Application note

Human calorimetry

Achieving highly precise metabolic measurement during human calorimetry with the Thermo Scientific Prima PRO Mass Spectrometer

Keywords
Human calorimetry, respiratory quotient, oxygen uptake rate, carbon dioxide evolution rate, diet induced thermogenesis, rapid multi stream sampler, magnetic sector analyzer

Figure 1: Typical human calorimeter chamber (courtesy of Fuji Medical Science).

Introduction

Two of today's major public health concerns are obesity and diabetes, with warnings of global epidemics being published with increased frequency. While the answer to these problems appears to be simple, eat less and exercise more, dietary control is also important. However, it has become clear that there is a lack of understanding about how the body utilizes food. Part of the research into this area involves calculations of energy expenditure based on measurements of air samples (indirect calorimetry) from a respiration chamber containing a human subject.

The human calorimeter (or room calorimeter) consists of an airtight chamber in which a subject will spend several hours resting, sleeping or taking physical exercise. The chamber is purged with a constant volume of fresh air. The air entering and exiting the chamber is analyzed continuously for the composition of oxygen and carbon dioxide. Typically, the room includes a bed, a desk, a toilet, exercise equipment and a television, enabling the volunteer subject to undergo normal activities for periods up to one day while participating in the studies. The balance of oxygen consumption and carbon dioxide evolution varies according to activity and the utilization of different foods by the body (i.e., carbohydrates, fat and protein). Accordingly, the room calorimeter measures not only a human's energy expenditure but also evaluates the subject's ability to utilize different types of food. This evaluation is done by assessing the Respiratory Quotient (RQ), the ratio of carbon dioxide molecules evolved to oxygen molecules consumed.

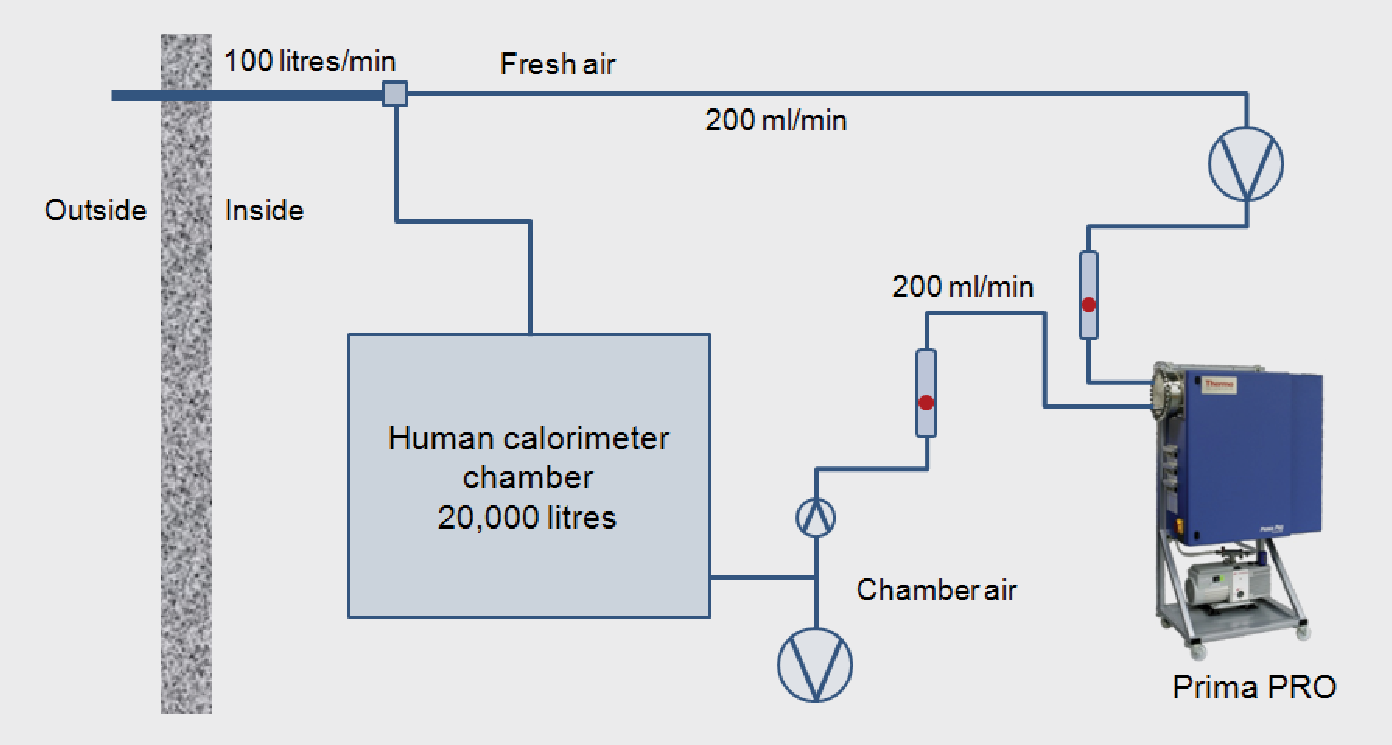

Figure 2: Typical human calorimeter chamber with the Prima PRO Mass Spectrometer.

Human calorimetry analytical requirements

Since the 1990s, biologists and nutritionists have used the best available mass spectrometers to monitor human calorimeters; an example of a typical human calorimeter is shown in Figure 1. The high-precision measurements of O_2 and CO_2 achieved by a mass spectrometer help monitor human metabolisms in real time, enabling the body's response to nutrition and exercise to be studied.

Additional experiments include determining the relationship between sleep patterns and energy expenditure, as well as that of oxidation of carbohydrates and fat. The typical setup of an indirect whole room human calorimeter is shown in Figure 2.

Since the quantities of gas involved in human breathing are very small, with respect to the large volume of the chamber (typically 20,000 litres), a very precise method of gas analysis is required. While it would be easier to measure respiratory changes if the chamber volume and air purge were smaller, this would lead to abnormal O_2 and CO_2 concentrations. In turn, smaller O_2 and CO_2 concentrations would disturb the respiratory patterns of the patient. The long-term precision of the O_2 and CO_2 measurements is a critical analytical specification and must be better than 0.002 mol% (absolute). The Thermo Scientific™ Prima PRO Mass Spectrometer (MS) has been designed with this application in mind.

It is important that both inlet and outlet air streams are measured with high precision since biology occurs both inside and outside the chamber. External effects must be accounted for as the levels of O_2 and CO_2 that are included in the purge air will depend on the activity in the area from where the air is drawn. This activity will vary significantly from night to day, and from variations in sunlight. Figure 3 shows typical data measured with Prima PRO MS that illustrates this temporal effect.

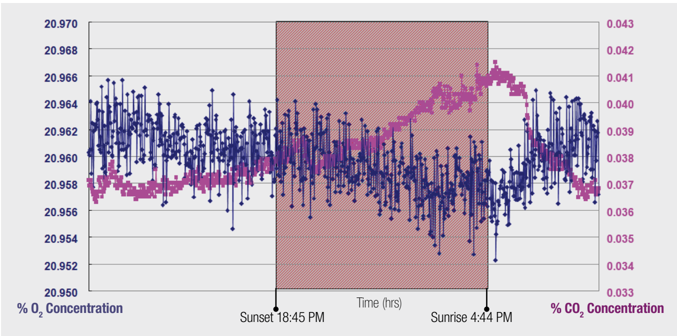

Figure 3: O_2 (dark blue data set) and CO_2 (pink data set) variation of ambient air over 24 hour period, as monitored by the Prima PRO MS.

One of the main interests of research in human calorimetry is in measuring influences on the metabolic rate. For example the 'thermic' effect of a meal can last up to several hours, a phenomenon referred to as Diet Induced Thermogenesis (DIT). Depending on the person's age, condition and genetics, as well as the composition of the meal, there is an increased metabolic rate after consumption. This increase is physiologically beneficial to the person, helping reduce obesity and diabetes.

Biochemically, this increase is a small metabolic increase, which is why a very precise analyzer, capable of measuring very small changes in the gaseous state, is required.

The base metabolic rate is typically 0.2 liters/minute in terms of O_2 consumption, but DIT can increase this rate to between 0.22 and 0.25 liters/minute. This effect can only be seen by a highly precise mass spectrometer that is capable of providing long term (i.e. greater than 24 hours) precision of greater than 0.002 mol%.

The reason the observed changes are so small is that the chamber volume causes significant dilution of the gases. For example the production of 0.2 litres/minute of gas over 30 minutes yields an increase in concentration of just 0.03 mol%. Calculations are performed to derive the actual rates of CO_2 evolution and O_2 consumption based on the measured concentrations and knowledge of the air flow rate and the chamber's internal volume. The equations used are:

$$O_2 \text{ Consumption Rate} = (flow \times 0.01 \times ([O_2\ in]-[O_2\ out])) - (chamber\ volume \times 0.01) \times (d[O_2\ out]/dt)$$

$$CO_2 \text{ Evolution Rate} = (flow \times 0.01 \times ([CO_2\ out]-[CO_2\ in])) + (chamber\ volume \times 0.01) \times (d[CO_2\ out]\ /dt)$$

Prima PRO Mass Spectrometer data quality

The Prima PRO analyzer provides highly precise measurements; this is shown by the oxygen stability plot and the associated statistics in Figure 4. The 17 ppm standard deviation in the analysis of oxygen at 20.95% indicates the analyzer can easily detect oxygen consumption below 50 ppm when the room calorimeter is purged with dry air

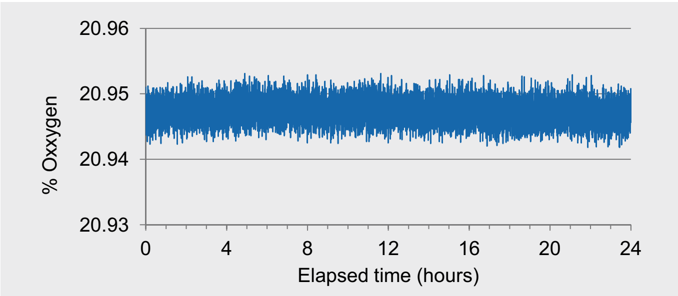

Figure 4: Oxygen stability plot as measured by the Prima PRO MS. Sampling interval is eight seconds. 5,240 total data points depicted with a mean of 20.9485±0.00169%.

The standard deviation is a measure of the reproducibility as observed over 24 hours, assuming constant concentrations, and is demonstrated using a certified calibration gas. The standard deviation is defined as follows: $\sigma_{n-1} = \{\Sigma(x_i - x_m)^2/(n-1)\}^{1/2}$, where n refers to the number of measurements to which the statistical determination is to be applied, x_i refers to the individual measurements and x_m is the mean value.

Prima PRO results

Typical results from an exercise test are shown in Figures 5 and 6. Following a period of rest the human subject exercises for 30 minutes with an exercise bicycle, achieving a pulse rate of 100 beats per minute, then resting again. Figure 5 shows the actual concentrations of O_2 and CO_2 measured with Prima PRO MS, while Figure 6 shows the Oxygen Uptake Rate (OUR) & CO_2 Evolution Rate (CER) calculated by the Thermo Scientific™ GasWorks Software.

The concentration changes during exercise tests are relatively large. However, the equivalent changes during DIT are extremely small and require measurement with an analyzer of the highest precision, typically better than 0.002% by volume. These small changes are shown in Figure 7.

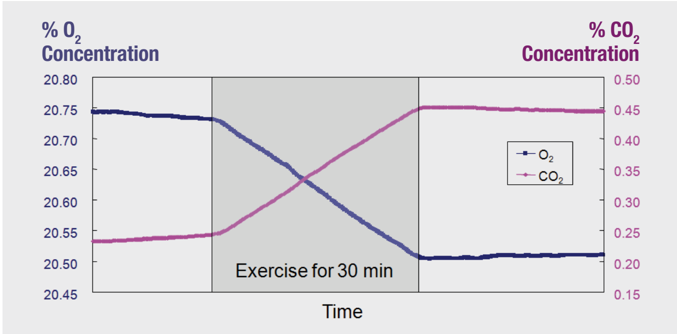

Figure 5: Exercise course variation of O_2 and CO_2.

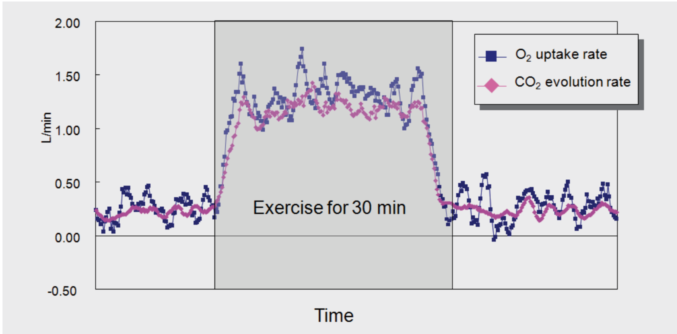

Figure 6: Exercise course variation of oxygen uptake ratio (OUR) & CO_2 evolution rate (CER).

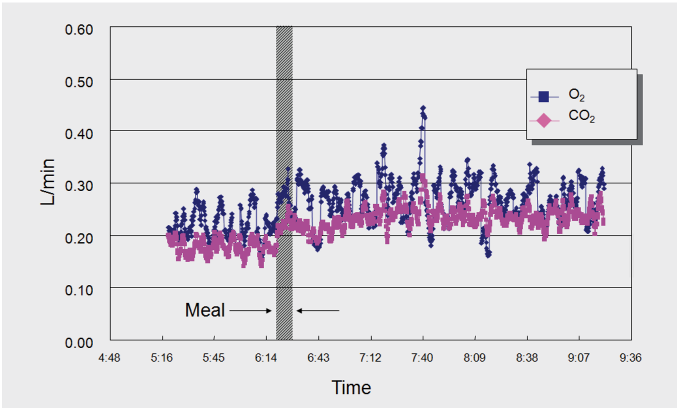

Figure 7: Example plot of Diet Induced Thermogenesis (DIT).

Technology overview

The Prima PRO MS is a high-performance gas analyzer based on a powerful and flexible scanning magnetic-sector mass spectrometer. The platform has been designed to deliver superior analytical performance with high reliability and minimal maintenance.

Mass spectrometers operate by ionizing neutral sample gas molecules and separating the resulting charged particle components according to their molecular weight. In most commercial gas analysis mass spectrometers, the ionization is conducted by bombarding the gas sample with an electron beam that is produced by a hot filament. In order to prevent collisions, the various ions need to be separated in a vacuum.

The technique chosen to separate the ions in the Prima PRO analyzer is the scanning magnetic sector where the trajectory of the ions is controlled by a variable magnetic field, allowing ions of interest to be sequentially collected onto a single detector. Thus the analyzer is able to scan the entire gas sample to accurately quantify known constituents and identify unknown ones.

The output signal from the scanning magnetic sector is a series of flat-topped peaks, each with amplitude proportional to the number of ions at each mass. Perfectly symmetrical peaks are readily achieved in modern magnetic sector mass spectrometers. Accurate intensity measurements do not rely on accurate mass peak jumping since each peak provides a large target with consistent amplitude. Since even relatively large errors in mass position will not result in any significant errors in amplitude measurement, the magnetic sector can be described as intrinsically fault-tolerant.

Furthermore, the sector uses a relatively high-energy ion beam (1kV typical) which is not easily deflected by the inevitable local surface charges and/or insulating layers that result from contamination. This stability further enhances the fault-tolerant nature of the Prima PRO analyzer when compared to the very low ion energy of the alternative quadrupole technology. Finally, the high energy ion beam ensures that extraction from the ionization region is faster (in comparison to a quadrupole analyzer), resulting in reduced ion-molecule interaction and therefore improved linearity. Long-term reproducibility data to 0.01% relative can be easily achieved.

Multi-stream sampling

The Prima PRO analyzers are equipped with a rapid multi-stream sampler (RMS), a highly reliable device for switching sample streams without compromising the quality of the sample presented to the analyzer. The RMS has a long track record of high reliability and is proven to switch streams six million times a year (year after year) with little or no maintenance. This capability allows a single analyzer to monitor up to 63 sample points. The stepper-motor driven device diverts one sample stream at a time to the mass spectrometer and, in turn, records the flow for each stream. The device can be heated to 120 °C (248°F) and has been designed to ensure rapid response to polar species such as methanol, ethanol and ammonia. Alternatively, the Prima PRO analyzer can be supplied with a solenoid manifold system that provides six sample ports and six calibration ports at a lower cost.

Conclusion

The Prima PRO MS from Thermo Fisher Scientific is making an invaluable contribution to the field of human calorimetry studies. Its ability to detect changes in oxygen consumption at ppm levels allows researchers to monitor extremely small transients and subtle changes in human metabolism. These studies improve our understanding of how body weight is regulated, how the pattern of food intake affects human energy metabolism and the impact of exercise on energy expenditure. This not only helps combat health issues such as obesity and diabetes, it plays a vital role in sports medicine, improving athletes' performance and endurance while protecting them from the harmful effects of over-exertion.

The Prima PRO MS has proven to be extremely reliable—its fault tolerant design combined with extended intervals between maintenance and simplified maintenance procedures ensures maximum uptime. In the clinical setting this is important, ensuring real-time respiratory metrics of human calorimeters are acquired. A great example of how the Prima PRO analyzer is being used for respiratory gas analysis is for the Fuji Human Calorimeter at the Fuji Medical Science in Japan. The Fuji Human Calorimeter is the world's most advanced human calorimeter in terms of accuracy, resolution and stability. Fuji Medical Science is the world's leading manufacturer and system integrator of health and metabolism related research systems and products. More information on the Fuji Human Calorimeter is available on the Fuji Medical Science web site, www.fujiika.com.

Prima PRO Process Mass Spectrometer.