Laboratory exercise: study of digestive and regulatory processes through the exploration of fasted and postprandial blood glucose

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Hopper MK, Maurer LW. Laboratory exercise: study of digestive and regulatory processes through the exploration of fasted and postprandial blood glucose. *Adv Physiol Educ* 37: 254–263, 2013; doi:10.1152/advan.00172.2012.—Digestive physiology laboratory exercises often explore the regulation of enzyme action rather than systems physiology. This laboratory exercise provides a systems approach to digestive and regulatory processes through the exploration of postprandial blood glucose levels. In the present exercise, students enrolled in an undergraduate animal physiology course select to participate in either an oral glucose tolerance test (OGTT) or one of the following three meal treatments based on typical student breakfasts: 1) high glycemic load (HGL), 2) moderate glycemic load (MGL), and 3) low glycemic load (LGL). The caloric value of the meals is 540 kcal. An Accucheck glucometer is used to determine fasted and 30-, 60-, and 120-min postprandial blood glucose levels. Students discover that postprandial glucose levels peak similarly for the OGTT and HGL group (137 ± 7.1 and 145 ± 4.7 mg/dl) and remain higher than MGL and LGL groups over the 2-h period. Between sampling, vibrant discussion covering such topics as glucose and cognitive function, insulin resistance, epigenetics, and fat diets occurs. The postlaboratory assignment requires students to discuss the importance of glucose homeostasis, graphically summarize their findings, review the literature to describe results in light of published data, and describe relationships between hyperglycemia and hyperinsulinemia and disease processes. Students evaluated this laboratory as highly effective and one of the top three experiences of the course.

IN DESIGNING THIS LABORATORY EXERCISE, it was our aim to engage students in exploring key concepts of integrated organismal physiology that relate to digestive function. Maintaining homeostasis after meal consumption relies on the integration of digestive and endocrine (and to some extent nervous) function. Therefore, the regulation of blood glucose, both fasted and fed, was ideal to meet our objective.

Overview

Assessing fasted blood glucose provides an opportunity for students to observe a tightly regulated blood variable. Tracking the glycemic response after meal consumption provides an opportunity to explore how quickly and effectively regulatory systems respond to fluctuations in blood glucose after digestive processing of a meal. Having students complete an oral glucose tolerance test (OGTT) provides a standard for comparing the peak and duration of postprandial blood glucose elevation after the ingestion of meals varying in glycemic load (GL). Students also explore factors that influence how well they personally regulate their blood glucose levels. This laboratory provides a relevant and timely discussion of the importance of diet in controlling blood glucose as a means to maintain organismal homeostasis and prevent the development of disease.

Background

Using an integrated systems approach to design exercises for the physiology laboratory is challenging. This approach has been more effective for systems such as the cardiovascular and respiratory systems, where measures such as heart rate, blood pressure, breathing frequency, and lung volumes can be obtained noninvasively. Other systems, such as the digestive system, present particular challenges. Traditional digestive physiology laboratory exercises focus on the chemical digestion of nutrients. Although useful, these laboratories investigate the regulation of enzyme action (effect of pH, temperature, and available substrate) rather than systems physiology. Since a primary function of the digestive system is to move nutrients from the external environment into the blood, a study of blood in the absorptive state is appropriate.

Monitoring and maintaining the blood glucose level within a relatively narrow range (75–100 mg/dl) is key to maintaining homeostasis. Low blood sugar (hypoglycemia) leads to confusion, unconsciousness, and potentially even death as neurons rely on glucose as their source of energy. Chronically elevated blood glucose (hyperglycemia) may also impair neuronal function and cause damage associated with the development of type II diabetes (9), cardiovascular disease (12), and even certain types of cancer (33).

Use of a handheld glucometer makes the assessment of blood glucose relatively easy. Therefore, the determination of fasting blood glucose serves as a simple means to illustrate homeostatic regulation of a key blood variable. Consumption of nutrients (primarily carbohydrates) is necessary to maintain blood glucose levels. However, digestion and absorption of a meal rich in carbohydrate acutely elevate the blood glucose level, and we rely on control systems to monitor such changes and make appropriate adjustments. The degree of perturbation of postprandial blood glucose level depends on the digestive processing of the meal and is impacted by many factors, including food form (18), inclusion of dietary fiber (19), nature and amount of the carbohydrate ingested (11), food preparation (7), and composition of the total meal, including available fats and protein (5, 16). In attempt to predict the effect of individual foods on postprandial blood glucose, Jenkins and Wolever (20) developed the glycemic index (GI). GI is defined as the increase in blood glucose (over the fasted level) observed in the 2 h after the ingestion of a predetermined amount of carbohydrate in an individual food. This value is then compared with the response to a reference food (glucose or white bread) containing an equivalent amount of carbohydrate. However,
metabolism within the cell. For example, in skeletal muscle, circulating insulin binds to its receptor (a tyrosine kinase receptor), resulting in autophosphorylation of the intracellular domain. Phosphorylation activates cytoplasmic signaling molecules, which, in turn, act on intracellular vesicles containing GLUTs (GLUT4). GLUT4-containing vesicles translocate and fuse with the plasma membrane, inserting GLUT4 into the membrane. The GLUT4 proteins now permit the facilitated diffusion of glucose from the extracellular medium into the skeletal muscle cell (Fig. 2). Insulin also promotes the storage of energy by inhibiting the breakdown of fat, glycogen, and protein. Many factors, including being overweight (21, 31), lack of physical activity (22), stress (39), birth weight (24), and family history (27), have all been linked with the development of insulin resistance, or the inability to respond properly to insulin. Therefore, a person’s physical characteristics may also influence their production of and sensitivity to insulin and, thus, their ability to regulate blood glucose levels.

Learning Objectives

After completing this activity, the student should have acquired the following content knowledge and be able to perform the following process skills:

1. Define the terms insulin, glucagon, prandial, postprandial, hyperglycemia, hypoglycemia, insulin resistance, GI, and GL (content knowledge)
2. Describe how the blood glucose level is regulated (content knowledge)

3. Explain the mechanisms by which a) insulin is released from the pancreas and b) insulin stimulates glucose transport in peripheral tissues (primarily skeletal muscle) (content knowledge)

4. Analyze personal blood glucose values in relation to defined normal values (process skill)

5. Predict the postprandial blood glucose level based on the GL of a meal (process skill)

6. Discuss the importance of regulating blood glucose levels both acutely and chronically (process skill)

Activity Level

Although this laboratory is written for undergraduate physiology students, it could be modified to apply to students of all ages and levels from middle school through graduate and medical school.

Prerequisite Student Knowledge or Skills

Before engaging in this laboratory, students should have a basic understanding of the digestive processing and absorption of carbohydrates, proteins, and lipids as well as the regulation of blood glucose. It is also useful for students to understand the concepts of GI and GL. Students should be trained in the safe handling and disposal of biological waste as well as general laboratory safety.

Time Required

Completion of this laboratory as written requires 2.5–3 h. To shorten the time required, students could be asked to eat at a set time before class or assess postprandial sugars for a shorter time period.

METHODS

Equipment and Supplies

Available over the counter. The following equipment and supplies are available over the counter (anywhere diabetic supplies are sold, such as commercial pharmacies and online):

1. Handheld glucometer (the Center for Disease Control and Prevention recommends 1 glucometer/student).

2. Glucometer batteries (always have spare on hand).

3. Glucometer test strips used for testing glucose in whole blood. Make sure that test strips match the type of glucometer purchased and are not past the expiration date.

4. Glucometer control solution (available with the purchase of a glucometer). Solutions will be used to test the glucometer before use.

5. Lancets and a lancet pen. Lancets and pens are often packaged and sold with the glucometer. Lancets can also be purchased separately. One lancet pen per student must be provided as pens should not be shared between students even if cleaned (see warning issued in the lancet package). Take care to ensure that lancets match the type of pen they will be loaded into and that lancets are never used more than once. Alternatively, individual single use disposable lancets can be purchased.

6. Alcohol swabs. These will be used to clean the fingertip before the finger stick.

Available through a scientific laboratory supply company. The following equipment and supplies are available through a scientific laboratory supply company (some of these items may be available through a commercial pharmacy):

1. Biohazard sharps containers. These will be required for the disposal of lancets after use.

2. Red biohazard (autoclave) bags. Use appropriately labeled bags for the collection of blood-contaminated materials (bench paper, glucometer strips, alcohol swabs, and band-aids).

3. Laboratory coats and eye protection. Care should be taken to allow students to work in an independent space. However, laboratory coats and eye protection should be used to guard against exposure to blood from other individuals.

4. Disposable laboratory safety (exam) gloves. Although students will work with ONLY their own blood, the instructor may need to wear gloves and provide assistance.

5. Food-grade dextrose (glucose) anhydrous (granular powder). There should be sufficient quantity to mix the required number of oral glucose solutions (75 g/person). Oral glucose solution (75 g) can also be purchased premixed.

Available through a commercial grocery. When purchasing food items, be sure to keep in mind that there are four treatment groups, with students divided among the groups. For example, if you have 20 students, there will be 5 students/treatment. Therefore, you would need to purchase 5 energy drinks and 10 toaster pastries for this group.

1. Energy drinks (small size, ~8 oz, 1 energy drink/student).

2. Toaster pastries (any flavor, frosted, 2 toaster pastries/student).

3. Bananas (medium size, ripe, 1 banana/student).

4. Medium-size bagels (whole wheat or white, 1 bagel/student).

5. Creamy peanut butter (enough to provide 2 oz/student).

6. Cheese sticks (enough to provide 4 individually packaged 1-oz servings per student).

7. Deli sliced ham [enough to provide 16 thin slices (200 g)/student].


9. Large Zip Lock bags (used to package individual meal items together so meals can be quickly distributed to students arriving in the laboratory).

10. Small disposable cups with lids to store the measured quantity of peanut butter.

11. Bottled water [sufficient volume to mix the required number of oral glucose solutions (330 ml/person)].

12. Sugar-free drink flavoring (used to flavor the oral glucose solutions). Mix this with water according to the package directions.

13. Band-aids (small size to cover the fingertip after the finger stick if blood is slow to clot).

14. Bleach (diluted 1:10). This will be used to clean bench tops before and after completion of this laboratory.

15. Antiseptic hand cleanser and/or hand soap and paper towels. Students should thoroughly cleanse their hands both before and after participation in this laboratory. When antiseptic hand cleansers or towlettes are used, hands should be washed with soap and running water as soon as feasible.

Human Subjects

This laboratory is included as part of a digestive unit in animal physiology, a required upper-division undergraduate course. Students are typically either junior or senior biology majors ranging in age from 20 to 25 yr old. Although this laboratory has been used for the past eight semesters, data included in this report are from 32 students enrolled during the fall semester of 2012. The protocol was approved by the Institutional Review Board, and all subjects gave informed consent (see Supplement 1 in the Supplemental Material). Adopters of this activity are responsible for obtaining permission for human or animal research from their home institution. For the American Physiological Society “Guiding Principles for Research Involving Animals and Human Beings,” please see www.the-aps.org/mm/Publications/Ethical-Policies/Animal-and-Human-Research.
Instructions

One week before the laboratory. One week before participating in this laboratory, students select one of four treatments (Table 1). Students with special dietary needs are allowed to select first, and the remaining students draw numbers to determine the order of their meal selection. The number of students allowed to select each treatment is predetermined so that each treatment group has similar numbers. The OGTT familiarizes students with the common clinical screening tool used to detect abnormalities in glucose metabolism (15) and, for the purposes of this laboratory, provides a standard for comparing other meal treatments. Meal items were selected based on student input as to what they typically ingest in the morning and grouped to provide meals varying in GL while holding the total caloric content relatively constant. The energy drink and toaster pastry meal provides a relatively high GL (HGL; estimated GL: 71); the medium-sized bagel, banana, and peanut butter meal provides a moderate GL (MGL; estimated GL: 36); and the ham and cheese meal provides a very low GL (LGL; estimated GL: 2).

Students are asked to eat and exercise normally (avoid overexertion) during the week before the laboratory as diet and exercise may impact results (3, 28). Students are also instructed to complete a predetermine fast by eating only a light breakfast at least 6 h before class on the day they will participate in this laboratory. Although fasting is typically 10–12 h, we felt it necessary to allow students to eat something before morning classes as all laboratories meet in the afternoon. Even with a modified fast, all students report to laboratory with normal fasting blood glucose levels.

Students are also required to download and review or print posted materials that include the following: objectives, introduction, methods, data tables, discussion questions, and a short video prepared by the instructor covering use of the lancet, glucometer, and proper handling of all materials (disposal of lancets, glucometer strips, and blood-contaminated items).

One day before the laboratory. Laboratory staff purchase food items through a commercial grocery, premeasure individual meals, divide meals into Zip Lock bags, and place the bags in the refrigerator. Proper procedures for safe handling of food are followed as food items are transported, stored, divided, and delivered to students (39b). The glucose solution for the OGTT treatment group is mixed according to World Health Organization’s recommendations for adults (75 g glucose mixed in 330 ml water) (15), flavored with sugar-free zero-calorie drink mix, and stored in the refrigerator until consumed. An estimate results (3, 28). Students are also instructed to complete a

Laboratory day. The following steps are performed on the day of the laboratory:

1. Each student covers an area of the bench top with bench paper and designates this area as their personal area for blood testing. The following materials are provided centrally at each bench and shared: Accu-check Compact Plus Glucometer with lancet device, lancets, glucometer test strips, alcohol wipes, hand sanitizer, sharps bucket, bench paper, biohazard disposal bags, 10% bleach solution for cleaning up, and graph paper.

2. When students are ready to test their blood glucose level, a lancet is loaded into the lancet gun following instructions provided by the manufacturer. Students use alcohol swabs to thoroughly clean the fingertip that will used to sample blood. (Students should use a different finger for all subsequent samples.) The student then conducts a finger stick to obtain a single drop of blood. Following the directions provided with the glucometer, students analyze their blood to determine the fasted blood glucose level. Used glucometer strips are placed in the red biohazard bags. Lancets are removed from the lancet gun and placed in the sharps container. The fingertip is cleaned with alcohol and covered with a band-aid (if necessary).

3. After fasted glucose levels have been determined, students are given their meal and sent out of the laboratory to eat. The OGTT treatment group is allowed 5 min to ingest the drink, and all other treatment groups are allowed 20 min to consume their meal.

4. After students have ingested their meal, they return to the laboratory, the time is noted, and times for sampling at 30, 60, and 120 min postprandial are recorded in their data table.

5. Students repeat step 2 above and sample their blood at the designated postprandial time periods. Blood glucose levels are recorded in the data table (Table 2).

6. Upon completion of the laboratory, students anonymously post data and clean up. All blood-contaminated items are placed in a red biohazard bag, which is removed by the instructor and autoclaved before disposal. Bench tops are cleaned with bleach solution. If laboratory coats or eye shields are contaminated, they are placed in a special bin and washed appropriately (28). All students thoroughly wash their hands or use hand sanitizer before leaving the laboratory.

Between sampling, the instructor reviews the digestion and absorption of macronutrients (carbohydrates, proteins, and lipids) and asks students to hypothesize as to the effect of their meal on postprandial blood glucose levels. Requiring students to write their hypotheses addresses the objective of having students predict postprandial blood glucose levels based on the GL of the meal. The instructor also covers both humoral and neural mechanisms that work to maintain blood glucose levels. Illustrations are used to present key concepts including

Table 1. Treatment nutritional information

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Description</th>
<th>Total Kilocalories</th>
<th>Carbohydrate, g</th>
<th>Fat, g</th>
<th>Protein, g</th>
<th>Fiber, g</th>
<th>GL</th>
</tr>
</thead>
<tbody>
<tr>
<td>OGGT</td>
<td>75 g glucose in 330 ml water</td>
<td>300</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>300</td>
<td>75</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>75</td>
</tr>
<tr>
<td>HGL</td>
<td>8.3 fluid oz energy drink</td>
<td>115</td>
<td>26</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>21</td>
</tr>
<tr>
<td>2 frosted toaster pastries</td>
<td>406</td>
<td>76</td>
<td>10</td>
<td>4</td>
<td>1</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>521</td>
<td>102</td>
<td>10</td>
<td>5</td>
<td>1</td>
<td>71</td>
</tr>
<tr>
<td>MGL</td>
<td>Medium bagel</td>
<td>280</td>
<td>56</td>
<td>2</td>
<td>11</td>
<td>2</td>
<td>33</td>
</tr>
<tr>
<td>Medium banana</td>
<td>105</td>
<td>27</td>
<td>0</td>
<td>1</td>
<td>3</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>2 tbsp peanut butter</td>
<td>180</td>
<td>6</td>
<td>16</td>
<td>8</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>565</td>
<td>89</td>
<td>18</td>
<td>20</td>
<td>7</td>
<td>44</td>
</tr>
<tr>
<td>LGL</td>
<td>16 slices deli ham (200 g)</td>
<td>240</td>
<td>8</td>
<td>8</td>
<td>36</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>4 single servings (1 oz) of string cheese</td>
<td>280</td>
<td>4</td>
<td>20</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>520</td>
<td>12</td>
<td>28</td>
<td>72</td>
<td>0</td>
<td>2</td>
</tr>
</tbody>
</table>

Values are from Ref. 13. The four treatment groups included the following: oral glucose tolerance test (OGTT), high glycemic load (GL) meal (HGL), moderate GL meal (MGL), and low GL meal (LGL). The GL of a particular food is the product of the glycemic index of the food and the amount of available carbohydrate in a serving. The total GL of the meal is the sum of GLs of each food item in the meal.
Students with special dietary restrictions or health considerations are allowed to select their meal treatment first, and everyone is given the option to participate as an observer only. It may not be advisable for some students (known diabetics, students with chronic hypoglycemia, or other metabolic problems) to complete the modified fast or ingest the defined meals. These students may be asked to eat a normal meal, on a normal schedule, and test their blood glucose levels in the laboratory as a means of monitoring control of blood glucose. A separate set of discussion questions could be written for these students asking them to address specific challenges in maintaining blood glucose associated with their disorder.

Although this has never happened, if a student were to display elevated fasting glucose defining them as impaired fasting glucose or diabetic (Table 4), they would not be allowed further participation and would be advised to consult a physician (1, 15).

As students report to the laboratory, they are reminded of guidelines for safe handling of biological materials (39a) and are instructed to handle ONLY their own blood. Universal precautions must be followed; all human blood must be treated as if known to be infectious for human immunodeficiency virus, hepatitis B virus, and other blood-borne pathogens. Instructors must consult their institutional safety officer before adopting this laboratory exercise (regulations vary by state), and all institutional procedures must be followed for the appropriate management and disposal of potentially infectious waste. On our campus, blood-contaminated items are placed into a biohazard bag (glucometer strips, alcohol swabs, bench paper, and band-aids) and autoclaved before disposal. Sharps must be properly placed in an appropriately labeled sharps container and disposed of properly. Sample management and disposal plans are available online (32). Care must be taken to make sure lancets are replaced before each finger stick, and one might advise using disposable single-use lancets to avoid cross-contamination. Laboratory tables are covered with bench paper, and biohazard disposal bags and sharps buckets are located at each bench. Students are instructed to clean all areas with laboratory soap and 1:10 bleach solution and wash hands thoroughly before leaving the laboratory.

Occasionally, a student experiences an elevation in postprandial glucose above acceptable levels. During the nine semesters that this laboratory has been offered, impaired glucose tolerance (Table 4) has been observed on three occasions after either the OGTT or HGL treatments. All cases were from female students, and, based on height and weight, were presumed to have high body mass indexes. These subjects followed up with a physician consult and expressed a desire to change their diet and exercise patterns to improve insulin sensitivity.

Safety Considerations


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Sourcebook of Laboratory Activities in Physiology

LABORATORY EXERCISE: FASTED AND POSTPRANDIAL BLOOD GLUCOSE

Table 2. Summary of data by treatment for all participants

<table>
<thead>
<tr>
<th>Sex</th>
<th>Fasted</th>
<th>30 min</th>
<th>60 min</th>
<th>120 min</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>OGTT</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>70</td>
<td>134</td>
<td>143</td>
<td>139</td>
</tr>
<tr>
<td>Male</td>
<td>96</td>
<td>148</td>
<td>132</td>
<td>102</td>
</tr>
<tr>
<td>Male</td>
<td>82</td>
<td>123</td>
<td>159</td>
<td>93</td>
</tr>
<tr>
<td>Female</td>
<td>74</td>
<td>106</td>
<td>104</td>
<td>100</td>
</tr>
<tr>
<td>Female</td>
<td>77</td>
<td>149</td>
<td>170</td>
<td>80</td>
</tr>
<tr>
<td>Female</td>
<td>83</td>
<td>172</td>
<td>185</td>
<td>132</td>
</tr>
<tr>
<td>Female</td>
<td>80</td>
<td>117</td>
<td>146</td>
<td>104</td>
</tr>
<tr>
<td>Female</td>
<td>82</td>
<td>149</td>
<td>169</td>
<td>139</td>
</tr>
<tr>
<td><strong>HGL</strong></td>
<td>80 ± 2.5</td>
<td>137 ± 7.1</td>
<td>151 ± 8.4</td>
<td>111 ± 7.4</td>
</tr>
<tr>
<td>Male</td>
<td>79</td>
<td>129</td>
<td>108</td>
<td>105</td>
</tr>
<tr>
<td>Male</td>
<td>93</td>
<td>158</td>
<td>143</td>
<td>103</td>
</tr>
<tr>
<td>Male</td>
<td>92</td>
<td>141</td>
<td>116</td>
<td>107</td>
</tr>
<tr>
<td>Male</td>
<td>87</td>
<td>128</td>
<td>105</td>
<td>101</td>
</tr>
<tr>
<td>Male</td>
<td>77</td>
<td>154</td>
<td>146</td>
<td>109</td>
</tr>
<tr>
<td>Female</td>
<td>72</td>
<td>152</td>
<td>175</td>
<td>145</td>
</tr>
<tr>
<td>Female</td>
<td>95</td>
<td>166</td>
<td>135</td>
<td>109</td>
</tr>
<tr>
<td>Female</td>
<td>77</td>
<td>135</td>
<td>118</td>
<td>114</td>
</tr>
<tr>
<td><strong>MGL</strong></td>
<td>84 ± 2.9</td>
<td>145 ± 4.7</td>
<td>131 ± 7.8</td>
<td>112 ± 4.7</td>
</tr>
<tr>
<td>Male</td>
<td>86</td>
<td>123</td>
<td>112</td>
<td>86</td>
</tr>
<tr>
<td>Female</td>
<td>79</td>
<td>159</td>
<td>147</td>
<td>91</td>
</tr>
<tr>
<td>Female</td>
<td>77</td>
<td>100</td>
<td>98</td>
<td>89</td>
</tr>
<tr>
<td>Female</td>
<td>76</td>
<td>88</td>
<td>92</td>
<td>101</td>
</tr>
<tr>
<td>Female</td>
<td>77</td>
<td>124</td>
<td>103</td>
<td>86</td>
</tr>
<tr>
<td>Female</td>
<td>83</td>
<td>115</td>
<td>121</td>
<td>115</td>
</tr>
<tr>
<td>Female</td>
<td>89</td>
<td>105</td>
<td>122</td>
<td>120</td>
</tr>
<tr>
<td>Female</td>
<td>71</td>
<td>87</td>
<td>100</td>
<td>114</td>
</tr>
<tr>
<td><strong>LGL</strong></td>
<td>80 ± 1.9</td>
<td>113 ± 7.8</td>
<td>112 ± 5.9</td>
<td>100 ± 4.7</td>
</tr>
<tr>
<td>Male</td>
<td>80</td>
<td>89</td>
<td>77</td>
<td>80</td>
</tr>
<tr>
<td>Male</td>
<td>84</td>
<td>89</td>
<td>91</td>
<td>79</td>
</tr>
<tr>
<td>Male</td>
<td>80</td>
<td>81</td>
<td>82</td>
<td>84</td>
</tr>
<tr>
<td>Male</td>
<td>83</td>
<td>94</td>
<td>94</td>
<td>85</td>
</tr>
<tr>
<td>Male</td>
<td>92</td>
<td>102</td>
<td>95</td>
<td>79</td>
</tr>
<tr>
<td>Male</td>
<td>76</td>
<td>91</td>
<td>86</td>
<td>99</td>
</tr>
<tr>
<td>Male</td>
<td>88</td>
<td>94</td>
<td>85</td>
<td>79</td>
</tr>
<tr>
<td>Female</td>
<td>78</td>
<td>87</td>
<td>83</td>
<td>78</td>
</tr>
<tr>
<td><strong>Means ± SE</strong></td>
<td>83 ± 1.8</td>
<td>92 ± 2.2</td>
<td>87 ± 2.1</td>
<td>83 ± 2.3</td>
</tr>
</tbody>
</table>

Treatment groups included the following: OGTT, HGL, MGL, and LGL. *Significantly different from the OGTT group at the same time period (P < 0.05). Differences in meal treatments are shown in Table 5.

the release of insulin from the pancreas (Fig. 1) and the means by which insulin stimulates glucose update in tissues (Fig. 2). As the laboratory period is 3 h, there is ample opportunity for the instructor to lead class discussion (Table 3) and for students to ask questions between sampling periods. Discussion is always vibrant, and students are encouraged to use both laboratory computers and personal devices to gather information pertinent to the conversation.

Troubleshooting

Proper working order of the glucometers is checked each semester. Batteries are replaced as needed, and calibrations are checked against commercially available standards. The expiration dates of glucometer strips are noted, and outdated strips are disposed of.

Although the evaluation of A1C is a better indicator of glycemic control (and currently the recommended instrument for the diagnosis of diabetes), simple evaluation of fasting and 2-h plasma glucose remain valid as well (Table 4) (1).
Table 3. Topics for oral and written discussion

Possible Discussion Questions for Students

- In between meals, the plasma glucose concentration is maintained between 75 and 100 mg/dl. Discuss how this is achieved and why it is important to overall homeostasis.
- How are carbohydrates digested and absorbed? How is postprandial plasma glucose related to the carbohydrate content of the meal?
- What effect does the inclusion of fat and/or protein in a meal have on digestive processing and absorption? On postprandial glucose and insulin levels?
- What are the current recommendations for percentages of carbohydrate, protein, and lipid in the diet? How do you think these recommendations were established? Are these recommendations advised for everyone?
- What is insulin and when is it secreted? Once released from the pancreas, what is the role of insulin?
- What effect does insulin have in skeletal, adipose and liver tissue? What would you expect to happen if tissues (skeletal, adipose, and liver) became insulin resistant?
- What is metabolic syndrome and how does it relate to insulin resistance?
- What is an OGTT? What is the purpose of this test?
- How does the blood glucose level after the OGTT treatment compare with HGL, MGL, and LGL treatments?
- Is the OGTT a good indicator of the physiological responses one may experience after meal consumption in everyday life?
- How does the physical state of the meal (solid vs. liquid, cooked vs. uncooked, high fiber vs. low fiber) affect digestion and absorption rates?
- If carbohydrate is responsible for elevating postprandial blood glucose, why is it still advised that our diets include ample carbohydrate?
- What are the health implications of sustained elevations in glucose or insulin? Is there a link between glycemia, insulinaemia, and the development of disease(s)?

Students may describe feelings of hunger, fatigue, and “nervousness.” Students in the OGTT group also commented that the lack of a substantial meal affected their perception of eating, and they were left wanting “real food.” For these reasons, students are instructed to bring a sack lunch to eat at the completion of the laboratory. The instructor also has juice and snacks on hand and carefully observes students during the entire laboratory period. Students are also instructed to monitor others in their laboratory group. Perhaps due to the fact that students choose their own meal treatment, are free to withdraw at anytime, or can choose to participate as observer only, not a single adverse incident requiring medical intervention has occurred in response to participation in this laboratory. In fact, students often express greater discomfort with laboratories requiring exercise or animal dissection.

Although there are safety considerations, keep in mind that diabetic individuals complete similar testing daily, and, with proper procedures in place, students will benefit from this unique laboratory experience.

Statistical Analysis

For these sample data set, all values are reported as mean ± SE. Although students are not required to determine statistically significant differences between meals, they do use paired t-tests to determine differences between time periods within treatment. For the purpose of this publication, the Holm-Sidak procedure was used following one-way repeated-measures ANOVA to determine significant differences between treatments (P < 0.05; Table 5) (38). Each meal was independently compared with the OGTT group, and significant differences between the meal and OGTT groups at each time point is shown in Fig. 3.

Table 4. World Health Organization-defined glucose levels

<table>
<thead>
<tr>
<th>Glucose Levels in Venous Plasma</th>
<th>Fasting</th>
<th>OGTT (2 h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Impaired fasting glycaemia</td>
</tr>
<tr>
<td>mmol/l</td>
<td>&lt;6.1</td>
<td>≥6.1 and &lt;7.0</td>
</tr>
<tr>
<td>mg/dl</td>
<td>&lt;110</td>
<td>≥110 and &lt;126</td>
</tr>
</tbody>
</table>

Values are from Ref. 14.
Table 5. Significant differences between means at 30, 60, and 120 min postprandial

<table>
<thead>
<tr>
<th>Comparison</th>
<th>30 min Difference of means</th>
<th>P value</th>
<th>60 min Difference of means</th>
<th>P value</th>
<th>120 min Difference of means</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>HGL vs. LGL</td>
<td>53.9</td>
<td>&lt;0.00*</td>
<td>OGTT vs LGL</td>
<td>64.4</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>OGTT vs. LGL</td>
<td>45.87</td>
<td>&lt;0.00*</td>
<td>HGL vs. LGL</td>
<td>44.1</td>
<td>&lt;0.001*</td>
<td></td>
</tr>
<tr>
<td>HGL vs. MGL</td>
<td>32.8</td>
<td>0.016*</td>
<td>OGTT vs. MGL</td>
<td>39.1</td>
<td>0.003*</td>
<td></td>
</tr>
<tr>
<td>OGTT vs MGL</td>
<td>24.6</td>
<td>0.071</td>
<td>MGL vs. LGL</td>
<td>25.2</td>
<td>0.054</td>
<td></td>
</tr>
<tr>
<td>MGL vs. LGL</td>
<td>21.1</td>
<td>0.097</td>
<td>OGTT vs. HGL</td>
<td>20.2</td>
<td>0.103</td>
<td></td>
</tr>
<tr>
<td>HGL vs. OGTT</td>
<td>8.1</td>
<td>0.432</td>
<td>HGL vs. MGL</td>
<td>18.9</td>
<td>0.070</td>
<td></td>
</tr>
</tbody>
</table>

No differences existed between fasting levels, and therefore fasting levels are not reported. *Overall significance level = 0.05 (by the Holm-Sidak method).

from 30-min levels. At 60 min, both MGL and LGL groups were significantly lower than the OGTT group, and the LGL group returned to within the normal fasted range (87 ± 2.1). Between 60 and 120 min, blood glucose levels declined for all groups, with the HGL and OGTT groups demonstrating nearly identical levels at 120 min (111 ± 4.7 in the HGL group vs. 111 ± 7.4 in the OGTT group). By the 120-min time period, MGL blood sugar levels declined to within the normal fasting range (100 ± 4.7). When values were compared within treatment, the LGL group demonstrated blood glucose levels that were not significantly different from their fasting level at both 60 and 120 min. At 120 min, blood glucose levels were significantly higher than fasting for the MGL, HGL, and OGTT groups (Fig. 3). Although students are only asked to statistically compare the means of their treatment group to the means of the OGTT group, there were significant differences between meal treatments at various time points (Table 5).

3. Postprandial blood glucose levels after the ingestion of the HGL meal will not differ significantly from the OGTT group. Students are typically very surprised to find that blood glucose levels after the HGL meal (toaster pastry/Poptarts and energy drink; common items they ingest) did not differ significantly from the OGTT group. In this sample data set, HGL values were not significantly different from OGTT values at any time period. Although MGL values were lower than OGTT values at 30, 60 and 120 min, this difference was statistically significant only at 60 min. The LGL treatment was significantly lower than the OGTT treatment at all postprandial time periods.

4. Occasionally, students may experience values that are not considered normal. The individual response to meal treatment depends not only on the composition of the meal but also on personal characteristics that influence insulin sensitivity. Of the 32 participants in this sample data set, 1 female student participating in the HGL treatment experienced a 120-min blood value that exceeded the 140 mg/dl cutoff described as impaired glucose tolerance (Table 4). Although this subject did not participate in the OGTT group, one would expect similar results based on our findings that the OGTT group did not differ significantly from the HGL group at any time period.

Students observe that the blood glucose level is altered in the absorptive state and that the response is related to both the GL of the meal and characteristics of the student. Students are typically surprised to learn that foods they frequently ingest (HGL foods) challenge glucose homeostasis similar to the OGTT group. Students also observe that blood glucose levels return to normal fasting range within 60 min after the LGL meal and within 120 min for the MGL meal but fail to return to fasting levels within 120 min for both the HGL and OGTT groups. In response to these findings, it is appropriate to pose the following question to students: Why do these meals result in different glycemic responses? Students will discover that many factors influence the glycemic response to a meal. Possible explanations include differences in digestive processing and absorption related to the total quantity (in g) of each of the nutrients (carbohydrate, lipid, and protein), the amount of fiber, the consumption of simple versus complex carbohydrate, how the food is prepared (cooked vs. uncooked), and the physical nature of the food (solid vs. liquid). Upon evaluation of the nutrient composition of the meals (Table 1), students realize that the meals ingested vary greatly in total carbohydrate, fat, protein, and fiber content. Students have previously learned that carbohydrate is digested and absorbed primarily as glucose (or converted to glucose by the liver). Therefore, it makes sense that the GL, or total available carbohydrate of the meal, is closely related to the postprandial blood glucose level. Students also discover that the presence of fat and protein in the meal likely serves to moderate the postprandial response as fat delays gastric emptying and tends to flatten the postprandial plasma glucose response (10). Depending on the type of protein ingested, β-cell secretion may increase (37), whereas the glucose response is decreased (17, 26). Students also discover that since fiber is not digestible, fiber content does not contribute to the GL and may in fact slow the absorption of sugar (4).
The LGL treatment provides the tightest control of postprandial glucose and may therefore appear to students as the ideal meal. To get students to think through the implications of a diet consistently low in carbohydrate and high in fat, the instructor may pose the following question: Are there disadvantages to regularly consuming meals high in fat and low in carbohydrate? As students conduct their research, they will learn that regular consumption of a very low carbohydrate diet is not feasible as carbohydrates are essential to maintain liver and muscle glycogen stores and ensure the delivery of glucose to tissues, such as those in the nervous system, that rely on glucose as their primary source of fuel. Students will also discover that such a diet lacks the essential vitamins, minerals, and fiber necessary to maintain health over the long term (8, 41). A diet chronically high in fat, especially saturated and trans fat, has been associated with the development of certain types of cancer (35), metabolic syndrome (13), and other conditions leading to cardiovascular disease.

Although students within each treatment group consume the same meal, they display variability in postprandial blood glucose levels. Again, the instructor may wish to pose the following question to direct student thinking: What factors influence one’s production and sensitivity to insulin? Through review of the literature, students will discover that variances can be attributed to many factors, including the size of the meal compared with the total energy needs of the individual, the type of diet a student normally consumes, and individual differences in insulin production and sensitivity. Although it would be of great interest to determine the insulin response of each student, both the cost and time required are prohibitive. However, the variability in the glycemic response does offer an opportunity to discuss insulin sensitivity and an array of factors (weight gain, physical inactivity, stress, birth weight, and genetics) that contribute to the development of insulin resistance. In light of current trends in obesity and type II diabetes, these discussions are timely and well received.

Evaluation of Student Work

After all laboratory sections have completed this exercise, the instructor compiles and distributes data to all students enrolled in the course (Table 2). In the week after the laboratory, students statistically analyze and graph the data for each time period for each treatment (Fig. 3). Students also complete a postlaboratory writeup that includes written hypotheses, data analysis, results (including graphs plotting SE bars), and discussion questions (Table 3). Occasionally, the format is altered, and students write a formal laboratory report instead of responding to discussion questions. Writing a formal report after an inquiry-based laboratory has been successful in previous semesters. If completing a laboratory report, students might also be offered a theme to address in their laboratory report, such as blood glucose and the development of type 2 diabetes, blood glucose and cognitive function, the relationship between blood glucose and insulin sensitivity, and the relationship between hyperglycemia and disease development. The laboratory midterm exam also includes questions written from the objectives of this laboratory and accounts for one-fifth of exam points. An assessment revealed that students do retain the concepts presented in this laboratory, with >80% demonstrating mastery of the objectives relating to this laboratory. When presented graphs tracking postprandial blood glucose levels, students successfully interpreted the graphs and identified “abnormal” values as well as predicted the meals most likely to be associated with the resulting blood glucose levels.

Inquiry Applications

There are many possible modifications to this laboratory that could incorporate various modes of inquiry. Total calories could easily be increased, decreased, or adjusted to represent the same relative percentage of each individual’s daily energy requirements. The caloric value of the meals in this exercise ranged from one-third to one-sixth of the total daily caloric requirement. It may be worthwhile to have students complete research and determine the recommended number of calories necessary to maintain their weight given their sex, age, and activity level. Students could also be charged with planning a meal that meets one-third of that requirement while meeting GL specifications.

Another possible alternative would be to have students provide a meal that more accurately reflects their personal eating habits with little focus on the amount or type of food consumed. Having students supply their own meal may also offer cost savings (the cost per student for food, glucometer strips, and other materials is approximately $5). After the laboratory, students could be asked to conduct research and describe the characteristics of the meal as well as personal characteristics that influence their results.

Students could also be required to work in groups to develop original, testable hypotheses and design an appropriate experiment to conduct. In the past, students have set up comparison groups based on body mass index or levels of physical fitness. Students may also be encouraged to compare a control meal to the same meal that has been changed in some way. Perhaps students could add a caffeinated beverage, a fiber capsule, or an over-the-counter “fat-blocking” product. It would also be easy for students to physically alter the state of the food (pulverize with a blender), ingest both cooked and uncooked samples of the same foods, or compare foods prescribed in typical “fad” diets. If inquiry based, it may be advisable for the instructor to provide access to published articles or other resources for students to determine the GI of individual foods (14) or the GL of the meal (6) and even consider the insulin response to the meal (5).

Additional Information

Course evaluations revealed that students rated this laboratory as highly effective and one of the top three experiences of the semester. Evaluation comments included the following:

“The best part of this lab is its applicability to daily life. This lab gave me the physiological reasoning behind food choices.”

“I have a family history of diabetes, and it was very enlightening to actually see how foods impact blood sugar.”

“This hands-on lab made the material more real—I loved it!”

“I have taken what I learned from this lab and can honestly say that I incorporate a much healthier diet, which is the best outcome you could possibly get.”
Summary

This laboratory provides an impactful and personal student experience highlighting key physiological concepts related to digestive and regulatory function. Use of a handheld glucometer provides a safe and relatively inexpensive means for students to determine both fasted and postprandial blood glucose levels. Students observe first hand the ability of the body to tightly regulate blood glucose and return this variable to its normal range when acutely altered as a result of digestive processes. This highly modifiable laboratory exercise provides the opportunity for undergraduate students to write hypotheses, collect and analyze data, and draw conclusions from a data set that has visual clarity when graphed. Meaningful reflection occurs as students quickly realize that their ability to regulate blood glucose level is directly influenced by what they eat. Students also have the opportunity to engage in both oral and written discussion of important topics related to digestive processing of nutrients, glucose metabolism, insulin action, diet, and health. Although not a stated objective, this laboratory exercise also contributes to the University of Southern Indiana’s mission to prepare students to live wisely as students learn how personal dietary choices may influence their health.

Additional Resources

The following are articles from Advances in Physiology Education:


The following articles are available through the American Physiological Society Archive of Teaching Resources:


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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: M.K.H. conception and design of research; M.K.H. performed experiments; M.K.H. analyzed data; M.K.H. interpreted results of experiments; M.K.H. and L.W.M. prepared figures; M.K.H. drafted manuscript; M.K.H. edited and revised manuscript; M.K.H. approved final version of manuscript.

REFERENCES