

Network: Obesity and Related Disorders**Project: Identification of Natural Genetic Variation Linked to High Fatness in Selected Mouse Lines**Gudrun A. Brockmann - Humboldt Universität zu Berlin - gudrun.brockmann@agrar.hu-berlin.de**Introduction**

Our subproject is aimed at the identification of candidate genes affecting obesity in a polygenic mouse model. The focus on natural genetic variation for obesity in mice is based on the fact that many human obesity genes were originally identified in mutant mice. However, monogenic types of obesity are likely to account for only a small fraction of human obesity. Most genetically controlled types of obesity seem to be influenced by multiple gene loci. Each gene has only a small effect, but the interaction of many genes causes the extreme phenotypes (Brockmann and Bevova, 2002). In humans, the genetic analysis of such polygenic traits is difficult due to the effects of the environmental and genetic backgrounds. Long-term selected mouse lines and their inbred derivatives offer the possibility to identify individual genes and to elucidate the complex network of interacting genes of the selected trait under controlled environmental conditions. Depending on the genetic variability in the particular founder population, the selected mouse lines differ not only in their phenotypes but also in their specific set of genes and gene variants. Several different mouse lines from heterogeneous base populations have been selected for body weight or fat content (e. g. Bünger et al., 2001), for instance the lines LG/J, DU6, M16, NZO, KK, and F mouse lines. Crosses between inbred derivatives from diverse growth selected mouse lines have revealed many quantitative trait loci (QTLs) for body weight and obesity (Perusse et al., 2004). However, crosses of different line pairs revealed a diverse pattern of QTLs. Moreover, most of the used mouse lines were selected primarily for high body weight. High body weight consists not only of fat but also to a considerable proportion of lean mass. It is therefore very likely that many QTLs merely affecting fat accumulation are still unknown. Thus, the development of new animal models to identify genes responsible for common obesity is still important.

We have established three novel mouse models for obesity, the Berlin Fat Mouse Inbred (BFMI) lines BFMI856, BFMI860, and BFMI861, which are collectively termed BFMI lines in this report. The BFMI lines were derived from the selection line BFM, which had been selected for low body weight and high fatness for over 50 generations. For phenotypic characterization, animals of the BFMI lines and the unselected line C57BL/6 (B6), which served as a control, were systematically analysed between the age of three and 20 weeks. To describe the physiological distribution of fat in different depots related to obesity, body composition was measured applying the quantitative magnetic resonance (QMR) technique to live animals. Furthermore, body weight and feed intake in response to standard breeding and a high fat diet were recorded on a weekly basis.

Results/Project Status**Body weight, adiposity and growth rates**

We considered body weight, body fat weight and an adiposity index as indicators of obesity in mice. The body length and body lean weight are given as a general growth parameter. At the age of three weeks, there were no significant differences in body weight, body length, body fat and body lean mass between the animals within each line. Even at this age all three BFMI lines had significantly more body weight, body fat and body lean mass and were longer than B6. During the time of investigation, animals of the BFMI lines gained more weight and grew faster than B6. Likewise, the body fat content and the fat gain were significantly higher in the BFMI lines than in B6 animals

irrespective of the diet. For lean mass and gain of lean mass, differences were negligible when compared to the body fat mass, however all three BFMI lines had significantly more lean mass and a higher gain of lean mass than B6. Within BFMI mice, in lines BFMI856 and BFMI861 males gained more body weight than females and the males of these lines were also longer than females. Although the sexes did not differ in their body fat weight in either line, there were significant differences in the rate of fat gain between males and females in lines BFMI856 and BFMI861. Generally, males had higher lean masses than females, but the gain of lean mass was significantly higher in males than in females only in line BFMI856. The diet had no significant effect on body weight itself in either line over the whole period of analysis. However, males of line BFMI860 fed the high fat diet grew faster than males fed the standard breeding diet. In these mice the high fat diet led to longer body. At high fat diet mice of lines BFMI860 and BFMI861 became more obese, although this was only significant for BFMI860 males. No diet-specific differences of lean mass could be seen in all BFMI lines, but the gain of lean mass was affected by high fat diet in BFMI860 males.

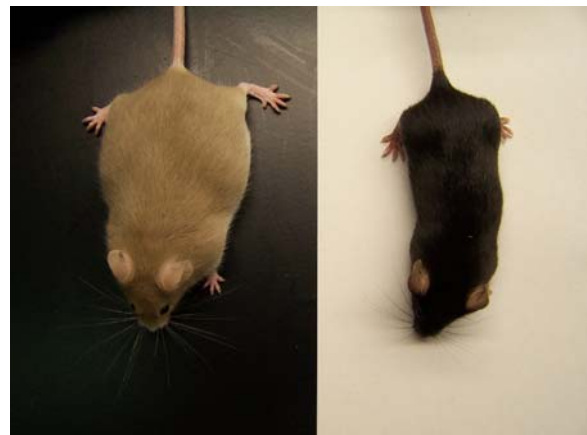


Fig 1: Female mice of the lines BFMI860 (left) and B6 (right) at the age of 12 weeks.

At the age of 20 weeks mice of the selected inbred lines were 1.5 - 1.8 times and 1.4 - 2.0 times heavier than B6 animals at standard breeding and high fat diet, respectively. The BFMI animals weighed 44 - 47 grams (males) and 34 - 41 grams (females) at standard breeding and 40 - 56 grams (males) and 34 - 46 grams (females) at high fat diet, with males being heavier than females within all BFMI lines, but, most distinctly in line BFMI856, where the difference between the sexes was 13 grams. In response to high fat diet, animals weighed the same (BFMI861) or more (BFMI860) than on standard breeding diet at the age of 20 weeks. At this age, mice of the BFMI lines had at standard breeding diet 3.4 - 6.5 times and at high fat diet 2.4 - 3.3 times more relative body fat (percentage of body weight) than B6 animals. Based on the QMR measurements the animals of the BFMI lines had a relative fat content of 22 - 30 % (males) and 28 - 37 % (females) at standard breeding and 30 - 34 % (males) and 31 - 40 % (females) at high fat diet, respectively. Females had more relative body fat than males in lines BFMI860 and BFMI861, and both sexes had an

increased fat content in response to high fat diet. Line BFMI856 was resistant to high fat diet and, moreover, males and females had the same fat percentages. Lean masses of mice of the selected inbred lines were only 10 - 50% higher than B6 animals irrespective of the diet, and males had more lean mass than females. In response to high fat diet, animals of line BFMI860 had more lean mass compared to animals at standard breeding diet at the age of 20 weeks. However, less lean mass at high fat diet compared to animals on standard breeding diet was found in both sexes of lines BFMI856 and BFMI861. This decrease was most distinct in males of BFMI856. This finding is consistent with the observation that 20 weeks old BFMI856 males had lower body weight but the same adiposity at high fat diet. However, none of these diet effects were statistically significant. The course of the adiposity index reflected mostly the variations of body fat content between and within the lines. However, the differences given by the adiposity index were more distinct than those of the fat content alone. Independent of age and sex, the adiposity index was higher for BFMI lines than for B6. In contrast to the body weight and the body fat mass, the analysis of the adiposity index revealed a sex effect in animals of line BFMI860, with females having a higher adiposity index than males. In lines BFMI856 and BFMI861, the adiposity index did not differ between the sexes, although males were heavier than females. The animals of these two BFMI lines showed no (BFMI856) or only a marginal (BFMI861) response to high fat diet. In line BFMI860 the response to high fat diet was much higher in male than in female mice and therefore only significant for males. The biggest difference in the adiposity index between animals on standard breeding and high fat diet was seen at about 12 weeks. The curves of the adiposity index converged towards the end of the experiment.

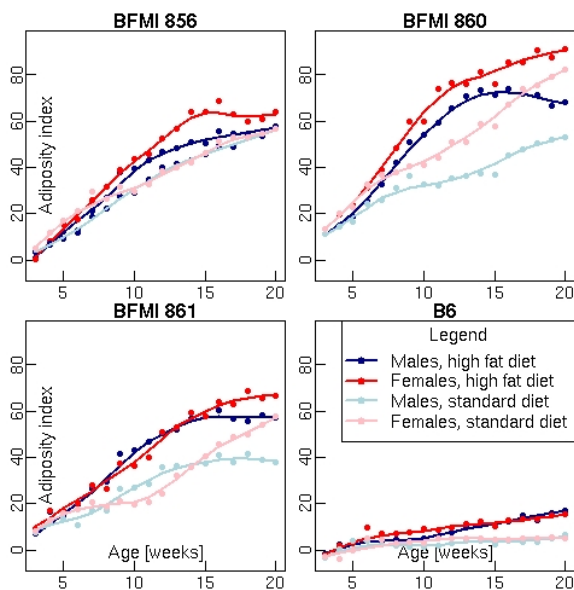


Fig 2: Adiposity index of BFMI lines and B6 from 3-20 weeks of age at standard breeding and high fat diet.

Fat distribution pattern

Under standard breeding diet conditions, the animals of the BFMI lines disposed of 5 - 13 times more total white fat tissue than B6 animals, when animals were dissected at the age of 20 weeks. The relative mass of all white fat tissues to body weight from animals on standard breeding diet were about 16% (BFMI856 and BFMI860) and 11% (BFMI861) in males and about 17% (BFMI856 and BFMI861) and 23% (BFMI860) in females. B6 mice had about 3% relative total white fat tissue in both sexes. The differences in total white fat masses between selected inbred lines and B6 were highly significant. The pattern of total white fat tissue within each

line was similar to that obtained by QMR measurements in mice at the age of 20 weeks, even though the absolute fat content was higher according to QMR determination. Significant sex or diet effects could not be detected within each BFMI line.

The distribution of fat accumulation in the different fat depots showed that the reproductive fat pad was the heaviest fat depot in the selected inbred lines and likely served as main fat depot. As a result of high fat diet, the additional fat was merely deposited in subcutaneous fat tissue.

Feed intake and feed efficiency

Feed intake within each line diet did not vary much over 13 weeks of the experiment (week eight to 20). Within this period, animals of the BFMI lines consumed more food and correspondingly more energy than B6 mice fed the standard breeding diet. All BFMI lines showed sex-specific differences in food and energy intake with males consuming more than females in lines BFMI856 and BFMI860 and with females eating more than males in line BFMI861. At high fat diet food intake was increased in males and females of line BFMI860, in females of lines BFMI856 and B6, and in males of line BFMI861, which developed hyperphagia. The other animals (males of lines BFMI856 and B6, females of line BFMI861) consumed the same amount of food irrespective of the diet. As a consequence, energy intake was increased in all mouse lines under high fat diet conditions.

Recording fat gain weekly permitted us to calculate the ratio of fat gain per energy intake over the time. This factor showed that mice of the selected inbred lines, which were fed the standard breeding diet, had 5.1 - 18 times, and those fed the high fat diet 1.8 - 6.8 times higher average efficiency in fat deposition than B6 mice. However, the average feed efficiency, which refers to body weight gain per consumed kilojoules, were only about 1.1 - 3.5 times higher in BFMI lines than in B6.

Outlook

The BFMI lines had more body weight, body fat, and lean mass than the control line B6 independent of age, sex, and diet. Within the BFMI lines, the line BFMI860 exhibited the highest adiposity index and the strongest response to high fat diet. Differences in obesity related phenotypes despite same living environment ensure that their differences can be traced back to different sets of fixed alleles contributing to fat accumulation. Between the BFMI lines, presumably, minute alterations in the genome are responsible for the phenotypic differences. Given that BFMI mice and their obesity phenotypes are of polygenic nature, the BFMI lines are excellent models for the study of obesity in humans.

Crosses of the BFMI lines with other lines (e.g. B6) make it possible to identify quantitative trait loci (QTLs) affecting obesity. The combination of obesity related QTLs with differentially gene expression analysis will lead to new candidate genes for obesity. At present, an F2-population for QTL analysis is being set up by crossing the lines BFMI860 and B6 and mice for differential gene expression experiments are being generated. Sequencing of the obtained candidate genes will reveal candidate SNPs in these genes. Functional analyses of identified candidate genes harbouring genetic variants will be performed and analysed bioinformatically. The identified murine candidate genes/polymorphisms will be provided for further analyses in human populations.

Lit.: 1. Brockmann GA and Bevova MR. Using mouse models to dissect the genetics of obesity. Trends Genet. 2002 Jul; 18(7): 367-376. 2. Büniger L et al. Inbred lines of mice derived from long-term growth selected lines: unique resources for mapping growth genes. Mamm Genome. 2001 Sep; 12(9): 678-686. 3. Perusse L et al. The human obesity gene map: the 2004 update. Obes Res. 2005 Mar; 13(3): 381-490.