 years of age or below. The Hong Kong Centre for Food Safety said that 99% of victims were aged under 3 years. Ten Hong Kong children were diagnosed with kidney problems, at least four cases were detected in Macau, and six in Taiwan.

Manufacturer, Sanlu, part-owned by New Zealand's Fonterra Cooperative, recalled all of its powdered milk products in China's north-west province of Gansu. However, twenty-two brands, including China Mengniu Dairy Co and Inner Mongolia Yili Industrial Group, of milk powder were quickly identified as containing melamine. "The majority of afflicted infants ingested Sanlu-brand milk powder over a long period of time, their clinical symptoms showed up three to six months after ingesting the problematic products," Health Minister Chen Zhu told Bloomberg Asia.

So, what is melamine and how does it spoof the protein levels in baby formula milk?



# Protein Analysis



Melamine



Melamine is an organic compound with chemical formula  $\text{C}_3\text{H}_6\text{N}_6$ . Officially it is 1,3,5-triazine-2,4,6-triamine in the IUPAC nomenclature system. It has a molecular mass of just over 126, forms a white, crystalline powder, and is only slightly soluble in water. It is used in fire retardants in polymer resins because its high nitrogen content is released as flame-stifling nitrogen gas when the compound is burned or charred.

It is its high nitrogen level – 66% nitrogen by mass – that gives melamine the analytical characteristics needed to fake the nitrogen in protein samples.

The world needs to use better methods for measuring protein content!!! This scandal should have never happened. :(

Why do we need to eat Turkey?



# Protein Consumption

Most health groups insist on you eating protein daily! Turkey is usually on the list:

## Meal Ideas:

Here are some easy ways for you to get your daily protein!

| Breakfast  | Snack               | Lunch  | Dinner                                     |
|--|---------------------|--|--|
| Toast with peanut butter   | String cheese       | Cold-cuts with vegetables and cheese (in a sandwich or wrap) | Baked or grilled fish                      |
| Scrambled eggs (or egg substitute) with cheese and salsa, rolled in a tortilla | Yogurt              | Chili with cornbread   | Turkey burger                              |
| Poached egg on an English muffin   | Hummus with carrots | Bean burrito   | Stir-fried tofu or chicken with vegetables |

Remember: Try to include some protein at every meal. It will help keep your muscles strong!

# Protein Consumption

Why do we eat protein? The following are "Essential Amino Acids," amino acids that we can not synthesize ourselves.

| Essential         | Nonessential     |
|-------------------|------------------|
| Isoleucine        | Alanine          |
| Arginine*         |                  |
| Lysine            | Aspartate        |
| Methionine        | Cysteine*        |
| Phenylalanine     | Glutamate        |
| Threonine         | Glutamine*       |
| <b>Tryptophan</b> | Glycine*         |
| Valine            | Proline*         |
| Histidine*        | Serine*          |
| Tyrosine*         | Asparagine*      |
| Leucine           | Selenocysteine** |

## RDAs

| Amino acid                                    | mg per kg body weight | mg per 70 kg | mg per 100 kg |
|---|-----------------------|--------------|---------------|
| <b>I</b> Isoleucine                           | 20                    | 1400         | 2000          |
| <b>L</b> Leucine                              | 39                    | 2730         | 3900          |
| <b>K</b> Lysine                               | 30                    | 2100         | 3000          |
| <b>M</b> Methionine<br>+ <b>C</b> Cysteine    | 10.4 + 4.1 (15 total) | 1050         | 1500          |
| <b>F</b> Phenylalanine<br>+ <b>Y</b> Tyrosine | 25 (total)            | 1750         | 2500          |
| <b>T</b> Threonine                            | 15                    | 1050         | 1500          |
| <b>W</b> Tryptophan                           | 4                     | 280          | 400           |
| <b>V</b> Valine                               | 26                    | 1820         | 2600          |

# Protein Consumption

## Limiting Amino Acids: Stoichiometry applied to eating.

What type of protein should we eat? Our bodies use amino acids in a specific ratio to each other, so if a person doesn't get enough of one of them to match with the rest, the rest can only be used at a level to balance with that low one. Most of these amino acids are fairly easy to get in a reasonably well-balanced diet. However, if you only get protein from one source, you could be limited by the specific amino acid content of your food. Stoichiometrically limited! The three most typical limiting amino acids include the two sulfur-containing amino acids (methionine and cysteine), tryptophan, and lysine.

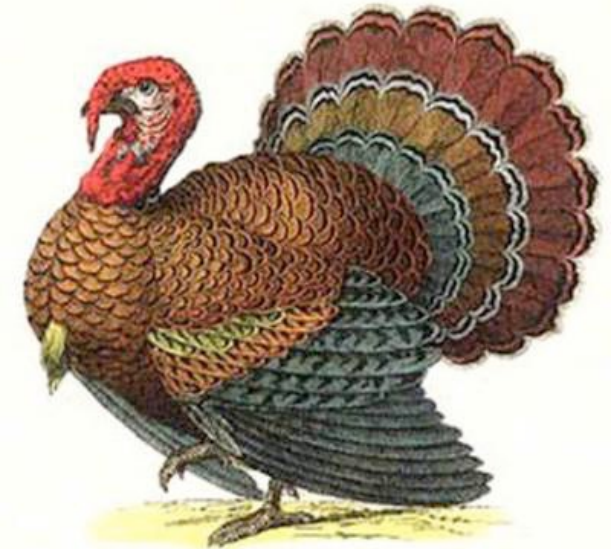
| Protein source | Limiting amino acid                        |
|----------------|--|
| Wheat          | lysine                                     |
| Rice           | lysine                                     |
| Legumes        | tryptophan or methionine (or cysteine)     |
| Maize          | lysine and tryptophan                      |
| Egg, chicken   | none; the reference for absorbable protein |

Turkey has plenty of all of the various amino acids, and lots of protein. So enjoy!

| Meat Type          | Calories | Total Fat | Protein  |
|--------------------|----------|-----------|----------|
| Breast with skin   | 194      | 8 grams   | 29 grams |
| Breast w/o skin    | 161      | 4 grams   | 30 grams |
| Wing w/skin        | 238      | 13 grams  | 27 grams |
| Leg w/skin         | 213      | 11 grams  | 28 grams |
| Dark meat w/skin   | 232      | 13 grams  | 27 grams |
| Dark meat w/o skin | 192      | 8 grams   | 28 grams |
| Skin only          | 482      | 44 grams  | 19 grams |

Resource: USDA Nutrient Data Laboratory – Turkey (Young Hen)

Data for a 100g sample.





Happy Thanksgiving!

