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C57BL/6JRj mice are protected against diet induced obesity (DIO)

Matthias Kern [a], Anja Knigge [a], John T. Heiker [a], Joanna Kosacka [a], Michael Stumvoll [a], Peter Kovacs [b], Matthias Blüher [a,c], Nora Klöting [a,d,*]

[a] Department of Medicine, Endocrinology/Diabetes, University of Leipzig, Germany
[b] Interdisciplinary Center for Clinical Research, University of Leipzig, Germany
[c] IFB AdiposityDisease, University of Leipzig, Germany
[d] IFB AdiposityDisease, Junior Research Group 2 "Animal models of obesity", University of Leipzig, Germany

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ABSTRACT

The C57BL/6 (B6) is one of the oldest inbred mouse strains. It has been widely used as control strain in metabolism research for many decades. Preliminary data from our lab indicated that C57BL/6JRj mice are not responding to diet induced obesity. Therefore, the aim of this study was to compare the two different B6 substrains, C57BL/6NTac and C57BL/6JRj, in regard to their response to diet induced obesity (DIO) and to investigate genetic differences which may explain such phenotypic differences. Sixteen male mice of C57BL/6NTac and C57BL/6JRj were fed a high fat diet (HFD) or standard chow diet (SD) for 10 weeks. Phenotypic characterization included measurements of bodyweight, physical activity, food intake and relative epigonadal fat mass. Genetic differences between both substrains were analyzed using a panel of 1449 single nucleotide polymorphism (SNP) markers. Our study revealed that C57BL/6JRj mice are protected against DIO independently from food intake and physical activity. Genetic SNP analysis among C57BL/6 mice identified genetic differences in at least 11 SNPs. Our data strongly support the importance of attention on the genetic background in obesity research.

1. Introduction

Experimental animal models offer a great opportunity to overcome genetic heterogeneity and various environmental factors influencing obesity and associated disorders. The C57BL/6 (B6) is one of the oldest inbred mouse strains. This strain is widely used as a control strain in metabolism research and serves as a background strain for spontaneous and induced mutant mice [1–3].

The C57BL strain was established by C.C. Little in the 1920s. In the mid 1930s the C57BL/6 strain was separated from the C57BL parent strain along with C57BL/10. More than nine C57BL/6 substrains were generated during the 1970s [4,5]. Substrains are defined as branches of an inbreed strain that are either known or suspected to be genetically different from the original inbred strain due to any of the following rules: (i) branches of a strain which were separated after 20 but before 40 generations of inbreeding, (ii) branches separated by more than 20 generations from a common ancestor, (iii) genetic differences between branches [6]. C57BL/6J of The Jackson Laboratory and C57BL/6N of the National Institutes of Health (NIH) are core substrains of C57BL/6 that were developed from the ancestral C57BL/6 line during the 1940s and 1950s [6–8]. C57BL/6JRj mice were transferred from Centre de Service des Animaux de Laboratoire (Orleans, France) to Janvier at F172 in 1993, according to the Janvier product catalog.

Preliminary data from our lab in a study on weight cycling [9] indicated that C57BL/6JRj mice did not respond to diet induced obesity. Phenotypic differences among C57BL/6 substrains have been reported with respect to behavior [10–12], glucose and insulin tolerance [13] as well as responsiveness to alcohol [14] and drugs [2–3,15]. Furthermore, genetic differences among C57BL/6 substrains have been investigated [1,2]. However, to our knowledge no study has combined both approaches to characterize genetic and phenotypic differences between C57BL/6 substrains, C57BL/6NTac and C57BL/6JRj.

Here, we demonstrate that the core substrains of C57BL/6, C57BL/6NTac and C57BL/6JRj, feature a remarkably divergent responsiveness to high fat diet induced obesity. We have further identified several SNPs between both C57BL/6 strains. These data provide a useful guide for biomedical researchers in selecting an appropriate substrain for metabolic studies and points out the relevance of the genetic background in engineering of experimental models such as transgenic mice.

* Corresponding author at: University of Leipzig, IFB AdiposityDisease, Junior Research Group 2, Liebigstr. 21, D-04103 Leipzig, Germany. Fax: +49 341 9713400.
E-mail address: nora.kloeting@medizin.uni-leipzig.de (N. Klöting).

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2. Methods

2.1. Animals

In 2007 breeding pairs from Taconic Farms, Inc. (C57BL/6NTac; Hudson, New York, USA, F198) and from Janvier (C57BL/6JRj; Le Genest Saint Isle, France, F142) were obtained and bred since that time in our own animal facility under standardized environmental conditions. Mice of both strains at the 11th inbred (F11) generation were used for these experiments. Animals were kept in groups of four in Macrolon cages (Size 2, Ehret, Emmendingen, Germany) under strict hygienic conditions. They had free access to food and water and were maintained at 12 h light and dark cycle (5 AM/5 PM).

All experiments were conform to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996) and were approved by the local authorities of the state of Saxony, Germany as recommended by the responsible local animal ethics review board.

2.2. Phenotyping

Eight male mice of each strain and diet group were fed a high fat diet with 40% kcal from fat (C1057, Altromin, Lage, Germany) or standard chow diet (Ssniff, Soest, Germany) with 9% kcal from fat for 10 weeks beginning at the age of 8 weeks. Bodyweight was recorded weekly, food intake and physical activity were measured for 5 days in week 16. At the end of the observation period relative visceral fat mass was analyzed.

2.3. Physical activity

We investigated wheel-running activity of both mouse strains under different diets to gain insight into physical effort. Mice of each strain were housed for 5 days in cages containing activity wheels (Intellibio, Nomeny, France). Wheels were connected to a system that used magnetic sensor to record wheel rotations interfaced with a PC. The mice had free access to the wheel and the following parameters were recorded: speed, acceleration (average and maximum), and distance (mean/run and over 24 h period).

2.4. Single nucleotide polymorphism (SNP) genotyping

Genomic DNA was extracted from tail tips using the DNeasy kit (Qiagen, Hilden, Germany). Genotyping of 1.449 SNP loci covering the genome was carried out using Golden Gate assay (Illumina Inc., San Diego, California, USA) [1]. SNP genotyping was performed by Taconic, Albany-Molecular Analysis Laboratory, New York, USA. 129S1/Sv, Balb/cJ and F1 cross between C57BL/6NTac and C57BL/6JRj served as control strains.

2.5. Data analysis and statistics

Data are given as means ± SD. Datasets were analyzed for statistical significance using a two-tailed unpaired *t* test, or differences were assessed by one-way ANOVA using the Statistical Package for Social Science, version 18.0 (SPSS, Chicago, IL). *p*-values <0.05 were considered significant.

3. Results

3.1. Phenotypes

As shown in Fig. 1A C57BL/6JRj mice are protected against diet induced obesity whereas C57BL/6NTac mice exhibit a strong DIO

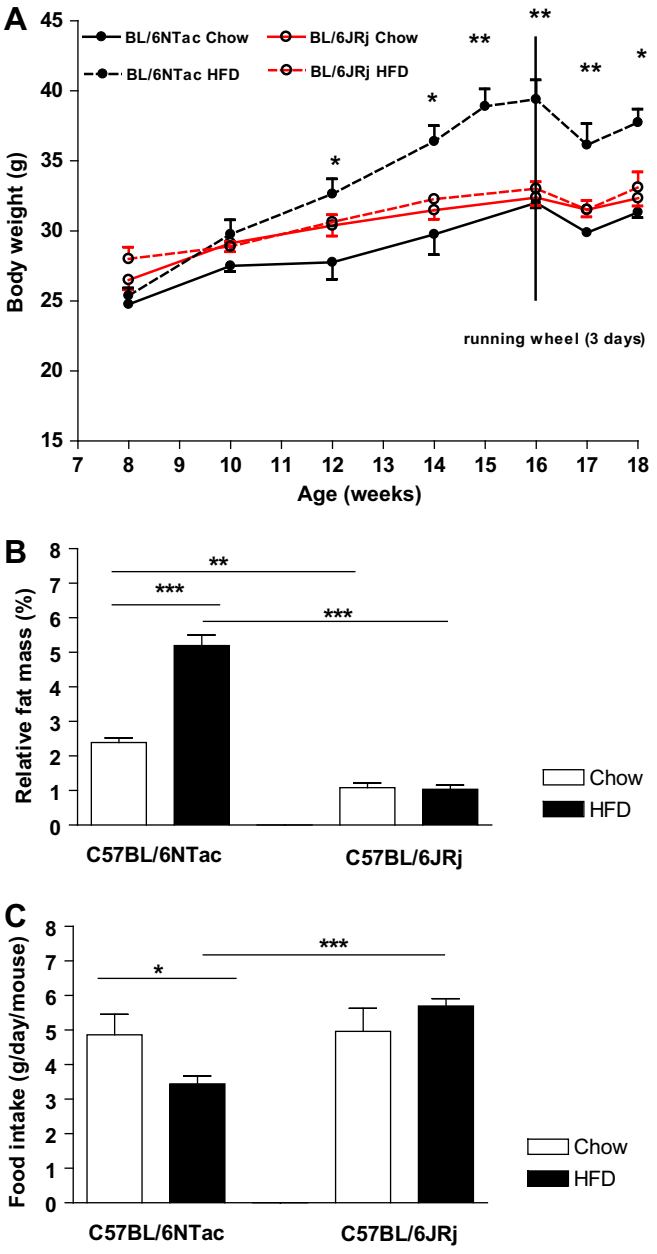

Fig. 1. Phenotype. Body weight gain (A), relative epigonadal fat mass (B), and food intake (C) of C57BL/6JRj and C57BL/6NTac mice under chow and HFD conditions. Significantly different at *0.05, **0.01, and ***0.001 *p*-value level.

responsiveness under HFD. In contrast, body weight gain of the C57BL/6JRj strain was comparable between standard and HFD groups. Standard chow fed C57BL/6NTac mice already featured a significantly higher relative epigondadal fat mass compared to the C57BL/6JRj mice and relative fat mass was significantly increased only in C57BL/6NTac under high fat diet conditions (Fig. 1B). Under HFD C57BL/6NTac mice showed reduced daily food intake by about 40% whereas food intake of C57BL/6JRj was indifferent of the diet (Fig. 1C). Physical activity, measured as running distance, duration and average speed and acceleration per day, was not different between both strains and diet conditions (Table 1).

3.2. Genetics

SNP genotyping was carried out for 1.449 SNP loci. 1430 (98.7%) targeted loci were successfully genotyped. As shown in Table 2, 11