Assembling and computerizing an indirect calorimeter

Final Design Report
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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 aberrant activities of the genes present in the pathways. Indirect Calorimetry is a valuable tool used to monitor phenotypic changes in metabolism between normal and experimental test subjects. The goal of this design project is to assemble an indirect calorimeter for mice and set up a data acquisition system to store data on a computer. Ideally, two identical systems with five cages per system would be constructed. Extensive testing would be necessary to ensure proper functioning of the calorimeter. Due to time and monetary constraints, our design focused on setting up a one cage system that serves as a proof of concept which would require extensive calibration and perhaps new, functioning parts in order to be useful in an experimental setting.

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 indirect calorimeter will be used with mice and a data acquisition system will store data on a computer. Ideally, two identical systems with five cages per system would be constructed; four cages containing knockout mice, and one cage containing an unaltered control mouse. Extensive testing and calibration would be necessary to ensure proper functioning of the calorimeter. The specific design constraints have been developed through discussions with Dr. Cai and Dr. Keesey, as well as thorough 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.

Client Biography

Assistant professor in the department of physiology Dongsheng Cai, MD, Ph.D., 2000, Shanghai Jiaotong University, is the client who has provided us with this project. His research focuses on investigating the roles of intracellular stress and inflammation pathways for physiological regulation and pathological dysfunction of metabolic homeostasis, with the long-term mission of identifying molecular mechanisms and developing therapeutic avenues for metabolic diseases particularly obesity and diabetes. He would like an indirect calorimeter to conduct experiments on knockout mice and search for specific genes which may have an effect on metabolism.

Professor Emeritus in the department of psychology Richard Keesey, Ph.D. 1960, Brown University, also helped us with the set up of the indirect calorimetry system. Most of the components of the system were originally purchased by Dr. Keesey years ago to assemble a similar system which he used for testing. His knowledge of the system served as a valuable resource over the course of the semester.

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. Proper metabolism relies on efficient interplay between these pathways. Aberrations in these pathways result in several disorders known today. Obesity, which affects 60 million Americans, results from abnormal metabolism [1]. 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 [2].

Dr. Cai is interested 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 may 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 Calorimetry measures the rate of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) to measure the Respiratory Exchange Ratio (RER) (See Equation 1). The RER is used to calculate the amount of heat produced by an organism using Equation 2. The RER also gives insight on the types energy fuels used to generate the heat. As seen in Figure 1, 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.

Figure 1: 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]

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 [4]. The goal of our project is to assemble one such indirect calorimeter for Dr. Cai.
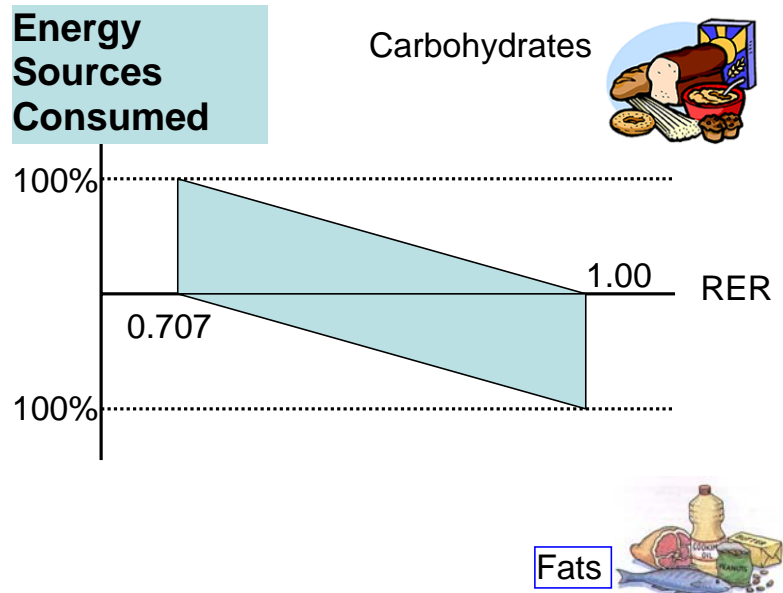
$$RER = \frac{VCO_2}{VO_2}$$ **Equation 1**

$$\text{Heat (kcal/hr)} = [3.815 + (1.233 * RER)] * VO_2$$ **Equation 2**

Design Constraints

Measurements

The principal measurements which need to be made are the volume of oxygen consumed (VO_2) and the volume of carbon dioxide produced (VCO_2). In order get the most relevant data from the experiments, two conditions must be met; (1) the levels of gases should be measured about every five minutes, and (2) 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, as they are also 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. All of these parts were 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. Since 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. An alterative solution to this problem is the construction of a self contained testing tank, or small enclosed room, with its own ventilation system and environmental control of temperature and humidity.

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 years, so the longevity of the parts, or the ease of the replacement of parts is critical. The final system should be set up in such a way that all parts are easily accessible for replacement, repair, and other modifications which may become desirable. Because Dr. Cai has all the parts of the calorimetry system he would like to limit spending on this project to only $2,000. 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.

Critical to the success of the final system is the acceptance of the methods and techniques that the indirect calorimeter employs by the scientific community. Dr. Cai has expressed a keen desire to model his indirect calorimeter after a well accepted system in order to make sure the results are unquestionable.


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 2) 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 CO_2 consumption and O_2 production. All the data is automatically sent to a data acquisition system with statistical spreadsheet capabilities for analysis. This system

Figure 2: The CLAMS system. This is a state of the art indirect calorimetry system [4].

unfortunately costs about $150,000 which is out of Dr. Chai's price range, however as will be explained further in the "Project Timeline" Dr. Chai did decide to purchase this system.

Design Alternatives
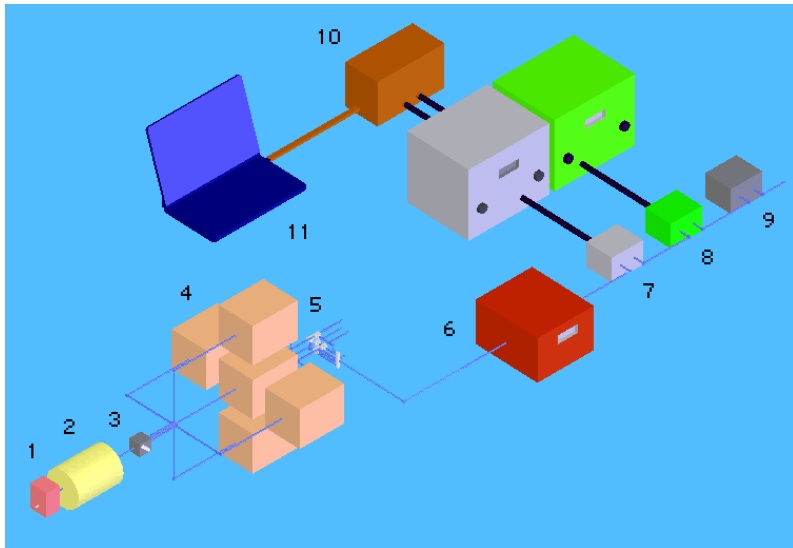
1. Diaphragm Pump
2. Air expansion chamber
3. Manual flow control
4. Animal chambers
5. Solenoid valve/relay system
6. Dehumidifier
7. CO2 sensor and analyzer
8. O2 sensor and analyzer
9. Pump
10. Data acquisition device
11. Computer for analysis

Figure 2: Setup of the entire push-pull system. The first pump pushes air through the cages. The second pump pulls air through the sensors for analysis. Solenoid valves control which cage's air is being measured.

Push-Pull System

The push-pull system has a number of positive aspects which make the design very plausible as seen in Figure 3 (on previous page). First, the system allows for accurate flow rates. This is achieved through the use of pumps which control the flow. Also in this setup the air expansion chamber allows for dehumidification air, which will be a secondary dehumidification process, as well as a means to reduce the oscillations of air pressure produced by the diaphragm pump. Also a constant flow of air will be achieved with the use of a manual valve connected to the air expansion chamber. With the use of the second pull pump, the amount of air needed to pass through sensors/analyzers can be controlled. The cost, a hugely important feature, would be low if no new equipment is needed. Although there are many parts to this system each is necessary to every system, except for the air expansion chamber, and cannot be left out. Therefore, the size constraint must be dealt with by efficient system setup.

Previous indirect calorimetry systems have used this method of moving air, including one by Dr. Keesey who is available to advise the construction of the system. Because the push-pull system uses two pumps to move air, there is an elevated risk of wrong airflow. The system will need to be periodically checked for leaks to ensure that accurate measurements will be taken. Significant amounts of electronics are needed for the system to function properly including solenoid valves with relays, and a data acquisition device. The Dasylab software (which was provided) has the potential to be used for all the electronics but further research and programming must be performed in order to ensure the system functioning correctly.

Pull System

The positive aspects of the pull system are very similar to the push-pull system and will only be briefly mentioned leaving the few differences to be emphasized. The pull system uses all

the same equipment as the push-pull system, with the exception of the air expansion chamber

(see Figure 4). Eliminating this element from the system does save space, a fairly important

consideration. However, if the system is evaluated as a whole, the space saved by eliminating

the chamber would be very small. There is a modest amount of literature about using a pull

system for indirect calorimetry and results using this method would be generally accepted. Dr.

Keesey is unfamiliar with the nuances of this type of system but would still be willing to help in

any way he could.

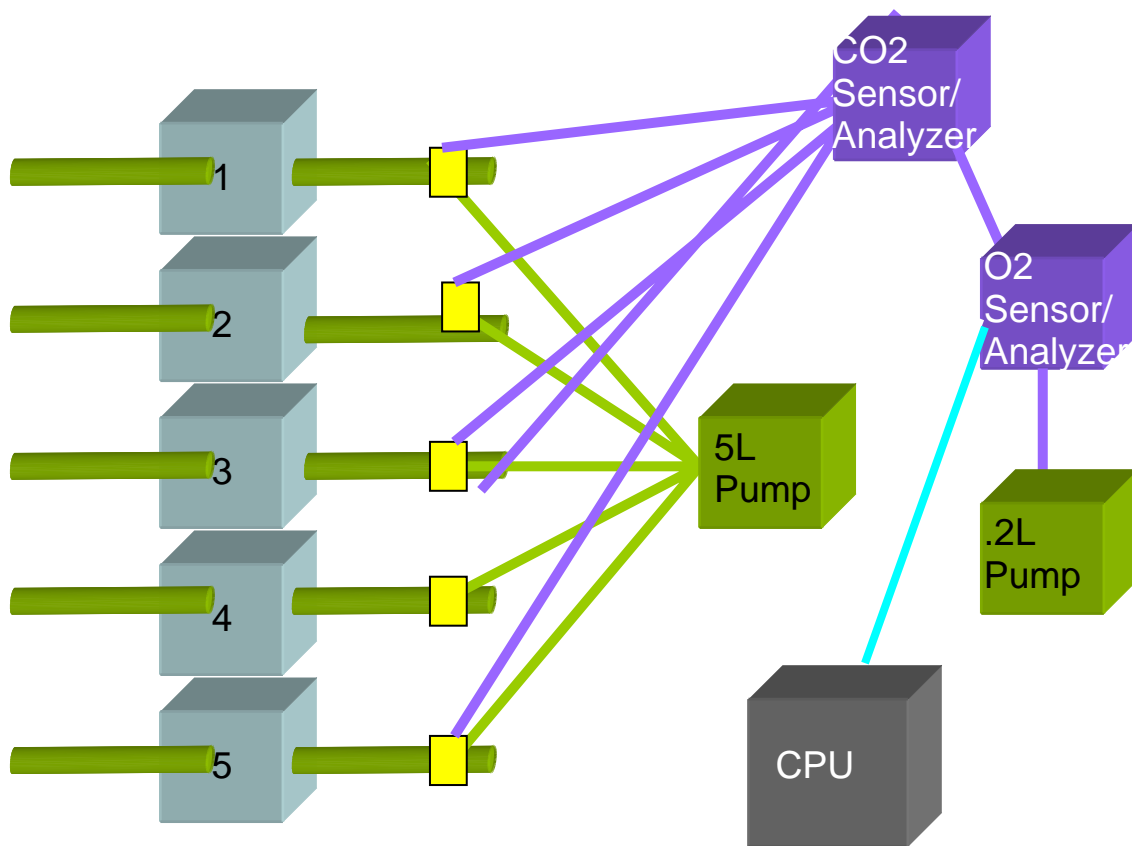**Figure 3**: Pull system. Both pumps in this system are used to pull air. The first 5L pump pulls air through the cages, and the second 0.2L pump pulls air through the analyzers and sensors.

One to One System

With the use of the one to one system, continuous monitoring would be achieved instead

of only measuring each cage every 5 minutes (see Figure 5). Although this is not necessary, it

would simplify the system because there would be no need for the solenoid valves and the electronics to control them and would allow for more accurate data to be obtained. Unfortunately, in order for this to happen every cage would need its own set of equipment. 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 easily.
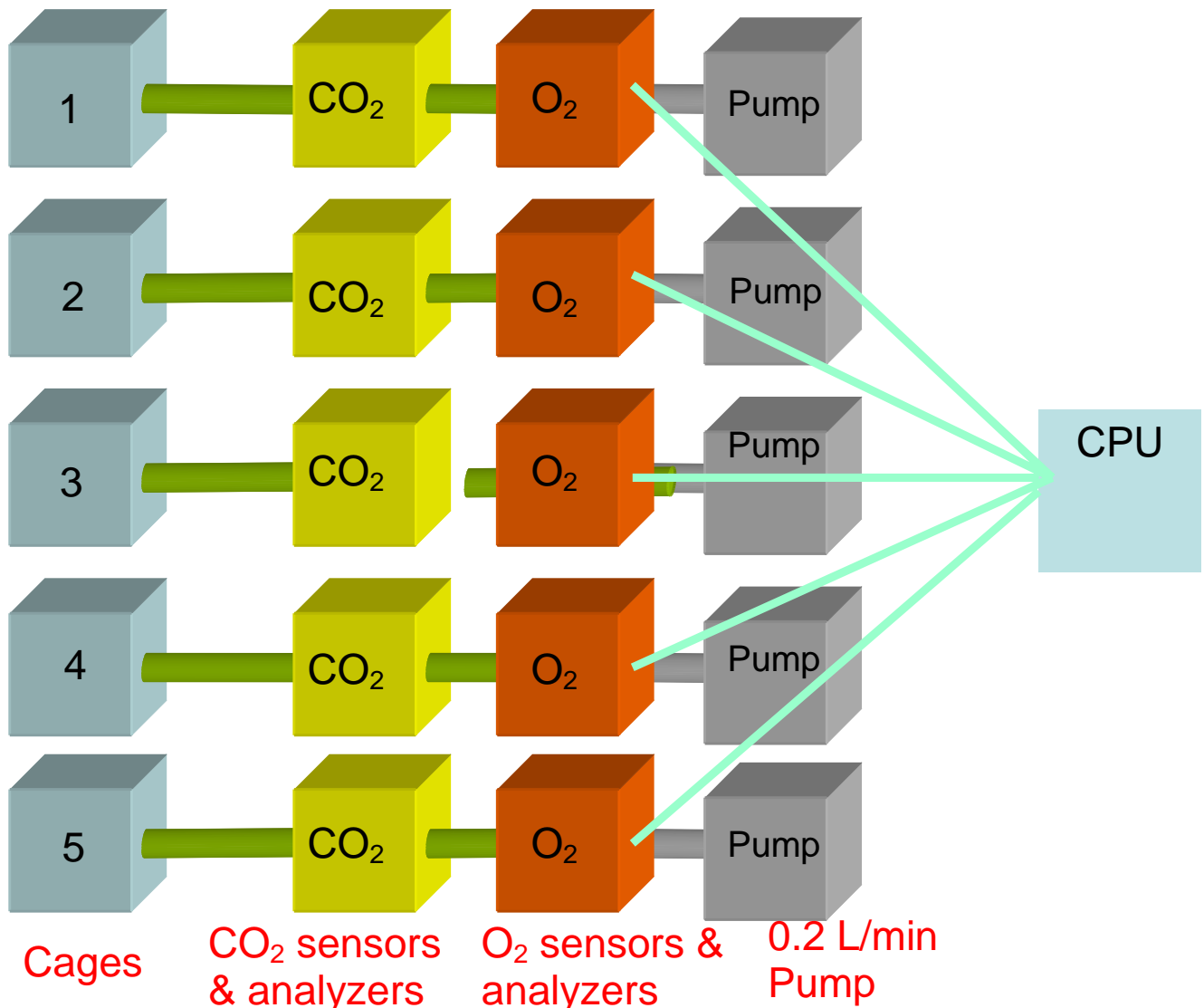
Figure 4: One to one system. In this system each cage has its own corresponding O2 and CO2 sensors/analyzers and pump. The pump is used to pull air through the cage and analyzers/sensors at the desired rate.

Design Matrix
Table 1: Design matrix for three designs

	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

After evaluating each of the three designs with the aid of the design matrix, our group decided to further pursue a push-pull system. While each of the three designs were based on the same idea of measuring concentrations of gas using the same equipment, the push-pull system separated itself from the others by a variety of factors including its lower cost and its established scientific acceptance as detailed earlier.

Project Timeline

Our plan of action had to be altered midway through the semester, as Dr. Cai informed us that the University would be purchasing a CLAM system and he was no longer interested in funding the construction and testing of a second system. However, each of the steps will be explained below to detail the steps which would have been taken.

After examining all of the equipment which was given to us, it was determined the best course of action would be to test and calibrate all of the O_2 and CO_2 sensors and analyzers. Two tanks of gas would be needed to form the linear relationship between the voltage output and percent gas. Ideally the two canisters, one with 21% O_2 and 0.04% CO_2 and another with 19.60% O_2 and 1.00% CO_2 would be used for calibration. This is due to the need for accurate values in the physiological range within which we would be working. If any of the sensors were

not in working condition, they would either need to be repaired, or new sensors would be need to be obtained.

The next step in the process would be the completing of a one cage setup as follows: pump➔ expansion chamber➔manual valve➔cage➔water condenser➔CO_2 sensor➔O2 sensor. This setup would allow us to ensure that all the equipment is working properly together. Also, calibration measurements need to be taken to obtain an accurate flow rate through the cages. These flow rates will be achieved by making the cages smaller to account for the size difference between a mouse and a rat (the cages with which we were provided were previously set up for rats). Testing will have to be performed to ensure the flow rate is not too fast, which would cause not enough gas buildup (thus undetectable changes), and not too slow, which would cause too much gas to build up (putting the test mouse in danger). A similar study performed by Molero, et al. used flow rates of 0.6 L/min with a chamber size of 20 cm x 10 cm x 12.5 cm [3].

Once one single cage is optimized, the signal acquisition stage of the setup will be performed. The voltage output will be taken from the O_2 and CO_2 analyzers, through a BNC connector, to a conversion box with allows a connection to be made between the BNC connector and the IOtech DBK80. The IOtech DBK80 is a 16-channel differential voltage input card, which will allow the analog signal to be transferred to the data acquisition system, the DAQBoard/2000. The DAQBoard/2000 connects to a computer through the PCI slot and runs with the provided DasyLab software.

Once the computer connection is made, the IOtech DBK25 will be incorporated into the process. The IOtech DBK25 is an eight channel relay output card which will be used to control the solenoid valves. The control of DBK25 will rely on the programming package built into the DasyLab software. Care must be taken in order to ensure that once the solenoid valve switches

enough time is provided to flush out of the system of the previous cages air. The previous system worked on a 15 second delay before recording took place. The solenoid values will be connected to the four additional cages to provide a five cage system. Once the valves are connected to the cages, the final design will be setup as seen in Figure 3. Once one entire setup is complete the second setup will be completed so that a total of 2 systems with 5 cages each will be used. These systems will be tested to ensure the accuracy of the data. The final step of the process is the construction of a cart which will allow the system to be transported from room to room. Each system will be mounted to its own cart for easy connections.

Final Prototype

Despite the difficult circumstances encountered by our design team, we managed to design a prototype. The prototype was based on the single-cage setup discussed previously in the midsemester report. However, unlike the midsemester report where the single-cage setup was not equipped with data acquisition, our prototype also incorporated data acquisition.

As discussed in the project timeline, our initial goal was to calibrate the CO_2 and O_2 analyzers. Since we did not have any funds to purchase the gas canisters, we proceeded with setting up the single-cage setup assuming that the analyzers were functional. Figure 6 on the following page shows this single-cage setup.

The air is pumped into the cage with the help of a diaphragm pump. The air then exits the cage and enters the flow control. The flow control ensures that the air entering the sensors is about 200 mL/min. This flow rate assures that the sensors provide accurate O_2 and CO_2 concentrations. The air enters the CO_2 sensor first and then enters the O_2 sensor. The O_2 sensor contains a furnace that heats up the incoming air in order to analyze O_2 concentration.

The sensors are then connected to the analyzers. The analyzers are connected to the ELVIS system via BNC cables. The ELVIS system acts as an Analog to Digital Converter (ADC), which allows discrete time data sampling [5]. The sampled data is then stored as an excel file with the use of a LABVIEW program, which can be seen in Appendix B [6]. The excel file contains two columns – Time (in seconds) and Voltage (in volts). The availability of gas canisters would have made it possible to convert the voltage values to O_2 and CO_2 concentrations.

1. Diaphragm Pump
2. Cage
3. FlowControl
4. CO_2 Sensor
5. CO_2 Analyzer
6. O_2 Sensor
7. O_2 Analyzer
8. BNC Cables
9. ELVIS system (ADC)
10. Computer
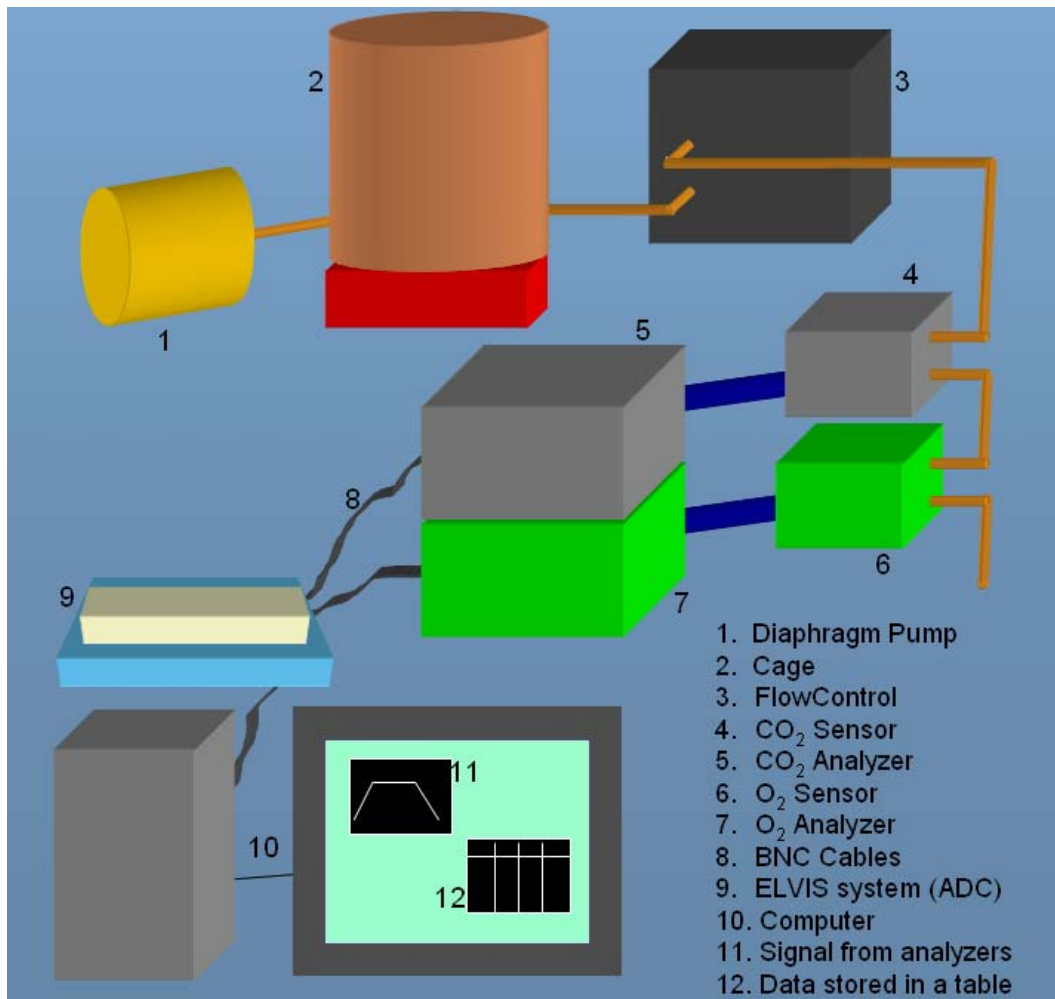11. Signal from analyzers
12. Data stored in a table

Figure 5: The single-cage setup. The system allows for continuously measurements of O_2 and CO_2 concentrations in a given air flow. The setup also allows for recording of data for further data analysis.

A simple test was done to validate our system setup. One of the members of the team exhaled into the tubing connected to the CO_2 sensor. After doing this we were able to see an increase in the voltage values displayed on the computer. The change was seen after a delay of about 5 seconds, which was expected since there is some delay between the moment the air is sensed by the sensor and the time it takes for the analyzer to detect the change. To summarize, the single-cage setup showed that the CO_2 analyzer was functional and that the data from the analyzers can be continuously monitored using an ADC and can be saved for further data analysis.

Cost Projection

There are many components involved in our indirect calorimetry system that we either know for a fact are non-functional, or were unable to properly test, therefore we must assume they are non-functional. Taking this into consideration we decided to do a detailed cost projection of what the total cost would be if we were to replace all non-functional elements of the system. A list of the parts that would potentially need to be purchased or replaced and their approximate costs follows:

Needed to be Replaced:
- Tygon Tubing 1/8" ID (50 ft) $15.80
- Gas tanks for calibration (MDS)   <$100.00
- Movement cart for system to be placed on   ~$400.00

Possible Replacement Parts:
- Thomas Industries 107CAB18 Diaphragm pump $179.95
- Sable FC-10a Oxygen Analyzer/sensor $5,745.00
- Sable Ca-10a Carbon Dioxide Analyzer/sensor $5,995.00
- Omega FMA6500 Series Mass Flow Control $1495.00
- (5) Humphrey Model 31E1-12VDC solenoid valves $221.75
- (7) Swagelok SS-OVS2 manual valves $361.20
- Dwyer Instruments VA10411 Flow meter $101.00

All price estimates were found online, and the specific parts were chosen according to the parts of a similar indirect calorimetry system used in many other projects cited in journal articles. The cart estimation could be far different from this depending on the layout of the final calorimetry system. This estimate is simply for a large moveable cart that would be capable of holding all of the cages, analyzers, and pumps required.

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.

Conclusion

The design process did not follow the path we envisioned, as our client loss interest in the project half way through the semester. Despite this lack of financial support, we were still able to put together a prototype that modeled how a fully functional indirect calorimetry system would operate. We were also able to write a LabView program that would allow us to track and store data on a computer if the prototype were functional. Nonetheless, we appreciate the opportunity that was provided by Dr. Cai, and also the guidance that was provided throughout the semester by Dr. Gentry.

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Product Design Specification
Indirect Calorimeter
Updated: May 8, 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, CO_2 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, CO_2 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, CO_2 sensors, air flow controls, mouse chambers, pipes, wires, a computer, all types of switches and controls, software, and instruction manual.

Design Requirements:

1. Physical and Operational Characteristics
a. Performance Requirement: Must be able to record data for CO_2, O_2, 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: CO_2 and O_2 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.

LabVIEW Program

Block Diagram