

**Network: Obesity and Related Disorders****Project: Metabolic Phenotyping of Mouse Models for Obesity**

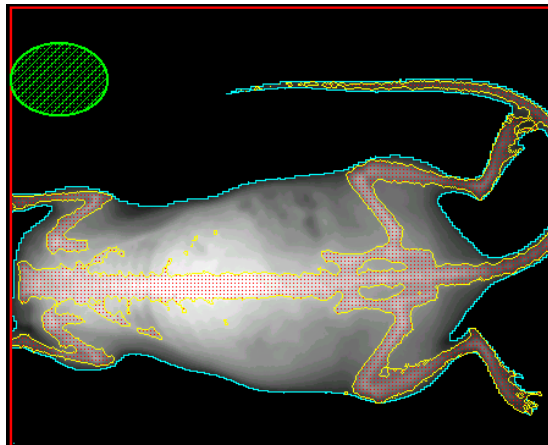
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**Introduction**

We are investigating the metabolic and endocrine physiology of mouse models with disorders in energy balance and body weight regulation, including phenotypically relevant ENU (ethyl-nitroso-urea) induced mutants, inbred mouse strains and transgenic mice in close collaboration with the SMP in Munich (German Mouse Clinic, GMC). Currently under investigation in our laboratory and at the SMP in Munich are overweight mouse mutants and inbred mouse strains with differential susceptibility to diet-induced obesity.

**Project Status****Methods for metabolic phenotyping**

A step-wise metabolic phenotyping protocol was established in our laboratory at the Philipps University Marburg (PUM) and is up and running successfully at the GMC in Munich. Mice are phenotyped in a standardized procedure, using 7 males and 7 females, aged  $18 \pm 1$  week. The mice are housed at an ambient temperature of  $23 \pm 1^\circ\text{C}$  in a light:dark cycle of 12:12 h. For a total observation period of two weeks, they are kept singly on grid panels, which allow collection of scattered food and feces.



**Fig 3:** Body composition is analysed by Dual Energy X-Ray Absorptiometry (DEXA) revealing body lean mass, fat mass and bone mineral content & density.

During the first week mice are fed *ad libitum*. Body weight, rectal temperature and food intake are measured daily. In parallel, feces production and fecal energy content is monitored in three day intervals to calculate the average daily metabolisable energy. Energy content of food and feces are determined by bomb calorimetry (IKA Calorimeter C7000). After this *ad libitum* feeding period mice are exposed to negative energy balance by restriction to 60% of *ad libitum* food intake. Measurements of body weight, body temperature and metabolisable energy are continued as during the first week in order to test whether the normal set of physiological responses is displayed in response to this challenge of energy balance.

Individually caged mice are placed inside an open respiratory system and their oxygen consumption and carbon dioxide production are measured at different ambient temperatures. Up to six mice can be measured in parallel. Data are monitored and stored continuously by a computer system.

Simultaneous measurement of oxygen consumption and carbon dioxide production allows the determination of the respiratory quotient and thus gives further insight into the combustion of preferred energy substrates. It further allows the determination of the thermoneutral zone, the analysis of basal and resting metabolic rate, metabolism related to activity, and thermal conductance. Components of the calorimetry device have been developed and built at Philipps-University of Marburg (PUM) and were transferred to the GMC.

The metabolic system is extended by a telemetry system for recordings of body temperature, heart rate, and locomotor activity patterns. Mice are implanted with transmitters into the abdominal cavity, and thus continuous recordings of body temperature can be obtained in unrestrained mice. Both systems are running in parallel and allow a complete monitoring of ultradian and circadian patterns of body temperature, heart rate, activity and daily energy budget.

Multiple recordings of body composition of mice are taken by non-invasive dual energy x-ray absorptiometry (DEXA, Piximus). Calibration of the DEXA system by comparison with chemical analysis of body composition has been established at PUM.

**SMA1 – small and obese**

A detailed phenotypic analysis of the dwarf obese ENU mutant SMA1, which exhibits a D167G amino acid exchange in growth hormone (GH), was completed in 2004<sup>1</sup>. The mutation causes impaired storage and/or secretion of pituitary GH, resulting in reduced plasma levels of GH and insulin-like growth factor 1 in a unique gene-dosage manner.



**Fig 1:** ENU induced point mutation in the growth hormone gene causes semi-dominant dwarfism and obesity. Wildtype  $Gh^{+/+}$ ,  $Gh^{+/sma1}$  and  $Gh^{sma1/sma1}$  mice are shown above.

Adult SMA1 mice accumulate excessive subcutaneous and visceral fat, with elevated plasma ghrelin levels indicating altered energy partitioning. We propose that the deranged

GH-axis function in SMA1 mice causes obesity via both, the lack of lipolytic GH activity as well as feedback mechanisms involving increased ghrelin secretion. Generation and identification of the SMA1 mouse exemplifies the power of the phenotype-driven approach in the identification of genes involved in the regulation of energy balance and body fat mass.

We furthermore utilized the SMA1 mouse model to analyse basic thermal and metabolic properties to detect metabolic alterations that can support the accretion of excess fat. With the exception of body temperature, all metabolic alterations observed in SMA1 reflect reduced size. The main outcome of this study is that analysis of gene effects on body weight and energy expenditure in mouse mutants must consider the appropriate allometric relationship between body mass and metabolic rate<sup>2</sup>.

### HWE007

Heavy weight mutant mouse lines generated in the ENU mutagenesis program at the Institute of Experimental Genetics (Munich) appear to be of even greater relevance for human obesity. The heavy weight phenotype of one mouse mutant line (Fig. 2) originating from the ENU screen for recessive traits has been verified in the intercross with B6 mice. In collaboration with the SMP in Munich, the HWE007 line is currently investigated in the primary and secondary screen (GMC) and heavy weight mice from the intercross are sampled for genomic mapping of the mutation. Preliminary results demonstrate that despite significant differences in body weight the daily food consumption and the daily amount of metabolised energy are not statistically different. Comparably, basal metabolic rate and daily energy expenditure determined by measurement of oxygen consumption is similar in mutant and wildtype mice, indicating that in relation to body weight energy expenditure is lower in overweight mice. A detailed analysis of the balance of energy intake and expenditure will follow to elucidate the physiological mechanism causing obesity in the mutant mouse line.



**Fig 2:** A heavy weight phenotype (right side) of one mouse mutant line originating from the ENU Screen in comparison to the control wildtype littermate.

### DIO and DR mouse strains

Human obesity is rarely caused by single gene mutations, but rather the result of interaction between multiple genes and environmental factors. The high-fat Western type diet is one of the major environmental factors promoting the development of obesity in the human population. The AKR/J mouse strain, a mouse model for diet-induced obesity (DIO), is prone to become obese and develops leptin resistance when fed a high fat diet. In contrast the SWR/J strain due to a different genetic background is resistant to DIO. In collaboration between our laboratory (PUM) and the SMP in Munich the metabolic phenotypes of these two mouse strains are compared to elucidate the metabolic disturbances that promote the development of obesity in response to a high fat diet.

Furthermore, we employ expression genetics to characterize alterations of gene expression profiles in selected tissues with the potential to rapidly assess a number of candidate genes. Many recently discovered obesity genes are predominantly expressed in the hypothalamus. Notably, the identification of melanin concentrating hormone in the regulation of food intake was primed by screening of hypothalamic RNA isolated from *ob/ob* and wildtype mice for differentially expressed transcripts. It is very likely that a number of genes involved in the DIO phenotype, especially those responsible for hypothalamic function, will show altered expression levels in response to high fat diet feeding.

### Human MC4R variants

Previous work on polymorphisms in the human melanocortin-4-receptor gene (*Mc4r*) merit a more detailed functional analysis in mouse models<sup>3</sup>. Two partners in our network are testing whether selected variants cause alterations in receptor function in transfected mammalian cell cultures. We are currently generating knock-in mouse lines carrying two different human *Mc4r* variants in order to investigate their phenotypic consequences on energy balance. Metabolic phenotyping of these knock-in mice will elucidate the sole contribution of the respective receptor variants on energy balance in a uniform genetic background of inbred knock-in mice. The required constructs have been generated in our laboratory at the PUM by PCR amplification of 6-8 kb of the *Mc4r* genomic region utilizing available BAC clones which cover the respective genomic region. This work as well as the homologous recombination and screening of ES cells, is conducted under the expert advice and service of the SMP in Munich. Knock-in mice will be generated by "NGFN services" at the SMP in Munich. We expect to start phenotypic analysis of the knock-in mice in 2006.

### Outlook

The comprehensive analysis of metabolic phenotypes in mouse models for obesity will increase our understanding of the fundamental physiological processes underlying the development of obesity. This knowledge will guide us in future investigations on gene functions in the regulation of body weight and energy balance.

*Lit.: 1. Meyer CWE et al. A novel missense mutation in the mouse growth hormone gene causes semidominant dwarfism, hyperghrelinemia, and obesity. Endocrinology. 2004 May;145(5):2531-41 2. Meyer CW et al. Gene or size: metabolic rate and body temperature in obese growth hormone-deficient dwarf mice. Obes Res. 2004 Sep;12(9):1509-18. 3. Farooqi IS & O'Rahilly S. Monogenic obesity in humans. Annu Rev Med. 2005; 56:443-58.*