STUDENT GUIDE
Lesson 2: Nutrient Detective Lab

Objective: To identify and quantify the presence of carbohydrates, lipids, and proteins in various health food samples.

Driving Question: Why might someone who exercises consume whey protein?

What do we still need to know to answer the Driving Question?

- What is whey protein?
- What are other types of proteins or macromolecules that exist?
- How do the components of milk impact exercise?

Part 1: Reading About Macromolecules

What are the three types of macromolecules?

The 3 main macronutrients are protein, carbohydrate, and fat.

Why are macromolecules important to athletes?

Nutrition can play a crucial role in optimizing training sessions as well as with recovery and metabolic adaptation. It is important for athletes to consume the optimal ratio of macronutrients in their diet based on their training goals, such as gaining muscle or losing fat while maintaining lean mass.



Part 2: Protocols for Testing Macromolecules

Simple Sugar Test: Benedict's Solution

In the presence of simple sugars, the clear blue solution turns green, yellow, orange or brick red. (See the color key to see what these represent.)

1. Add 40 drops of the sample to a test tube.
2. Add 15 drops of Benedict's solution to the test tube.
3. Heat in a hot water bath at 40-50 degrees Celsius for 10 minutes.
4. Note the color change.

Complex Sugar (Starch) Test: Lugol's Iodine Solution

In the presence of starches, the solution will turn a dark bluish/purple-ish black.

1. Add 30 drops of sample to an empty well in the paint tray.
2. Add 5 drops of iodine.
3. Note the color change.

Protein Test: Biuret Solution

In the presence of proteins, this blue solution will change color to pink-purple

1. Add 30 drops of sample to an empty well in the paint tray.
2. Add 5 drops of Biuret solution.
3. Note the color change.

Lipid Test: Sudan III Stain

In the presence of lipids, fat cells will turn red and form a fatty layer on top of the liquid's surface.

1. Fill a quarter of the test tube with the sample. Fill another quarter of the test tube with water. The test tube should be half full.
2. Add 10 drops of Sudan III stain to the test tube. Cover with your thumb and shake gently. Let sit.
3. A red-stained oil layer will separate out and float on the water surface if fat is present.

Complete Table 1: Positive & Negative Results when Testing for the Presence of Macromolecules

Macromolecule	Chemical Test Name	Positive Test Color	Negative Test Color
Carbohydrates: Simple Sugars	Benedict's Solution	Green, Yellow, Orange or Red	Blue
Carbohydrates: Starch	Lugol's Iodine Solution	Dark Blue or Black	Reddish Orange Yellow Brown
Proteins	Biuret Solution	Pink or Purple	Blue
Lipids	Sudan III Stain	Reddish Orange (will clump or separate into layers)	Orange (even distribution)



Part 3: Preparing Samples for Testing

To prep your samples, use the following directions:

Liquid - pour it into a test tube until it is ¼ of the way full (less than half). Add 20mL of water to the test tube. Seal the test tube with your gloved thumb and vigorously shake to mix. After 30 seconds of shaking, allow the contents to settle for 1 minute.

Solid - use a mortar and pestle to grind the food into a fine powder if it is not already a powder. Add your sample to a test tube and add 20mL of water to the test tube. Seal the test tube with a clean gloved finger (not one used already) and vigorously shake to mix. After 30 seconds of shaking, allow the contents to settle for 1 minute.

Once you have prepared your samples, use the pH paper to test the pH of each one. Use a new piece of pH paper for each sample. Discard the pH paper after it has been used. Write the pH number on the lines above next to each sample name and circle if it is an acid, base, or neutral.

Look at the food items available for you to test. Choose 4 items to complete the macromolecule tests for. List the items you are testing below:

Sample 1: Nuts pH 6-7 Acid, Base or Neutral (circle one)

Sample 2: Yogurt pH 4-5 Acid, Base or Neutral (circle one)

Sample 3: Granola Bar pH 6-7 Acid, Base or Neutral (circle one)

Sample 4: Fruit pH 3-5 Acid, Base or Neutral (circle one)



Part 4: Data Analysis

Sample 1: Nuts				
Chemical Test	Benedict's	Lugol's Iodine	Biuret	Sudan III
Macromolecule	Simple sugars	Complex carbs	Protein	Fat
Color?	Blue	Reddish Orange Yellow	Pink or Purple	Reddish Orange (clumps, layers)
Positive or Negative?	Negative	Likely negative (small, fiber)	Positive (peptide bonds)	Positive

Notes:

Sample 2: Yogurt				
Chemical Test	Benedict's	Lugol's Iodine	Biuret	Sudan III
Macromolecule	Simple sugars	Complex carbs	Protein	Fat
Color?	Green, Yellow,	Reddish Orange	Pink or Purple	Reddish Orange

	Orange or Red	Yellow Brown		(clumps, layers)
Positive or Negative?	Positive (lactose)	Negative	Positive (high)	Positive (high if full fat, low if low fat or fat free)

Notes:

Sample 3: Granola Bar				
Chemical Test	Benedict's	Lugol's Iodine	Biuret	Sudan III
Macromolecule	Simple sugars	Complex carbs	Protein	Fat
Color?	Green, Yellow, Orange, or Red	Dark Blue or Black	Pink or Purple	Reddish Orange (clumps, layers)
Positive or Negative?	Positive (sugar, honey)	Positive (oats, grains)	Positive (moderate, especially if it contains nuts of seeds)	Positive, (moderate, depends on ingredients - nuts, seeds, added oils)

Notes:

Sample 4: Fruit				
Chemical Test	Benedict's	Lugol's Iodine	Biuret	Sudan III
Macromolecule	Simple sugars	Complex carbs	Protein	Fat

Color?	Green, Yellow, Orange, or Red	Reddish Orange Yellow Brown	Blue	Orange (even distribution)
Positive or Negative?	Positive (fructose, fiber)	Negative, but may be positive for starchy fruits like unripened banana	Likely negative (very small)	Likely negative (minimal, except for avocados or olives)

Notes:



Part 5: Summary of Results

Analyze the data you have collected. Summarize your findings and discuss which health foods contain carbohydrates, lipids, and proteins.

The following items tested positive for containing simple sugars: Yogurt, Granola Bar, Fruit
The following items tested positive for containing complex carbohydrates: Granola Bar
The following items tested positive for containing lipids: Nuts, Yogurt, Granola Bar
The following items tested positive for containing proteins: Nuts, Yogurt, Granola Bar

How do the results of the macromolecule tests compare with the nutritional labels on the food packaging? Are there any discrepancies? What are some possible reasons if there are differences observed?

The nutritional label for nuts, like almonds, shows they contain fat, carbohydrates (fiber and sugar) and protein, but the carbohydrates did not show up in our testing. It might be that the amount of sugar in the particular nut we tested contains more sugar than an almond.
The nutritional label for plain, non-fat yogurt shows it contains simple sugar and protein. Because we did not use non-fat yogurt, we also got a positive result for fat, which makes sense.
The nutritional label for a granola bar with oats and honey shows that it contains fat, carbohydrates (fiber and sugar), and protein. This is consistent with the testing we did and there were no differences.

The nutritional label for an apple shows that it contains carbohydrates (fiber and sugar), very little protein (0.5 g) and no fat. This is consistent with the testing we did. With such a small amount of protein, it would make sense that it does not show up in our experimental results.

What are the limitations of the tests used in this lab for identifying macromolecules?

The Benedict's Test is limited because you have no way of knowing the type of sugar present. Also, you don't get a specific amount for its quantity in the color change that occurs.

For the Lugol's Iodine Test, it only detects starch and not other complex carbohydrates. Like the Benedict's Test, there is no way to know the amount present in the sample.

The Biuret Test does not indicate the types of proteins present in the sample. Because of this, we don't know that it will work for all types of foods that contain protein.

The Sudan III Test is hard to distinguish between a negative result and a somewhat positive result. If there is not a lot of fat in the sample, it can be difficult to tell if it is just forming a layer at the top or if it is clumping to the fat molecules in the solution. Because of this, it probably leads to inaccurate or misinterpreted results at times. Also, just like the other tests, there is no way to know the amount of fat present in the sample.

How can the information gained from these tests be used to make informed dietary choices?

If you are looking to include a particular type of macronutrient into your diet, you can use the results of these tests to know if the food you want to eat will meet that need. For example, if you are trying to incorporate protein into your diet more frequently, it would not be a good choice to eat only fruit, as they contain little to no protein. Similarly, if you are trying to eliminate sugar from your diet, it would be a better choice to eat a snack containing nuts instead of eating fruit.

How do the different macromolecules contribute to the overall caloric content of a food item?

Not all of the macromolecules contribute caloric content to a food item equally. According to their nutritional labels, a medium sized apple has 95 calories. Nearly all of those calories come from carbohydrates, as it is the only macromolecule present. A granola bar has nearly the same amount of calories (100) but the calories come from carbohydrates, proteins, and fats. This must mean that not all of the macromolecules are able to provide the same amount of energy to the body.

If a food sample tested positive for both reducing sugars and starch, what does this indicate about the carbohydrate composition of the food?

It means that the food item has many different types of sugars present. It might contain some type of simple sugar, like glucose or fructose, but also contain a complex carbohydrate, like fiber. Testing positive for starch could also mean that the food item might just contain a lot of glucose.

What are the possible health implications of consuming foods high in lipids but low in proteins and carbohydrates, or vice versa?

It depends on what the person is doing during the day. As we learned in the Fueling for Performance reading, the different types of macromolecules contribute to metabolic functions differently and their impact on the body's performance depends on many things:

- the composition and quality of foods consumed
- the timing of consumption
- whether or not other supplements are being taken
- the person's current body composition
- How much energy they require during the day

During exercise, the way your body uses energy depends on the types of activity you are doing. For people who are doing strength training should opt for a higher protein diet. People who are doing low-intensity endurance training should opt for a higher fat diet. Regardless of the types of activities you are doing and the energy your body is using, there can be some negative effects from high-protein diets for some people, such as kidney failure and osteoporosis. It is also important to consume the right type of fats, focusing on unsaturated fats and essential fatty acids instead of trans fat.

How might the results of these tests be used in a real-world setting, such as in the food industry or healthcare?

In a real-world setting in the food industry, the results of these tests could be helpful in developing more well-balanced, healthier food options.

In a real-world setting like health care, the results of these tests could help doctors or medical providers help people make more informed choices about the foods they consume and the impacts they might have on their bodies.

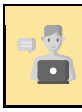
How do the roles of carbohydrates, lipids, and proteins differ in the body, and why is it essential to have a balanced intake of each?

Proteins are composed of amino acids, some of which only come from proper nutrition. They are important for body processes like building muscle, enzymes, and hormones. Healthy protein sources such as lean meats/fish, cottage cheese, and Greek yogurt are essential to ensuring that the body functions properly.

Carbohydrates are the main source of energy during high-intensity activity for the central nervous system as well as muscular work. Not all carbohydrates are created equal, so consuming whole grains, fruits, and vegetables that are rich in antioxidants, fiber, and vitamins/minerals is better for the body than foods with too much processed sugars.

Fats are needed for many body processes, like cell membrane structure, absorption of vitamins, and hormone regulation. There are different types of fats that the body needs during low-intensity endurance exercise as a source of fuel. However, trans fat and saturated fat should be consumed less than healthy sources of fat, which can be found in salmon, nuts and nut butters, as well as avocado oil.

Having a properly balanced intake of all of these macronutrients can keep the body's metabolic functions maintained and can provide many options for energy sources during the day, especially during exercise.



Part 6: Constructing an Explanation About Whey Protein Consumption

Use the findings from your investigation to construct an explanation to the Driving Question, ***Why might someone who exercises consume whey protein?***

Proteins are important for many body processes. Proteins are composed of both essential and nonessential amino acids, with the former only being fully obtained through proper nutrition as our bodies cannot produce them.

According to the Fueling for Performance reading, the dietary reference intake (DRI) for protein is 0.8 g per kilogram body weight (0.36 g/lb) for sedentary individuals. There are many instances where higher daily protein intake is beneficial for athletes; examples include strength training, maintaining lean mass in a caloric deficit, and during injury recovery.

Recommended sources of protein for athletes include lean meats and fish, cottage cheese, eggs, plain Greek yogurt, and protein shakes. If an athlete were making a protein shake to drink before or after a workout, a common type of protein available at stores is whey protein. The likelihood of someone who is not needing the extra protein to provide energy during exercise making a protein shake is low. Those individuals would likely consume whey protein in other ways or in smaller amounts, such as having yogurt for breakfast. It seems more likely that someone who works out would be supplementing their regular diet with other forms of protein and consider using whey protein.