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# Body fat distribution and organ weights of 14 common strains and a 22-strain consomic panel of rats

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ABSTRACT

The goal of this study was to determine the adiposity of a range of rat strains, including a panel of consomics, to estimate heritability. To that end, we assessed the body fat distribution and organ weights of groups of adult male rats from 3 outbred strains, 11 inbred strains and 22 consomic strains. We measured the weights of the gonadal, retroperitoneal, mesenteric, femoral, subscapular and pericardial white fat depots, the subscapular brown fat depot, the kidneys, liver, heart, spleen, and brain. Strains were compared for the measured weight of each of these adipose depots and organs, and also for these weights adjusted statistically for body size. All individual adipose depot and organ weights were highly heritable, in most cases $h^2 > 0.50$. The fourteen inbred and outbred rat strains were not very different in body length but there was a three-fold difference in body weight, and up to a twenty-fold difference in the weight of some adipose depots. Comparison of the FHH-Chr $n^{BN}$ consomic strains with the FHH host strain revealed 98 quantitative trait loci (QTLs) for body composition and organ weight, with the introgressed chromosome reducing weight or adiposity in most cases. These results can be used to guide the choice of appropriate rat strains for future studies of the genetic architecture of obesity and body size.



Obesity is determined by the amount of lipid stored in adipocytes, which aggregate with other cell types to form adipose depots, sometimes called fat pads. There are five large depots in the rat: the gonadal, retroperitoneal, subscapular, inguinal and mesenteric, as well as several smaller ones (e.g., pericardial) [1]. The gonadal, retroperitoneal and mesenteric depots are associated with the viscera of the abdominal cavity whereas the inguinal and subscapular depots are subcutaneous. In humans, there are well-described metabolic consequences, such as increased risk of high blood pressure and diabetes, related to storing fat in the visceral versus subcutaneous adipose depots [2]. Thus, investigators are interested in the mechanisms whereby increased abdominal (visceral) obesity leads to the metabolic syndrome. The pattern of gene expression differs by adipose depot location but how and why particular depots ultimately differ in weight, or how they might change whole-body metabolism, is unclear [3,4]. However, one contributor to differences in fat pad weight is genotype [5]. Thus a genetic approach to understand the controls of adipose depot weight will be useful, especially in the rat, which is a well-characterized model for the study of nutrition and metabolism [6].

The purpose of this study is twofold. In *Experiment 1*, we wanted to obtain comprehensive information about the distribution of body fat in rats of different strains. To this end, we used rats from 3 outbred and 11 inbred strains. We measured the weights of six depots of white fat (gonadal, retroperitoneal, mesenteric, femoral, subscapular and pericardial) and subscapular brown fat. We also weighed the kidneys, liver, spleen, and brain. In *Experiment 2*, we wanted to begin to understand the underlying genetic effects on body composition. To this end, we measured the same traits in a panel of consomic (chromosome substitution) rat strains. Comparison of strains with a substituted chromosome with control rats allowed us to determine whether genes and their alleles on a particular chromosome change fatness.

## 1. Methods

### 1.1. Choice of strains

Over 1000 rat strains have been bred for research, of which about half are inbred [7,8]. Several considerations went into the choice of the strains used here. In *Experiment 1*, we measured three outbred strains that have been used extensively in rat obesity research [9–11], although rarely compared [12] (SD, LE, and WI; see Table 1 for full strain names and abbreviations). The long-term goal of this work is to conduct genetic analyses, and so when we began this study in

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**Table 1**
List of rat strain names and abbreviations used in this study.

| Short abbreviation | Full strain abbreviation | Strain name |
|---|---|---|
| SD$^{o}$ | Crl:CD(SD) | Sprague Dawley CD (IGS) |
| LE$^{o}$ | Crl:LE | Long Evans |
| WI$^{o}$ | Crl:WI | Wistar |
| BN | BN/SsNHsdMcwiCrl | Brown Norway |
| BUF | BUF/CrCrl | Buffalo |
| COP | COP/CrCrl | Copenhagen |
| DA | DA/OlaHsd | Dark Agouti |
| Dahl | Dahl SS/JrHsdMcwi | Dahl-S (salt-sensitive) |
| F344 | F344/NTac | Fischer 344 |
| FHH | FHH/EurMcwi(Crl) | Fawn Hooded Hypertensive |
| LEW | LEW/SsNHsd | Lewis |
| Nob | Noble/CrCrl | Noble |
| PVG | PVG/OlaHsd | Piebald Virol Glaxo |
| SHR | SHR/NCrl | Spontaneously Hypertensive |
| FHH-Chr n$^{BN}$ | FHH/EurMcwi-Chr n$^{BN/SsNHsdMcwi}$ | FHH-BN consomic (on chromosome n) |

$^{o}$ = outbred strain. Vendor: Crl = Charles River, www.criver.com; Tac = Taconic, www.taconic.com; Hsd = Harlan, www.harlan.com. For Crl:CD(SD), CD = Cesarean-derived, IGS = International Genetic Standard system of breeding. The FHH-Chr n$^{BN}$/Mcwi strain set used here involves chromosomes from the BN/SsNHsdMcwi (BN) strain introgressed onto the FHH/EurMcwi (FHH) background. "Chr n" refers to any of 22 chromosomes (1–20, X or Y).

December 2005, we chose to test seven commercially-available inbred strains that formed the basis of a panel of single nucleotide polymorphisms (SNPs) [13] (DA, Dahl, F344, FHH, LEW, PVG, and SHR). We also wanted to include maximum genetic diversity in order to capture the widest range of phenotypes. To do this, we consulted a rat phylogenetic tree based on microsatellite markers [14], which led us to include four additional inbred strains (BN, BUF, COP, and Nob), with also the consideration that these were commercially available. Later, we obtained 22 strains of FHH-Chr n$^{BN}$/Mcwi consomic rats (including the BN and FHH progenitor strains), which we measured in a separate experiment (*Experiment 2*). Consomic strains are useful because differences in phenotype between a consomic and host strain can be attributed to genes in the introgressed chromosome (for reviews, see [15,16]). In our case, each of the 20 autosomes and the X and Y chromosomes from the BN/SsNHsdMcwi (BN) strain have been introgressed onto the FHH/EurMcwi (FHH) background. These strains were developed by Jacob and colleagues at the Medical College of Wisconsin [17], who have collected basic biochemical, cardiac, vascular, histological and renal data from members of the FHH-Chr n$^{BN}$ consomic set [18], but there have been no previous reports to our knowledge of body fat distribution.

Measurements were made on male rats because focusing on one sex has the advantage of simplifying genetic analyses. We are aware that body composition differs between male and female rats, and thus conclusions drawn about genetic architecture in one sex may not pertain to the other [19].

### 1.2. Maintenance

The experiment protocols were approved by the Animal Care and Use Committee of the Monell Chemical Senses Center. Rats were received at Monell when they were 47–59 days old and measured for some behavioral traits shortly thereafter [20,21]. All the rats were housed in the same vivarium, with an ambient temperature of 23 °C and fluorescent illumination between 0600 and 1800 h. Each rat was housed alone in a 25×18×19 cm hanging cage, with stainless steel back and side walls and a mesh front wall and floor. Powdered AIN-76A diet was available from a 4-oz glass jar that was attached with a stainless-steel spring to the front wall. AIN-76A is a semisynthetic diet containing by weight: 20% protein (casein), 65% carbohydrate (sucrose and cornstarch), 5% fat (corn oil), and 10% fiber (cellulose), minerals and vitamins. It has an energy content of ~15.9 kJ/g. The diet was purchased from Dyets Inc (Bethlehem, PA; catalog no. 100000; [22]). Deionized water was available from a 300-mL glass bottle equipped with a neoprene stopper and a stainless steel sipper tube. Food and water were always available except for brief periods when cups or bottles were replenished. Cardboard sheets under the rats' cages collected excrement and spillage, and were changed frequently.

### 1.3. Necropsy procedure

Rats in *Experiment 1* were 330–370 days old and those in *Experiment 2* were 123–162 days old when they were killed by injection of pentobarbital sodium and phenytoin sodium, and weighed (±0.1 g; these body weight data have been reported in publications focused on the behavioral test results [20,21]). Rats from Experiments 1 and 2 were of different ages when necropsied because those from Experiment 1 underwent more days of behavioral testing [20,21]. We used a ruler to measure body length, which we considered to be the distance between the bottom of the lower incisors to the anus. All organs were removed and weighed (±0.1 g). Landmarks for the gonadal, perirenal, retroperitoneal, mesenteric, femoral (inguinal), pericardial and subscapular (intrascapular) depots were based on dissection guidelines in the mouse [1]. Brown fat from the subscapular region was separated from white fat and weighed. Visceral fat weight was defined as the summed weight of intra-abdominal fat depots, i.e., gonadal, retroperitoneal and mesenteric. Total fat weight was the sum of the visceral fat weight plus the inguinal, subscapular and pericardial fat depot weights. For bilateral organs like the gonadal adipose depot and kidneys, both left and right organs were removed and weighed. The brain was transected at the brain stem distal to the cerebellum. Olfactory bulbs were removed and weighed with the whole brain.

### 1.4. Dependent variables and data analysis

Obesity depends on the proportion of fat mass relative to overall body size. We used two methods to assess this: (1) percent fat, and (2) standardized fat mass. Percent fat is the ratio of fat weight/body weight, and is frequently used as a measure of obesity in rodents, although it has limitations [23]. Standardized fat mass is fat mass computed after the variance associated with body size is removed [24] and, although more difficult to calculate, is more preferred from a statistical standpoint. We computed these two variables separately for visceral as well as total fat. Like fat mass, organ weight is usually considered relative to body size. Therefore, for individual organ weights including adipose depots, standardized values were obtained by adjusting for body size as described above.

Statistical analysis differed for the two experiments. For *Experiment 1*, differences among strains were evaluated using an ANOVA (body weight, body length, and percent fat), or a general linear model with body weight and length as covariates (fat mass, visceral fat mass, and individual organ weights), followed by Fisher's LSD post hoc tests to determine the pattern of strain differences. For *Experiment 2*, the parental strains and all consomic strains were compared by ANOVA or general linear models, as described above, but each consomic strain was then compared to the FHH host strain with post hoc tests. Significant differences between a consomic strain and the FHH strain were interpreted to mean that one or more QTL was present on that chromosome [25].

To determine the degree of genetic influence on the traits studied, the ratio of the between-strain sum of squares to the total sum of squares obtained from these analyses was used to estimate heritability ($h^2$) in the narrow sense [26]. These calculations were conducted separately using unadjusted and standardized values. The rationale for this procedure was that the unadjusted measures are most commonly used and thus allow comparison with other estimates, and the standardized measures provide information about the heritability