Assembling and computerizing an indirect calorimeter

March 15, 2007

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Abstract

Metabolic disorders affect millions around the world. The disorders are a result of aberrant activities in the metabolic pathways, specifically the aberrant activities of the genes present in the pathways. Indirect Calorimeter is a valuable tool to monitor phenotypic changes in metabolism between normal vs. experimental test subjects. The goal of this design project is to assemble and computerize an indirect calorimeter for mice. Two identical systems with five cages per system need to be setup. The data will be recorded into a computer via a data acquisition device. Extensive testing will be conducted to ensure proper functioning of the calorimeter.

Problem Statement

In order to obtain the physiologic data required to further the understanding of obesity and its consequences, an indirect calorimeter must be designed and assembled. The specific design constraints have been developed through discussions with Dr. Cai and Dr. Keesey, as well as though research of the existing literature relating to indirect calorimetry. These constraints have been organized into two categories, measurements and construction, but it should be noted that each category is influenced heavily by the other. As the project continues to develop, existing constraints will need to be modified and new constraints will be created. A complete list of the current constraints can be found in appendix A.

Background

Metabolism

Metabolism is the sum of all chemical reactions that occurs in an organism. However, metabolism mostly deals with nutrition and breakdown, storage, and mobilization of food sources. Metabolism, like many other major functions of the body, is controlled by intricate biological pathways. Figure 1 shows some of the metabolic pathways; it includes some of the fuel intermediates as well as signaling molecules involved in metabolism.

Proper metabolism relies on efficient interplay between these pathways. Aberrations in

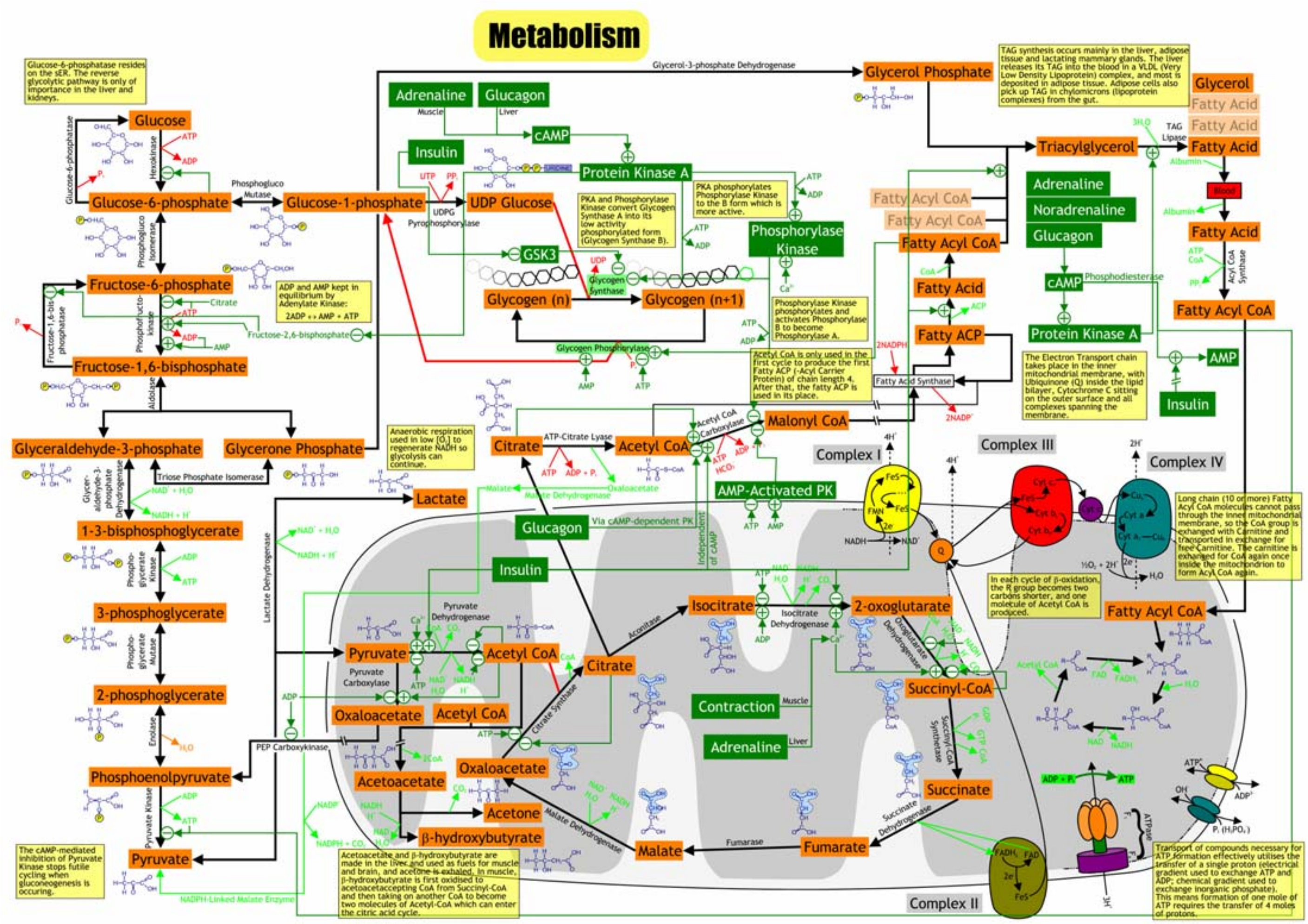

Figure 1. Some of the metabolic pathways. The pathways consist of several different signaling molecules and fuel intermediates.

Source: http://www.srcf.ucam.org/~ajm226/mp/metabolism.jpg these pathways result in several disorders known today. Obesity, which affects 60 million Americans, results from abnormal metabolism. Diabetes, a disorder characterized by the inability to produce insulin, is yet another result of abnormal metabolism. Researchers are focusing their attention to these biological pathways to decode the causes of these metabolic disorders.

Dr. Dongsheng Cai, an assistant professor in the Physiology Department at University of Wisconsin – Madison, is interesting in dissecting how metabolism is regulated by the stress and inflammatory pathways in the Central Nervous System (CNS). Dr. Cai's hypothesis is that the absence or lowered activity of some of the genes in these pathways impact metabolism. By using molecular biology techniques of gene knockouts and mutations in mice, Dr. Cai aims to find the significance of these genes and how lowered activity or absence of these genes lower mice's basal metabolism. One of the tools Dr. Cai wishes to use is an indirect calorimeter.

Indirect Calorimeter

Indirect Calorimeter measures the rate of oxygen consumption (VO_2) and carbon dioxide (VCO_2) production to measure the Respiratory Exchange Ratio (RER)[4] (See Equation 1). The RER value has many applications. It is used to calculate the amount of heat produced by an organism using Equation 2[4]. The RER also gives insight on the types energy fuels used to

$$RER = \frac{VCO_2}{VO_2}$$

Equation 1

$$\text{Heat (kcal/hr)} = [3.815 + (1.233 * RER)] * VO_2$$

Equation 2

generate the heat. As seen in Figure 2, an RER value of about 0.707 indicates that most of the heat produced by the organism is a result of carbohydrate metabolism. On the other hand, if the RER value is around 1.00, most of the heat produced by the organism is a result of fat metabolism. In addition to this, an indirect calorimeter also allows for monitoring for the RER value over time. This allows for observing any fluctuations in metabolism at different times of the day. The goal of our project is to assemble and computerize one such indirect calorimeter for Dr. Cai.

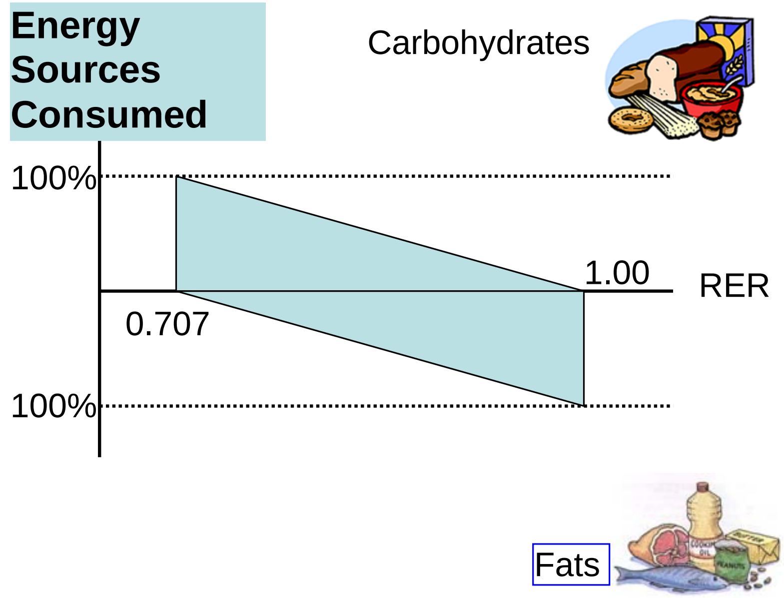

Figure 2. RER and energy fuel used to produce heat. A low RER value (~0.707) indicates that the majority of heat is produced by carbohydrate metabolism. A high RER value (~1.00) indicates that the majority of heat is produced by fat metabolism[4].

Design Constraints

Measurements

The principal measurements which need to be made are the volume of oxygen consumed (VO2) and the volume of carbon dioxide produced (VCO2). In order get the most relevant data from the experiments, two conditions must be met. These conditions are that the levels of gases should be measured about every five minutes, and be recorded with units of milliliters per hour (mL/hr). Based on these two criteria, flow rates through the calorimeter must be determined to produce measurements which adhere to the criteria. In addition, each of these measurements must have no more than 5 % error for each measurement. Besides dealing with gaseous measurements, food consumption, water consumption, waste excretion, and movement should be measured because they are physiologically relevant to Dr. Cai's experiments.

Construction

Two identical indirect calorimetry systems need to be constructed. One system will house the four 'knockout' mice while the second system will house the control mice. Each system will also need a cage without a mouse to act as the reference cage for the gas measurements which will be made. Dr. Cai has provided our design team with all the necessary parts to an indirect calorimetry system including: oxygen sensors, oxygen analyzers, carbon dioxide sensors, carbon dioxide analyzers, flow meters, pumps, tubing, cages, expansion chambers, and a variety of valves. Each of these parts was part of a working calorimetry system 20 years ago, but have not been used together since.

The animal testing must be done in the animal testing room which is located two floors above Dr. Cai's lab. Unfortunately, the animal testing room can only be reserved for short periods of time, meaning that the calorimetry system cannot be kept indefinitely in the room and must be transported between Dr. Cai's lab and the testing site. In order to accomplish this, Dr. Cai would like the calorimetry system to fit on a rolling cart to make transportation easy. Because the system must fit on a cart, both size (surface area) and weight of the calorimeter must be kept to a minimum to ease in the transportation.

Dr. Cai has indicated that he would like to run tests using the indirect calorimeter for at least 2 years and for as many as 5 year, so the longevity of the parts, or the ease of the replacement of parts is critical. Because Dr. Cai has all the parts of the calorimetry system he would like to limit spending on this project to only $2000. Dr. Cai realizes that if some of the expensive parts need to be replaces, for example the oxygen or carbon dioxide analyzers or sensors, the cost will be significantly higher.

Competition

Dr. Cai has expressed his desire have a calorimeter system much like a system produced by Columbus Instruments called the CLAMS system which stands for Comprehensive Lab Animal Monitoring System. The CLAMS system (See Figure 3) allows for 24-hour, automated, non-invasive collection of several physiological and behavioral parameters simultaneously. These include the ability to monitor animal activity, food and water consumption, urine and waste production, oxygen consumption and carbon dioxide production, metabolic performance and temperature. This system also has the ability to automatically calculate the Respiratory Exchange Ratio (RER) from the CO2 consumption and O2 production. All the data is automatically sent to a data acquisition system with statistical spreadsheet capabilities for analysis. This system unfortunately costs about $150,000 which is significantly more than Dr. Cai wants to spend.

Figure 3: The CLAM System

Design Alternatives

Push Pull Design

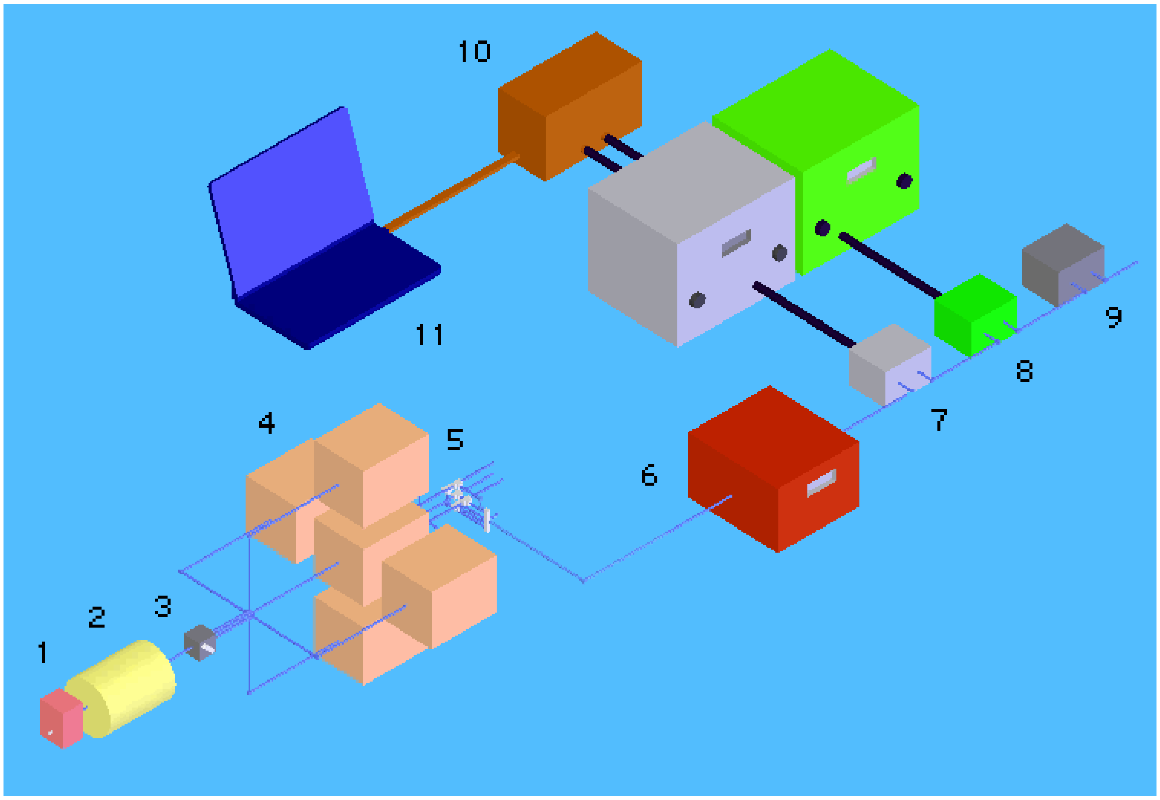

Figure 4: *Setup of the entire push-pull system*

The final design which will be pursued will be a combination push-pull system. The entire system can be seen in Figure 4**.** The system is named the push-pull system because air is first pushed through the system to obtain flow into the five cages, then air is pulled off of the main line to run the air through the analyzing sequence.

Pre-Cage

The system will be discussed in the order in which the air will flow. First air from the atmosphere enters the system by the means of a diaphragm pump (Figure 4.1). This pump will force air through the system. However, due to the oscillations of air which are caused by the diaphragm pump, an air collection chamber is needed (Figure 4.2). This collection chamber reduces the oscillations and also partially dehumidifies the air, due to the pressurization in the chamber. The collection of air, allows for the specific flow rates to be achieved. This is done through the use of a manual value (Figure 4.3), which controls the flow out of the chamber and into the five cages.

Post-Cage

Once the air exits the manual valve, the tubing splits and equal amounts of air are sent through the five cages (Figure 4.4). Air is continuously passed through the five cages, this is done to ensure that all the cages are constantly being flushed of air, which allows for healthy concentrations of oxygen and carbon dioxide in the air the rats will be breathing.

Even though air cages have air flowing through them, only one cage can be measured at a time. This is due to monetary and space considerations, which do not allow for the purchasing of five sets of analyzers/sensors. Each of the cages will be measured approximately every thirty seconds. This will be accomplished with the use of solenoid valve/relay system (Figure 4.5). The relay will periodically change the solenoid valves. The solenoid valves either act by sending air through the analyzing sequence or into the atmosphere. So for instance, the air from one cage will be sent to the atmosphere for 2 minutes and be sent to the analyzing sequence for 30 seconds. Also back pressure valves will be used so the pressure in the cages is not increased when the solenoid valve changes.

Analyzing Sequence

The first step for the air, which will be analyzed, is dehumidification (Figure 4.6). Dehumidification is needed because the analyzers/sensors need dry air in order to achieve accurate measurements. Once the air is dehumidified, the air is sent through the carbon dioxide sensor/analyzer combination (Figure 4.7). Next the air is sent through the oxygen sensor/analyzer combination (Figure 4.8). The oxygen sensor/analyzer needs to be placed after the carbon dioxide sensor/analyzer because the oxygen sensor heats up the air to considerable temperatures.

In the analyzing sequence, the amount of air which is flowing through the sensor/analyzer combination needs to be controlled. This is done through the use of a pump (Figure 4.9), which pulls a certain amount of air through the sequence. The pump is setup in parallel with the air sent through the solenoid valve, which allows a precise amount of air to be passed through.

Data Acquisition

Once the information is collected from the analyzer/sensor combinations, through the use of an analog to digital converter signal is sent to a data acquisition device (DAQ) (Figure 4.10). Once the information is sent through the DAQ, information will be uploaded to a computer (Figure 4.11) where further data analysis will be achieved.

Pull System

The only inherent difference between many of the different indirect calorimeters is the method by which they move air though the system. The pull system derives its name from the fact that air is drawn through the system from pumps which are located after the cages and oxygen and carbon dioxide sensors. A five liter pump draws a constant one liter of air through each cage (See Figure 5). Depending on the configuration of a solenoid value (depicted as the yellow square), air is either pumped through the combination of O2 and CO2 sensors and analyzers, or out into the atmosphere. One of the cages does not house an animal and is used as a reference cage for the data analysis.

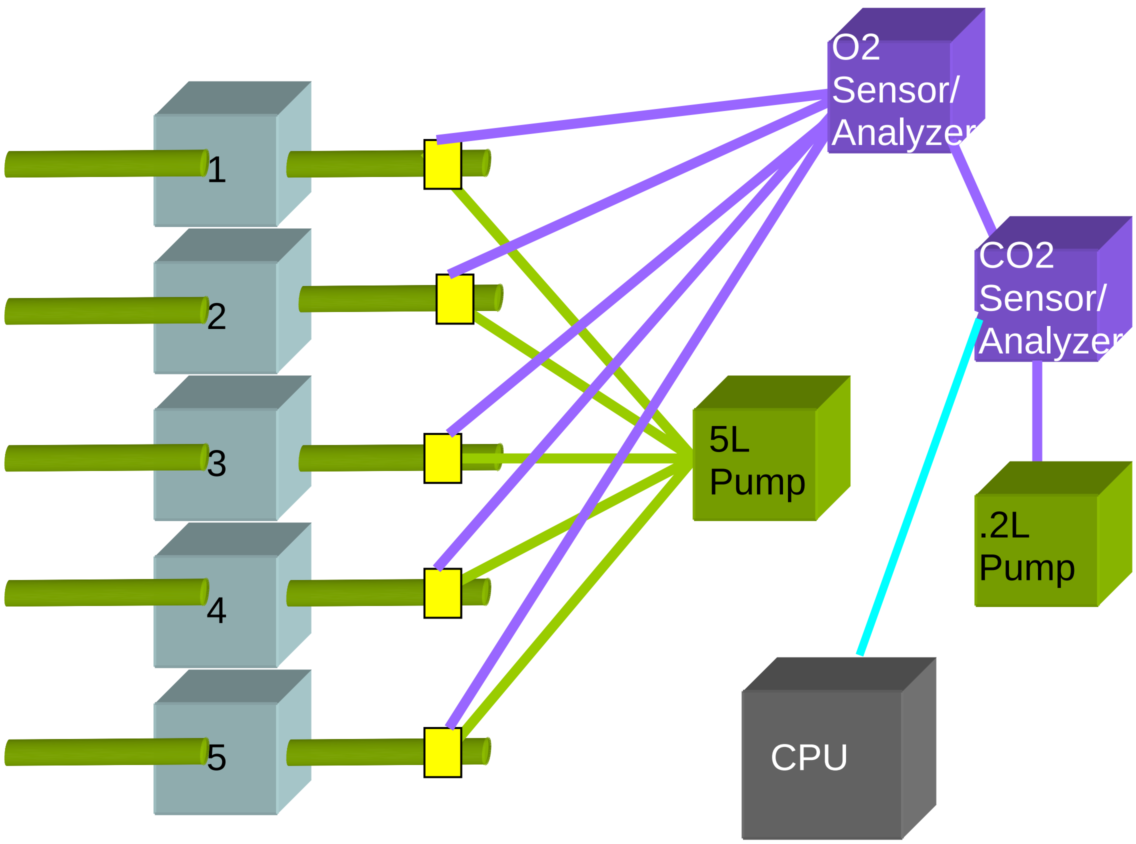

Figure 5: Pull System

One to One System

The One to One system has five identical combinations of equipment which moves air first through the cages and then into the oxygen and carbon dioxide sensors and analyzers to test the air (See Figure 6). A computer is interfaced with each sensor to compile all the data. As in the last system, one of the cages is empty and is used as a reference cage for the data analysis.

This type of system is very simple to set up because of the simplicity of all the connections; it does not require any of the solenoid values and switching between pumps like the previous system. Because of the need for 5 sets of sensors, analyzers, and pumps, this system would be very expensive and cumbersome to move.

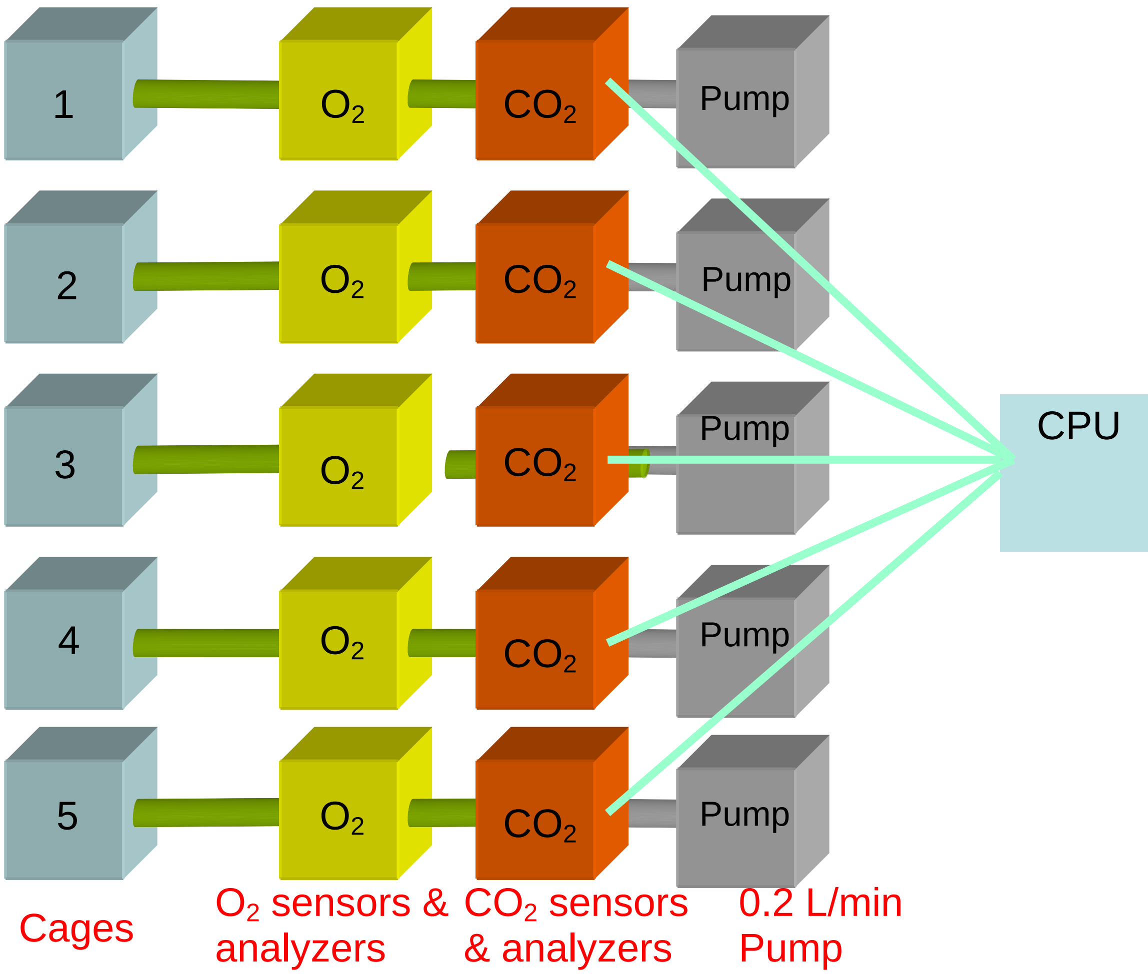

Figure 6: One to One System

Design Matrix

	Cost (1-10)	Knowledge Base (1-10)	Size (1-5)	Simplicity (1-5)	Total
Push-Pull	7	10	4	4	25
Pull	7	6	4	3	20
One to One	1	4	1	5	11

Table 1: Design matrix for three designs

Push-Pull System

The push-pull system has a number of positive aspects which make the design very plausible. First of which is the system allows for accurate flow rates. This is done through the use of pumps which control the flow. Also in this setup the collection chamber allows for dehumidification air, which will be a secondary dehumidification process. Also a constant flow of air will be achieved with the use of a manual valve connected to the collection chamber. With the use of the second pull pump the additional amount of air needed to pass through sensors/analyzers is achieved. The cost, size and simplicity of the system are feasible. However the information base for the push-pull system is much greater than the additional systems. Dr. Keesey has previously created a system similar to the push-pull design and could contribute to our project when needed.

One of the problems with the setup will be the size requirements of the system. With the additional collection chamber additional space will be needed. This is a problem as space is a concern for this project. Also the system will need to be periodically checked for leaks to ensure that accurate measurements will be taken.

Pull System

The positive aspects of the pull system are very similar to the push-pull system. However our knowledge base is not as large for this system compared with the push-pull system, which is why the push-pull system is chosen over the pull system. The negative aspects of this system are very similar to the push-pull system, except less space will be used because a collection chamber will not be needed.

One to One System

With the use of the one to one system continuous monitoring would be achieved. This would allow for more accurate data to be obtained. The cost of this system would be considerably larger than our budget would allow and the system would also impose size issues which could not be accommodated.

Future Work

Our team will pursue the push-pull system for our final design. Figure 7 shows the overall flowchart of our future work. The first step is to ensure that the CO_2 and O_2 sensors and analyzers are functional. If they are functional, we will calibrate the sensors and analyzers using known mixtures of gases. This

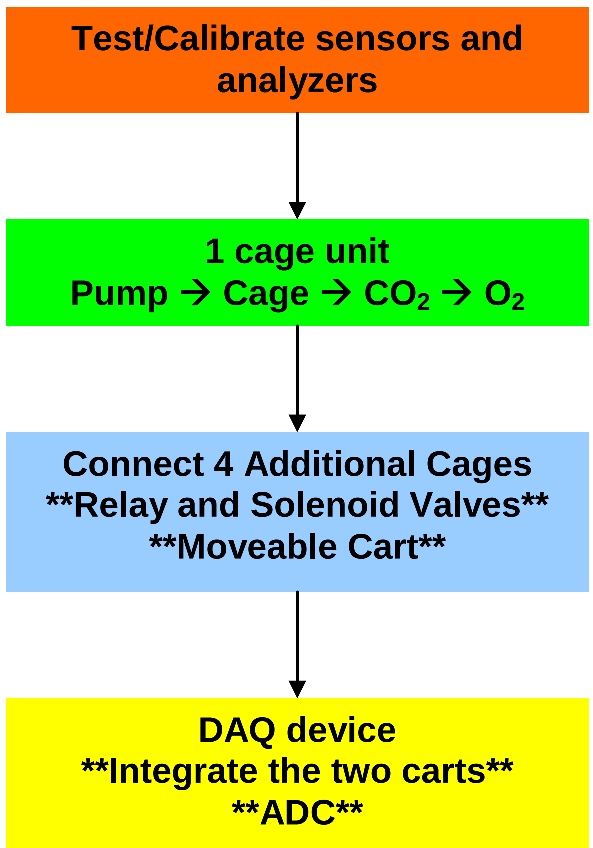

Figure 7: Future Work Flow Chart

mixture of gases will comprise of CO_2 and O_2 concentrations that are physiologically relevant; the expected VO_2 and VCO_2 from the mice cages will be within the range of these mixtures. If the sensors and analyzers are non-functional, new sensors and analyzers will be purchased. Two sets of CO_2 and O_2 sensors and analyzers will be tested and calibrated, since we have to setup two identical units.

After the sensors/analyzers are calibrated, a 1 cage unit will be set up. This setup will comprise of 1 pump pumping air into 1 cage and the cage connected to the CO_2 and O_2 sensors and analyzers. After the 1 cage unit is setup and fully functional, the other 4 cages will be incorporated. At this step, we will utilize relay and solenoid valves to sample the cages at different times. This whole setup will then be mounted on a movable cart. Since our client requires two setups – one for normal mice and the other for experimental mice, another similar cart will be assembled. The analyzers from both carts will be connected to a Data Acquisition (DAQ) device. The DAQ device will need to have two inputs for O_2 and CO_2 concentrations. Also these measurements will need to be taken at a frequency of 100 Hz. This will allow for sufficient accuracy of the system to be obtained. The output of the sensor/analyzer combination ranges from 0-7.5 volts, so the DAQ must be able to record values in this range. Additional components of the DAQ device need to be further researched. The DAQ device will also contain Analog to Digital converters (ADC) to convert the analog output of the analyzers into a digital input to the laptop or PC. The ADC must have a resolution of at least 12 bits, which will allow for (7.5 V)/(2^12)=1.83 mV accuracy.. The whole device will then be connected to the laptop or the PC. Our team will develop extensive testing protocol as well as a user manual for the indirect calorimeter.

Ethical Considerations

The main ethical consideration that our design team and client have to consider is animal testing. Detailed animal testing protocols must be created before any testing can be conducted and the protocols have to be approved by the Institutional Review Board (IRB). Once the protocol is approved, the researchers must strictly abide to the protocol; any changes or updates to the protocol have to be submitted to the IRB and re-approved before carrying out further tests. The violation of code of conduct on animals as stated in the IRB policy can result in serious consequences.

It is unlikely that intellectual property will become an ethical concern for our group, as very similar products (such as the CLAM system) are currently on the market. Despite this it is possible that we would create a unique design that requires a patent. However, before we can apply for a patent, we would have to ensure that our design is significantly different than similar designs that are currently available. To do so, we would get recommendations and suggestions from Wisconsin Alumni Research Foundation (WARF) about our prototype and also refer to them when completing application for a possible patent.

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Appendix A

Product Design Specification
Indirect Calorimeter

Updated: February 5, 2007

Team Members:

- Jon Baran: BSAC
- Dhaval Desai: Communicator
- Kyle Herzog: Team leader
- Tim Pearce: BWIG

Problem Statement:

All components of an indirect calorimetric are available in my lab. We look for student(s) who are interested in assembling the pieces into a functioning unit and computerizing the system with the existing software. This unit will be used to continuously collect the real-time (24~48 hours) data on mouse oxygen consumption, CO2 production, activity and food intake. This will be very useful instrument for us to study the genetic and pharmacological effects of our targets on the treatment and prevention of obesity-diabetes. We have all components of an indirect calorimeter, mainly including oxygen sensors, CO2 sensors, air flow controls, mouse chambers, pipes, wires, and computer, all types of switches and controls, software, manual instruction.

The commercial instrument that is similar to this unit is the CLAM system (costing $150,000), the principle and working mechanism of which can be found at the web site of Columbus Instruments.

Client Requirements:

- Use materials provided by Professor Cai to construct a calorimeter. These include oxygen sensors, CO2 sensors, air flow controls, mouse chambers, pipes, wires, a computer, all types of switches and controls, software, and instruction manual.
- Obtain measurements for water and food intake and urine produced.
- Be able to record and quantify the animals' movements.

Design Requirements:

1. Physical and Operational Characteristics

a. Performance Requirement: Must be able to record data for CO2, O2, and movement about every five minutes for a continuous period of 24-48 hours. The data should be recorded in units of mL/hr. Food, water, and waste must be measured every day.

b. Safety: Animal safety.

c. Accuracy and Reliability: CO2 and O2 measurements must be able to be measured to at least 5%.

d. Life in Service: For the duration of client's project, probably 2 to 5 years.

e. Operating Environment: The current environment is Professor's Cai's lab, but he is interested in moving to an environment that can withstand longer durations of testing. Environment is room temperature.

f. Ergonomics: Easy use by researchers that requires minimal training. A cart to push the cages and set-up around should be considered

g. Size and Shape: Small enough to fit on a rolling cart which can be moved from the animal room in the basement to the lab.

h. Weight: Not so heavy that transfer on a cart is undoable.

i. Materials: Must stand up to long testing cycles.

j. Aesthetics, Appearance, and Finish: Spatially efficient.

2. Product Characteristics:

a. Quantity: One device is required.

b. Target Product Cost: The prototype should cost less than $2000 to build.

3. Miscellaneous:

a. Standards and Specifications: The device should comply with all applicable laws, regulations, and policies governing animal testing.

b. Customer: The typical customer would be physiologists researching metabolism in small animals. We are designing it for Professor Cai's specific study, as opposed to producing it for mass production.

c. Patient-related concerns: No potential harm caused to animals subjects.

d. Competition: The CLAM system produced by Columbus Instruments.