Proceedings of

Satellite Meeting of the 13th European Congress on Obesity

Indirect Calorimetry in Rodent Models: Application to Studies of the Metabolic Syndrome

Prague, May 29-30, 2004

Meeting organized by Barbara Cannon, Pavel Flachs, Jan Kopecký and Jan Nedergaard

WHAT CAN INDIRECT CALORIMETRY REALLY TELL US?

Nedergaard J., Golozoubova V., Gao B, Backlund E.C., Cannon B.
The Wenner-Gren Institute, The Arrhenius Laboratories F3, Stockholm University, SE-106 91 Stockholm, Sweden

Indirect calorimetry is a black-box approach to metabolism. In its simplest form, we only have the "black box" (i.e. the animal), an influx of oxygen into the box and an efflux of oxygen, with the difference between these, i.e. the rate of oxygen consumption, as the only parameter measured. It may be thought that from this system, only little information could be extracted. However, with ample utilization of extra external parameters such a time and temperature, new insights into the metabolism of the animal can be obtained and combined with other measurements, notably carbon dioxide production, and with acute agonist and antagonist treatments, the amount of information from this integrative measurement approach increases impressively. We will illustrate these possibilities with examples from our analyses of mice that lack all their functional thyroid hormone receptors, and of mice that lack the uncoupling protein UCP1.

Some examples are found in:

Golozoubova, V., Gullberg, H., Matthias, A., Cannon, B., Vennström, B. Nedergaard, J. Depressed thermogenesis but competent brown adipose tissue recruitment in mice devoid of all thyroid hormone receptors. *Mol. Endocrinol.* 18: 384-401, 2004.

Golozoubova, V., Hohtola, E., Matthias, A., Jacobsson, A., Cannon, B. Nedergaard, J. Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *FASEB J* 15: 2048-2050, 2001.

SOMEDIC SYSTEM FOR INDIRECT CALORIMETRY

Johansson B.
Somedic, Hörby, Sweden

About 6 years ago, a project started at Somedic to develop a new Indirect Calorimeter (INCA) for small animals. The reason was a direct request from a major pharmaceutical company (Pharmacia, now Biovitrum) that could not find any commercial product meeting their needs for high-resolution calorimetry work.

There were five initial acquirements on the INCA:
1. It should allow measurements on rats and possibly also mice.
2. It should be simple to use, with output data in easily readable computer format.
3. Oxygen consumption should be the primary indicator of metabolic activity.
4. The results should be as accurate as possible, with high temporal resolution.
5. Measurements should be carried out in a constant temperature environment.

With time, the INCA has been further equipped with:
6. Measurement of carbon dioxide production, for calculation of the Respiratory Quotient.
7. Large range temperature regulation of the measurement chamber.
8. Measurement of animal core temperature and total locomotor activity, using non-contact methods.
9. Ergonomical and animal friendly design.

This presentation describes the development process and the present capacity of the INCA system.

SOME MATHEMATICAL AND TECHNICAL ISSUES IN OPEN-CIRCUIT INDIRECT CALORIMETRY

Arch JR[1], Hislop D[1], Wang SJ[1], Speakman J[2]
[1]University of Buckingham, UK; [2]University of Aberdeen, Uk

O_2 consumption (VO_2) by caged rodents can be calculated using open circuit indirect calorimetry if CO_2 is absorbed, knowing only the volume of gas leaving the cage (V^o) and the fall in its O_2 content (x). If CO_2 is not absorbed, VO_2 cannot be calculated from V^o and x alone because V^i (i=in) is less than V^o by an amount that depends on the proportions of macronutrients oxidised. Hence VO_2^i is not known. Fortunately, fat oxidation produces less energy than carbohydrate oxidation per unit VO_2, and a remarkable equivalence of errors allows energy expenditure (EE) to be calculated accurately from V^o and x. By using a CO_2 as well as an O_2 analyzer, not only VCO_2, but also VO_2 can be measured, because the volume of N_2 (and argon) leaving the cage can then be calculated. This equals VN_2^i and hence gives VO_2^i. Carbohydrate, fat and protein oxidation can be calculated from VO_2, VCO_2 and N excretion, although gluconeogenesis, ketogenesis and lipogenesis can obscure what is happening to substrate utilisation in the short-term. Such measurements have shown that β_3-adrenoceptor agonist-driven thermogenesis is dependent on fat oxidation. To obtain an instant measurement of VO_2, VCO_2, or EE, one must know the composition of air inside the cage (and ideally in the animal). This is important if these values are to be related to activity, or if maximum or minimum values are needed. When the VO_2 of the animals undergoes a step change, x approaches steady state with a rate constant of air flow/ volume and a half-life of 0.693/rate constant. Instant values of VO_2 etc. can be calculated by including a dx/dt term in the calculation, but better accuracy may be achieved by reducing cage size.

MAJOR BIOLOGICAL ISSUES IN PHENOTYPING RODENT MODELS FOR METABOLIC SUSCEPTIBILITY TO OBESITY

Dulloo A.G.
Department of Medicine/Physiology, University of Fribourg, Switzerland

After a decade of phenotyping rodents that overexpress or lack gene(s) implicated in the control of thermogenesis and lipid metabolism, we have learned to expect the unexpected. In some cases, the failure to demonstrate the 'expected' could be pinpointed to a lack of appreciation that differences between mutants and wildtypes - whether in body composition, energy expenditure, respiratory quotient or physical activity – can only be unmasked by appropriate challenges, by appropriate methodologies (techniques, study design, duration of measured parameters) and by more robust numerical and statistical analysis of data. Equally important from lessons learned during this past decade is the need to confront the issue of metabolic scaling and thermal regulation in the interpretation of data on energy expenditure and physical activity. Between a 30 g mouse and a 60 kg human, there is a 2000-fold difference in body weight, and a 300-fold difference in metabolic body size (body weight$^{3/4}$). Mice and rats are studied at laboratory temperature (21-24 °C), which is well below their zone of thermoneutrality (>30 °C), whereas humans live (and are generally studied) with their body kept in a microenvironment that is thermoneutral. An appropriate consideration of these biological issues in the design of indirect calorimetry studies in rodent models is of central importance in the elucidation of mechanisms that confer genetic and metabolic susceptibility to *human* obesity.

INDIRECT CALORIMETRY IN THE ASSESSMENT OF DIETARY INTERVENTIONS IN DIFFERENT RODENT MODELS

Klaus S.
German Institute of Human Nutrition, Potsdam-Rehbruecke, Germany

Aims: Evaluation of macronutrient diets and compounds in the development and prevention of obesity in rodent models.

Methods: Indirect calorimetry (IC) in combination with continuous measurement of body temperature and activity (transponders) was employed to assess the role of different diets in energy balance and body weight regulation.

Results: Energy expenditure (EE) of rodents is highly variable and affected by body mass, daily rhythms, environmental temperature, activity levels, food consumption, and diet composition. Respiratory quotient (RQ) as a reflection of substrate oxidation and turn over also shows large daily variations and is acutely affected by feeding versus fasting and diet composition. Epigallocatechin gallate (EGCG, the main catechin of green tea polyphenols) did not acutely increase EE in mice but decreased RQ during night only. Chronic dietary supplementation with EGCG prevented the development of diet induced obesity in obesity prone mice.

Conclusions: IC is a valuable tool in the assessment of energy and substrate metabolism in rodent models. However, measurements should be performed over a prolonged period with high temporal resolution and carefully controlled for above listed parameters in order to obtain useful results. Taking this into consideration we could show that the anti obesity effect of EGCG is not due to increased thermogenesis but rather to an effect on energy partitioning, probably a reduction of postprandial lipogenesis.

TYPE 2 ANGIOTENSIN RECEPTOR DEFICIENT MICE RESIST HIGH FAT DIET-INDUCED OBESITY AS A RESULT OF AN INCREASED LIPID UTILIZATION

Yvan-Charvet L[1], Quignard-Boulange A[1], Meneton P[3], Even PC[2]
[1]INSERM UR465, [2]INA UMR 914, [3]INSERM UR367, Paris, France

Aims: AT2 angiotensin receptor (*AT2R*) deletion is associated with hypotrophy of adipose cells suggesting a potential role of this receptor in limiting an excessive fat storage. To test the possible consequences on energy metabolism we measured the various components of energy expenditure (EE) in these mice under ad libitum feeding with a low-fat (LFD) or a high-fat diet (HFD), and in response to food restriction and refeeding.

Methods: Weaned male WT and *AT2R*-null mice were fed ad libitum a LFD or HFD for 10 weeks. The components of energy expenditure were measured continuously during 23 hours by indirect calorimetry in relation to spontaneous activity so that resting EE, total EE and EE with activity could be dissociated.

Results:. Daily total, resting and activity related EE were not different between WT and AT2 R-null mice under LFD as well as under HFD. In contrast, we observed that AT2 R-null mice had a reduced post prandial increase in RQ, a lower the RQ during the light period under LFD and a lower RQ at night under HFD. In contrast, the decrease in RQ during transition from the fed to the fasted state was the same in WT and AT2 R-null mice.

Conclusions: These results suggest that obesity-resistance in AT2 R-null mice result from alterations in energy partitioning in the fed state (as testified by periods of lower RQ) rather than from an increase in resting EE or activity, or from enhanced capacities to mobilize lipids when fasted (as testified by a similar decrease in RQ during transition from the fed to the fasted state).

UNDERSTANDING LEPTIN`S FUNCTION: THE ROLE OF USE, MISUSE AND MISSING USE OF INDIRECT CALORIMETRY

Schmidt I.
Max-Planck-Institut, Bad Nauheim, Germany

The role of leptin as adipostatic signal is widely accepted. In contrast, its ability to do so, not only by decreasing food intake (FI) but also by increasing metabolic rate (MR), is often assumed without appropriate experimental support. Thus, increases in sympathetic activity in anesthetized animals or increases in brown adipose tissue uncoupling protein (UCP) mRNA have been interpreted in this way. However, when determining all three parameters of the energy balance equation (FI, MR and changes of body energy content) while delivering leptin to the brain via the natural (i. e. peripheral) route, leptin has been shown not to increase MR, but only to normalizes it, when it was suppressed to save energy. Moreover, because UCP mRNA indicates thermogenic capacity rather than acute activity, it might increase although MR does not. However, when using indirect calorimetry to study changes in energy balance it is mandatory to consider changes in total MR, because the mass-specific MR must obviously increase, if animals loose fat mass. Moreover, attributing, as frequently done, changes in body composition to changes in MR, if changes in FI remain statistically non-significant, may reflect conversion of a type 2 error to a type 1 error. Only by continuous measurements of MR along with FI, the underlying causes for changes in body composition can be accurately identified. Thus, many conclusions not only about leptin`s funtion but also about downstream targets studied in genetically or pharmacologically manipulated animals have to be revised when indirect calorimetry is correctly used to unravel the causes of weight loss or weight gain.

EFFECT OF HIGH PROTEIN FEEDING AND DIETARY SELF SELECTION ON THE DAY-NIGHT CYCLE OF LIPOLYSIS-LIPOGENESIS IN THE RAT

Even P.C., Makarios L., Fromentin G., Tome D.
Laboratoire de Nutrition Humaine, UMR INRA-INAPG 914, Paris, France

Aims: The purpose of this study was to understand the metabolic changes responsible for the decrease in adiposity reported under dietary self selection and high protein feeding (1).

Methods: Male Wistar arts were adapted to a standard diet (14 % protein, P14), a high protein diet (50 % protein, P50) or let free to select between protein and a fat carbohydrate mix. They were then housed in an open circuit calorimeter for simultaneous measurement of respiratory exchanges, spontaneous activity and meal pattern.

Results: All rats showed a cycle of lipogenesis at nigh and lipolysis at day related to the level of food intake. However, lipolysis during the light period was repeatedly inhibited by the periodical occurrence of meals in the P14 but not in the P50 fed rats. Most of the rats under dietary self selection ingested predominantly protein during the light period, which also prevented the inhibition of lipolysis after feeding.

Conclusions: In rats fed a high carbohydrate diet, the meals that occur during the light period induce long-lasting inhibitions of the spontaneous lipolysis that normally characterize this period, an effect that is not observed in rats fed a high protein diet or in those under dietary self selection that select protein during the light period. This phenomenon may explain the lower adiposity index previously reported in rats fed a high protein diet or under dietary self-selection.

(1) Makarios L et al. Rats free to select between pure protein and a fat-carbohydrate mix ingest high-protein mixed meals during the dark period and protein meals during the light. *J Nutr* 134: 618-624, 2004

SR141716 (RIMONABANT) AN INVERSE CANNABINOID RECEPTOR TYPE I AGONIST DOES NOT INCREASE OXYGEN CONSUMPTION AND MOTILITY IN POST-ABSORPTIVE MICE LACKING CB1 ($CB1^{-/-}$)

Kunz I., Meier M., Künnecke B., Bourson A., Ozmen L., Roever S., Schilling W.
F. Hoffmann-La Roche Ltd, Vascular and Metabolic Research, Basel, Switzerland

Aims: To evaluate the acute effect of the CB1 inverse agonist SR141716 on oxygen consumption (OC), respiratory quotient (RQ) and motility (M) in mice lacking cannabinoid receptor type 1 ($CB1^{-/-}$) and in wild-type littermate mice ($CB1^{+/+}$).

Methods: Five months old $CB1^{-/-}$ and $CB1^{+/+}$ mice were adapted to the respiratory chamber for 24h with chow diet and water ad lib. Food was restricted to 75 % on the night prior to the dosing with SR141716 or vehicle at 8.00 am. Continuous measurement of gas exchange and M were performed for 9h on mice which received only water ad lib. Body composition was ascertained by MR relaxometry on the day after indirect calorimetry.

Results: In $CB1^{+/+}$ mice, OC and M were increased over baseline (corrected for vehicle) in the first 3h after dosing by 51 % and 164 % and after 9h by 40 % and 78 %, resp., whereas no effect on RQ was observed. In contrast, OC, M and RQ remained at baseline in $CB1^{-/-}$ mice. $CB1^{-/-}$ mice weighed less than their littermate (25.8±0.8 vs. 27.8±1.0 g) and had a significantly lower fat mass (3.6±1.1 vs. 5.3±1.3 g; $p<0.001$).

Conclusions: The increase in OC and M elicited by SR141716 is mediated via the CB1 receptor. The increase in OC can be explained with an increase in M. However, the differences in the duration of the effects may indicate an additional acute thermo-genic effect of CB1 inverse agonist SR141716.

ADEQUATE PROCEDURES TO OBTAIN THE DYNAMICS OF OPEN-RESPIROMETRY SYSTEMS

Chaui-Berlinck J.G., Bicudo J.E.P.W.
Laboratory of Theoretical Physiology – Biosciences Institute – University of São Paulo, Brazil

Open-respirometry systems are dynamical systems that attain the total amplitude of a given input only after a certain time interval. In face of this intrinsic feature, changes in oxygen consumption rate by the subject during experiments would not be immediate linearly mirrored by the changes in the sensor output (readings). Therefore, in order to obtain real oxygen consumption data, researchers must be ready to perform the mathematical reconstitution of the input from the output. To execute such a task the usual approach is by compartmental analysis and the time-constants of the system must be obtained during the calibration procedure that is, basically, to perturb a system and characterize the time evolution of the change in its state. An usual way is to inject a nitrogen bolus, supposedly acting as an impulsive function. Here we show that this procedure is only effective in achieving its primary goal if the system has its internal medium homogeneously and instantaneously mixed and the dynamics of the gas transit in the connecting tubing can be disregarded. In other words, the impulsive function can be employed only in systems enforced to behave with first-order dynamics, which is dictated by convection. On the other hand, the system dynamics can be dominated by diffusion-like gas movements, with substantial variance around a mean velocity of gas transit. Under such circumstances, the calibration procedure must be done by injecting a constant input to change the system from a steady-state to another one. Otherwise, the dynamics observed during the calibration would not resemble the dynamics that prevail during the experiments and signal reconstitution will be misleading. We demonstrate these differences based on gas kinetic theory.

EFFECT OF LEPTIN AND CHARACTERIZATION OF ENERGY EXPENDITURE IN MICE USING *IN VIVO* INDIRECT CALORIMETRY

Alberts P., Ekdahl Å., Sahaf N., Engblom L., Högberg H., Lidell V.
Biovitrum, SE-112 76 Stockholm, Sweden

Aims: To characterize energy expenditure under resting conditions and the influence of leptin using some normal, obese and/or diabetic, and PTP1B knock out mice.

Methods: Oxygen consumption and carbon dioxide production was measured at different environmental temperatures (15-32.5 °C). The lower critical temperature (LCT) of the thermo neutral zone, temperature sensitivity (TS), basal metabolic rate (BMR), and respiratory quotient ($RQ=VCO_2 / VO_2$) were calculated. The influence of the age of *ob/ob* mice on the parameters was studied. The effect of leptin on body temperature and RQ was tested in *ob/ob* mice.

Results: In all mice the LCT was in the range 26-30 °C and the BMR was 9.7-11.3 ml $O_2/min/kg^{0.75}$. In PTP1B knock out mice the oxygen consumption was almost twice as high as in the wild type mice at the lower environmental temperatures tested, which was reflected in doubled TS. The body temperature was not altered in any of the mice. Leptin lowered RQ and enhanced body temperature in *ob/ob* mice.

Conclusions: The increased TS in the PTP1B knock out mice suggests 'impaired insulation' or 'deliberately increased' heat loss. In addition to the well-known food intake reducing effect, leptin lowered RQ and enhanced body temperature. The compiled results support the suggestion that PTP1B and leptin have functionally opposing effects on energy metabolism.

INFLUENCE OF MOUSE STRAIN ON ENERGY BALANCE

Backlund EC[1], Nedergaard J[1], Cannon B[1]
[1]The Wenner-Gren Institute, The Arrhenius Laboratories F3, S-106 91 Stockholm, Sweden

Aims: The goal of this study was to compare the ability of different mouse strains (C57/BL6, 129sv, FVB/N, β_3-KO and the outbreed NMRI) to resist weight gain after voluntary eating either chow or a high fat/carbohydrate cafeteria diet (CD).

Methods: 6-8 mice of each strain were divided into two groups, chow or cafeteria, and put in single cages in 22ºC. Food intake, body weight gain and body core temperature during daytime were monitored regularly the first 3-4 weeks of the experiment. After three weeks, the resting metabolic rate (RMR) at 30ºC ambient temperature was determined. After 15 weeks, all mice where sacrificed and epididymal white adipose tissue (eWAT) and interscapular brown adipose tissue (iBAT) were dissected.

Results: NMRI, C57/BL6 and Fvb/N mice on cafeteria diet significantly increased their body weight. All strains on CD showed a tendency of increased body temperature compared to ND. Only NMRI mice on CD increased their RMR significantly, although the other strains indicted the same. C57/BL6, Fvb/N and NMRI on cafeteria diet had increased iBAT wet weight and NMRI and FVB/N significantly more eWAT. 129Sv showed a remarkable resistance to weight gain, in spite of consuming equal amount of calories to the other strains on cafeteria diet.

Conclusions: Our experiments emphasize that the genetic background is of great importance when choosing which mouse strain to use as a model system when studying obesity-related questions.