

**Network: Obesity and Related Disorders**

**Project: Functional Studies on the Interaction between Genetically and Environmentally Induced Obesity**

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**Introduction**

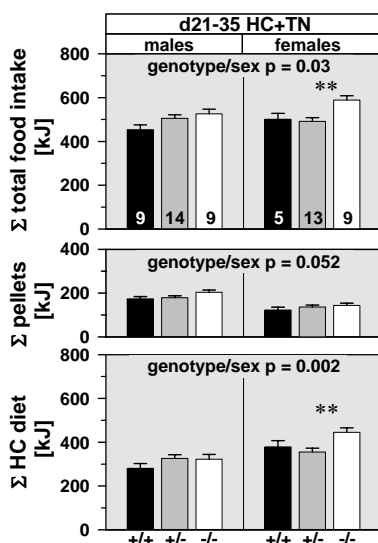
2-4% of extremely obese patients display relevant mutations in the melanocortin-4 receptor (MC4R). Hetero- and homozygous mutation carriers mostly had early onset obesity (1). MC4R-deficient mice were first described in 1997 (2). A previous study of our group showed that hyperphagia, not hypometabolism, causes early onset obesity in this mouse model (3).

We wanted to explore the genetic and environmental impacts on development of obesity in 35 and 56-day-old wild-types and MC4R-deficient mice. One group of each age was fed standard rodent diet and was housed at an ambient temperature of 22°C. To account for the human living conditions in industrialized countries, the other two groups additionally had access to a highly palatable high caloric food (white chocolate) and were housed at thermoneutral ambient temperature (34°C).

The hormone leptin is a satiety signal which is produced by the white adipose tissue in proportion to the body fat content. It is known that increasing leptin levels downregulate the orexigenic neuropeptides neuropeptide Y (NPY) and agouti-related protein (AGRP) and upregulate the anorexigenic neuropeptides pro-opiomelanocortin (POMC) and cocaine and amphetamine-regulated transcript (CART) (4). An influence of genotype (3) and age (5) on neuropeptide expression of MC4R-deficient mice was already detected.

Our current experiments analyze the influences of a supplementary high caloric food and of thermoneutral ambient temperature on body composition of wild-type and MC4R-deficient mice in comparison with mice under standard conditions. Statistical analyses were applied to verify the influences of rearing conditions, sex and genotype. We also analyzed the expression of NPY, AGRP, POMC and CART in the arcuate nucleus of 35-day-old mice depending on rearing conditions.

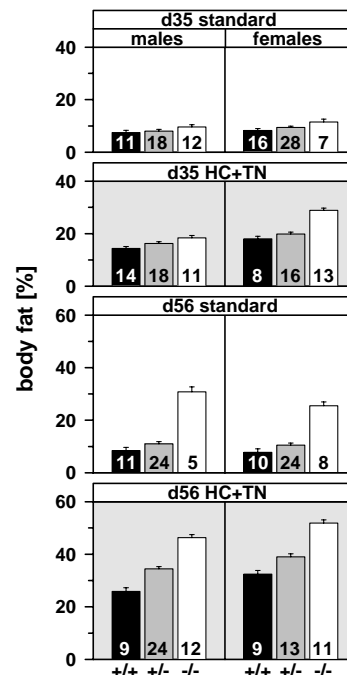
**Results**



**Fig 1:** The influence of genotype and sex on cumulative total food intake, standard diet intake and HC diet intake least square means ± SEM. The mice were reared with access to additionally high caloric food under thermoneutral conditions.

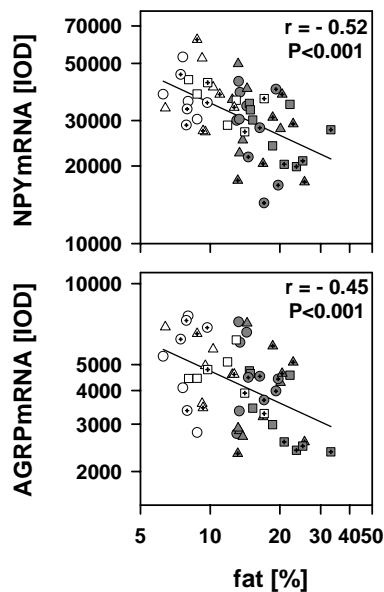
We determined total daily food intake, pellet intake and high caloric food intake in kJ from weaning until day 35 or 56. Statistical analysis of cumulative food intake over this period with 3-way ANOVA shows a significant influence of litter and genotype for the 35 day-old animals and also a significant genotype/sex interaction. There is no influence of sex on total food intake but the separate analysis of cumulative pellet and high caloric food intake shows a supplementary significant influence of sex in addition to litter and genotype influences (figure 1). The results for the 56-day-old mice correspond to those obtained in the 35-day-old mice, the significance levels are even higher, only the genotype/sex interaction disappears. Female animals have a higher preference for high caloric food than males. 62.8% of the cumulative food intake (d21-35) in males and 74% in females consist of the high caloric diet. From day 21-56 the fraction of high caloric food is increasing to 78.4% in male animals and 85.7% in female animals.

Comparison of body composition of mice living on standard conditions with those having access to high caloric food and living on thermoneutral conditions at 35 days of age reveals a significant influence of rearing conditions, sex and genotype on body mass, body fat and body fat content. The results for the 56-day-old mice are similar. For this age there is also a genotype influence on fat-free dry mass (FFDM), but there is no influence of rearing conditions on body mass and no influence of sex on body fat. 35-day-old mice with access to high caloric food under thermoneutral conditions show an about 20% lower FFDM than animals under standard conditions. The body fat content of these mice is doubled and is even 150% higher in -/- females compared to those living on standard conditions (figure 2).



**Fig 2:** Body fat content analyzed by 3-way ANOVA (factors: rearing conditions, sex and genotype) least square means ± SEM.

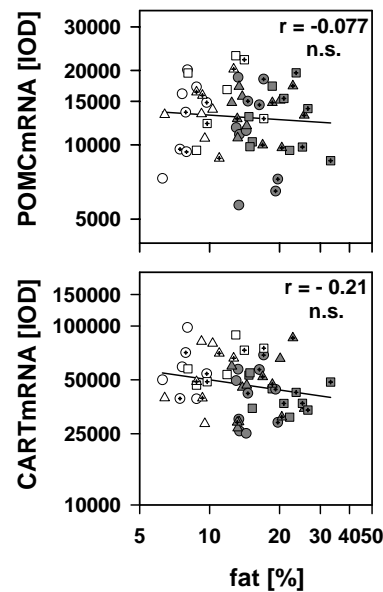
Like younger mice, 56-day-old animals with supplementary high caloric diet and exposure to 34°C exhibit a decrease in FFDM and an increase of body fat content. In +/- and +/- mice body fat content is about 200% higher in male and even 300% higher in female mice. MC4R-deficient -/- males show an increase in body fat content of only half of that developing in females. In our experiments the influence of the environment included both a change in food composition and a change in ambient temperature. Under thermoneutrality and access to high caloric diet the animals deposited excess fat but their motor activity was reduced and consequently muscle mass development was impaired.



**Fig 3:** Correlation of NPY and AGRP mRNA expression in the arcuate nucleus with body fat content. White symbols represent mice under standard conditions, grey symbols mice with access to supplementary high caloric food and thermoneutral ambient temperature; +/+ (circle), +/- (triangle), -/- (square) wild-type and MC4R-deficient mice. Female animals are marked with a cross.

Regression analysis of the expression levels of the orexigenic peptides NPY and AGRP in relation to body fat content are shown in figure 3. There were two experimental groups of 35-day-old wild-types and MC4R-deficient mice. One group had standard rodent diet at 22°C and the other group was fed supplementary high caloric food at 34°C. For each neuropeptide the slopes of the regressions for the animals on standard conditions and the animals with high caloric food at 34°C do not differ significantly. So the regressions are shown for both groups together. There are significant correlations between body fat content and both NPY and AGRP ( $P < 0.001$ ). Animals on standard conditions have a higher expression of orexigenic peptides.

The anorexigenic peptides POMC and CART do not show a significant correlation with body fat content (figure 4). Statistical analysis of the neuropeptide expression in relation to the rearing conditions with the Mann-Whitney rank sum test confirms that, with the exception of POMC expression, the differences between the mean values for the two rearing groups were significant. Using analysis of covariance (body fat content as covariate) for all four neuropeptides verified a significant difference between the two rearing conditions. Animals with supplementary high caloric food and thermoneutral ambient temperature show lower levels of NPY, AGRP, POMC and CART mRNA expression in the arcuate nucleus.



**Fig 4:** Correlation of POMC and CART mRNA expression in the arcuate nucleus with body fat content. White symbols represent mice under standard conditions, grey symbols mice with access to supplementary high caloric food and thermoneutral ambient temperature; +/+ (circle), +/- (triangle), -/- (square) wild-type and MC4R-deficient mice. Female animals are marked with a cross.

#### Outlook

Our results show that there is an influence of rearing conditions, sex and genotype on body composition of wild-types and MC4R-deficient mice. 35-day-old wild-types doubled and 56-day-old +/- females even quadrupled their body fat content under Western-style conditions as compared to standard conditions. We assume that in our mouse model primary genetic background effects are strongly modified by the environment. Additional experiments including in situ hybridization studies will help to separate the influence of food composition from that of ambient temperature. In future studies we will focus on NSCL-2 knockout mice, another model which is associated with the development of obesity.

*Lit.:* 1. Hinney A, Schmidt A, Nottebom K, Heibült O, Becker I, Ziegler A, Gerber G, Sina M, Görg T, Mayer H, Siegfried W, Fichter M, Remschmidt H, and Hebebrand J. Several mutations in the melanocortin-4 receptor gene including a nonsense and a frameshift mutation associated with dominantly inherited obesity in humans. *J Clin Endocrinol Metab* 84: 1482-1486, 1999. 2. Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, and Lee F. Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* 88: 131-141, 1997. 3. Weide K, Christ N, Moar KM, Arens J, Hinney A, Mercer JG, Eiden S, and Schmidt I. Hyperphagia, not hypometabolism, causes early onset obesity in melanocortin-4 receptor knockout mice. *Physiol Genomics* 13: 47-56, 2003. 4. Barsch GS, and Schwartz MW. Genetic approaches to studying energy balance: perception and integration. *Nature Reviews Genetics* 3: 589-600, 2002. 5. Arens J, Moar KM, Eiden S, Weide K, Schmidt I, Mercer JG, Simon E, and Korf H-W. Age-dependent hypothalamic expression of neuropeptides in wild-type and melanocortin-4 receptor-deficient mice. *Physiol Genomics* 16: 38-46, 2003.