Physiology & Behavior 135 (2014) 49–54

Contents lists available at ScienceDirect

Physiology & Behavior

journal homepage: www.elsevier.com/locate/phb

Macronutrient selection by seven inbred mouse strains and three taste-related knockout strains

Michael G. Tordoff *, Arnelle Downing, Anna Voznesenskaya

Monell Chemical Senses Center, Philadelphia, PA 19194, USA

HIGHLIGHTS

- Mice were offered a choice between sources of carbohydrate and fat
- Inbred strains differed, ranging from strong carbohydrate-likers to strong fat-likers
- Mice lacking T1R3 and their controls did not differ in their carbohydrate and fat choices
- Mice lacking ITPR3 or CALHM1 consumed more fat and less carbohydrate than did their controls

ARTICLE INFO

Article history:
Received 21 March 2014
Accepted 28 May 2014
Available online 6 June 2014

Keywords:
Mouse strain survey
Food choice
Gustation
Fat preference

ABSTRACT

Many animals thrive when given a choice of separate sources of macronutrients. How they do this is unknown. Here, we report some studies comparing the spontaneous choices between carbohydrate- and fat-containing food sources of seven inbred mouse strains (B6, BTBR, CBA, JF1, NZW, PWD and PWK) and three mouse models with genetic ablation of taste transduction components (T1R3, ITPR3 and CALHM1). For 8 days, each mouse could choose between sources of carbohydrate (CHO-P; sucrose-cornstarch) and fat (Fat-P; vegetable shortening) with each source also containing protein (casein). We found that the B6 and PWK strains markedly preferred the CHO-P diet to the Fat-P diet, the BTBR and JF1 strains markedly preferred the Fat-P diet to the CHO-P diet, and the CBA, NZW and PWD strains showed equal intakes of the two diets (by weight). Relative to their WT littermates, ITPR3 and CALHM1 KO mice had elevated Fat-P preferences but T1R3 KO mice did not. There were differences among strains in adaption to the diet choice and there were differences in response between males and females on some days. These results demonstrate the diverse responses to macronutrients of inbred mice and they point to the involvement of chemosensory detectors (but not sweetness) as contributors to macronutrient selection.

1. Introduction

Animals thrive when given appropriate choices of macronutrients to select [1]. How they do this is unknown. There have been several attempts to understand the physiological controls underlying macronutrient selection (e.g., [2–7]) and to assess environmental and behavioral contributions (e.g., [3,8]; reviews [7,9,10]), but there has been very little effort to characterize the genetic controls. Smith Richards and colleagues found diverse preferences among 13 strains of mice allowed to choose from separate sources of protein, carbohydrate and fat [11]. Based on a B6 × CAST F_2 intercross, they discovered three chromosomal regions linked to macronutrient preference [12] and characterized one, a locus on chromosome 17, in detail [12–15]. We demonstrated that a 21-gene region of chromosome 17 influenced macronutrient selection [16] and hypothesized that the causative gene was *Itpr3*, a component of the canonical G protein-coupled receptor (GPCR) taste transduction cascade.

Here, we used the two-cup choice method developed by Smith Richards et al. [11] to investigate the selection of carbohydrate and fat by 7 mouse strains. We also took advantage of three knockout (KO) mouse models, involving genetic ablation of T1R3, ITPR3, and CALHM1, to investigate the contribution of these taste transduction elements to macronutrient choice.

2. Methods

We conducted four experiments, which all used the same general test procedures. One experiment compared 7 inbred mouse strains; each of the other three compared mice with a genetic ablation of a taste-related gene to their wild-type (WT) controls. All procedures

* Corresponding author at: Monell Chemical Senses Center, 3500 Market St., Philadelphia, PA 19104, USA. Tel.: +1 267 280 3247.
E-mail address: tordoff@monell.org (M.G. Tordoff).

http://dx.doi.org/10.1016/j.physbeh.2014.05.039
0031-9384/

were approved by the Monell Chemical Senses Center Animal Care and Use Committee.

2.1. Subjects

The largest experiment compared 7 inbred mouse strains, with the following names, abbreviations, and Jackson Lab stock numbers: C57BL/6J (B6; 000664), BTBR *T + Itpr3*^{tf}/J (BTBR; 002282), CBA/J (CBA), JF1/Ms (JF1; 003720), NZW/LacJ (NZW; 001058), PWD/PhJ (PWD; 004660), PWK/PhJ (PWK; 003715). The mice were the progeny of founders obtained from The Jackson Laboratory (JAX). The JF1 mice originated from stock provided to us in 2001 (12 years ago; before access to these mice was restricted) so they have had many generations to diverge from the JF1/Ms strain and are best considered as a distinct strain, JF1/MsMon. The other mice tested were 1–3 generations descendant from the purchased founders, which allowed little opportunity for genetic drift. The strains were chosen for study because they have wide phylogenetic diversity [17,18] and were conveniently available in our laboratory because of our interest in calcium taste. The BTBR, JF1, PWD and PWK strains have anomalously high preferences for calcium [relative to 37 other strains [19]; see Mouse Phenome Project [20]]; the B6, CBA and NZW strains avoid calcium. We have tested the macronutrient preferences of the BTBR and NZW strains previously [16]; Smith Richards and colleagues have previously tested B6 mice and another 12 strains [11–13], which were not tested here.

We conducted three experiments involving mice with genetic ablation of the taste-related genes, *Tas1r3*, *Itpr3*, and *Calhm1*. The T1R3 KO mice were provided to us in 2007 by Dr. R. Margolskee (now at Monell), and the ITPR3 KO mice were made by us in 2012 according to procedures described elsewhere [21]. The CALHM1 KO mice were from stock provided in 2013 by Dr. Kevin Foskett (University of Pennsylvania) which, in turn, were from stock generated by Dr. Philippe Marambaud (Feinstein Institute for Medical Research; [22,23]). Each KO mouse line was backcrossed to the B6 strain for several generations and then maintained by crossing heterozygotes (+/−) so that homozygous wild-type (WT; +/+) and knockout (KO; −/−) mice could be obtained from the same litter. Genotypes were determined by a commercial assay service (Transnetyx, Inc). Heterozygotes were not tested (they were needed to breed mice for other experiments).

2.2. Test procedures

The mice were housed in a vivarium maintained at 23 °C with a 12:12 h light/dark cycle (lights off at 1900 h). They were weaned at 21–23 days and raised in groups of the same sex until ~7 days before testing, when they were individually housed in plastic "tub" cages (dimensions, 26.5 × 17 × 12 cm) with 5–10 mm pine shavings on the floor. Deionized water was available to drink from a 300-mL glass bottle with a stainless steel sipper tube, and the maintenance diet, pelleted AIN-76A (Dyets Inc, Bethlehem, PA; catalog no. 100000) was available to eat from a hopper built into the cage lid.

At the start of the 8-day test, each mouse was housed in a new cage with two glass jars (30-mL capacity; Fisherbrand, catalog no. 02-911-912) holding the diets described in Table 1. The distinctive feature of the CHO-P diet was that it contained corn starch and powdered sucrose, whereas the Fat-P diet contained vegetable shortening. Both diets contained casein (protein), minerals and vitamins. The diets were placed in the cage, with the CHO-P diet on the left and the Fat-P diet on the right. To prevent the two jars being knocked over, each was held upright in the center of a 3" diameter acrylic disk (U.S. Plastics Corp., catalog no. 44185) by three clear 8–32" × 7/8" screw fasteners (U.S. Plastics Corp., catalog no. 32016). Food spillage using these jars was minimal but any spillage was easily collected from the acrylic disk and was accounted for. In addition, the cage had a corrugated cardboard sheet on the floor (instead of pine shavings) to allow detection and collection of any far-flung spillage. Every 24 h, the food jars and spillage were weighed with 0.1-g precision and the positions of the two jars were switched. In order to maintain freshness, the Fat-P diet was replaced every other day and refilled on alternate days; the CHO-P diet was refilled as needed. Body weights were measured daily to the nearest 0.1 g.

Table 1
Composition of carbohydrate-and-protein (CHO-P) and fat-and-protein (Fat-P) diets given as a choice to mice.

Ingredient	CHO-P diet	Fat-P diet
Casein, g/kg	198.8	327.7
DL-Methionine, g/kg	2.9	4.9
Sucrose, powdered, g/kg	212.4	0.0
Cornstarch, g/kg	496.2	0.0
Primex (vegetable shortening), g/kg	0.0	519.3
Cellulose, g/kg	49.2	76.2
AIN-76A Mineral Mix #200000, g/kg	32.0	53.3
AIN-76A Vitamin Mix #300050, g/kg	10.0	15.3
Choline chloride, g/kg	1.8	3.1
Energy content, kcal/g	3.41	5.95
% Protein	20.8	19.7
% CHO	79.2	1.8
% Fat	0.0	78.5

Notes: Values are in g/kg diet. The small percentage of CHO in the Fat-P diet derives from sucrose used as the diluent for the DL-methionine, mineral and vitamin mixes. The diets were prepared by Dyets Inc, Bethlehem, PA (catalog nos. 103259 and 103260).

Because of limited equipment and difficulty breeding the mice, experiments were conducted in replications of 20–24 mice. The mice had a wide age range (albeit all adult; Table 2) but care was taken to test cohorts of the same age and, for the KO experiments, WT and KO mice from the same litters. Table 2 summarizes the number, sex, age and body weight of the mice.

2.3. Data analysis

The primary measures of each experiment were daily intakes of CHO-P diet and Fat-P diet. Preference for the Fat-P diet was derived in two ways: Preferences by weight were calculated as the ratio of Fat-P intake (in g) divided by total intake [in g; i.e., Fat-P intake/(Fat-P intake + CHO-P intake) × 100]. Preferences by energy were calculated using the same formula after weights (in g) were converted to kilocalories based on an energy density of 3.41 kcal/g for the CHO-P diet and 5.95 kcal/g for the Fat-P diet.

Intakes of each diet (in g), total intakes (in kcal), and Fat-P diet preferences were analyzed using mixed-design ANOVAs with factors of Group (i.e., strain or genotype), Sex and Day. Post hoc LSD tests were used to compare intakes of the groups on individual days when an interaction term was significant. The criterion for statistical significance was $p < 0.01$.

Table 2
Summary of number, age, and weight of mice tested.

	Males			Females		
Strain	*n*	Age range, days	Weight, g	*n*	Age range, days	Weight, g
B6	9	80–114	33 ± 1	9	106	20 ± 1
BTBR	11	97–128	39 ± 1	11	87–182	30 ± 1
CBA	8	90–101	27 ± 1	8	81–139	21 ± 1
JF1	9	62–172	23 ± 2	8	63–105	13 ± 0
NZW	11	72	30 ± 0	12	62	25 ± 1
PWD	8	192	20 ± 0	10	91–147	14 ± 0
PWK	8	62–164	16 ± 1	10	77–100	13 ± 0
T1R3 WT	8	51–58	23 ± 1	5	51–58	18 ± 1
T1R3 KO	5	51–58	19 ± 2	7	51–58	21 ± 2
ITPR3 WT	8	68–113	25 ± 1	19	68–194	24 ± 1
ITPR3 KO	8	83–99	28 ± 2	19	74–194	23 ± 1
CALHM1 WT	6	58–116	21 ± 1	8	58–116	19 ± 1
CALHM1 KO	6	58	23 ± 0	4	58–116	20 ± 1

Notes: age range gives youngest–oldest (in days); when only one value is given all mice were the same age.