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QTL analysis of self-selected macronutrient diet intake: fat, carbohydrate, and total kilocalories

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Richards, Brenda K. Smith, Brenda N. Belton, Angela C. Poole, James J. Mancuso, Gary A. Churchill, R. Li, Julia Volaufova, Aamir Zuberi, and Barbara York. QTL analysis of self-selected macronutrient diet intake: fat, carbohydrate, and total kilocalories. *Physiol Genomics* 11: 205–217, 2002. First published September 17, 2002; 10.1152/physiolgenomics.00037.2002.—The present study investigated the inheritance of dietary fat, carbohydrate, and kilocalorie intake traits in an F_2 population derived from an intercross between C57BL/6J (fat-preferring) and CAST/EiJ (carbohydrate-preferring) mice. Mice were phenotyped for self-selected food intake in a paradigm which provided for 10 days a choice between two macronutrient diets containing 78/22% of energy as a composite of either fat/protein or carbohydrate/protein. Quantitative trait locus (QTL) analysis identified six significant loci for macronutrient intake: three for fat intake on chromosomes (Chrs) 8 (*Mnif1*), 18 (*Mnif2*), and X (*Mnif3*), and three for carbohydrate intake on Chrs 17 (*Mnic1*), 6 (*Mnic2*), and X (*Mnic3*). An absence of interactions among these QTL suggests the existence of separate mechanisms controlling the intake of fat and carbohydrate. Two significant QTL for cumulative kilocalorie intake, adjusted for baseline body weight, were found on Chrs 17 (*Kcal1*) and 18 (*Kcal2*). Without body weight adjustment, another significant kcal locus appeared on distal Chr 2 (*Kcal3*). These macronutrient and kilocalorie QTL, with the exception of loci on Chrs 8 and X, encompassed chromosomal regions influencing body weight gain and adiposity in this F_2 population. These results provide new insight into the genetic basis of naturally occurring variation in nutrient intake phenotypes.

genetics; mouse inbred strains; food intake; energy intake; diet selection; body weight; fat pad; obesity

ALTHOUGH MACRONUTRIENT selection, the process of choosing to ingest varying amounts of fat, carbohydrate, and protein, has been studied widely, few studies have examined the possible genetic basis for macronutrient-specific and total calorie intake (12, 36). The contribution of genetic factors to the variation in human macronutrient intake has been difficult to demonstrate due to the nature of the phenotype. Cultural and environmental factors play a strong role in the acquisition and expression of food preferences, and the assessment of voluntary nutrient intake in a laboratory setting is considered cost-prohibitive and impractical. Therefore most investigators have relied on the determination of dietary intake by self-report. Based on subjective methodology such as questionnaires and food diaries, macronutrient intake appears to be partially heritable (for review, see Ref. 36). For example, 20% of the total phenotypic variance in carbohydrate and fat intake was attributed to a genetic effect in one population study that included a large number of twin pairs (31). However, there have been few studies investigating the amount and proportion of macronutrients in the diet by measuring nutrient intake under controlled conditions (12, 14). In a laboratory study employing a macronutrient self-selection paradigm, measures of fat intake ranged from 3–50% of energy in healthy, normal weight men (14). These results were consistent with the subjects' reports of habitual fat intake, thus providing evidence for variation in the preferred level of dietary fat intake. Results from another laboratory study, based on measures of calorie intake in twins during test meals, suggested that familial factors influenced macronutrient-specific intake, but more convincing evidence was found for genetic influences on total calorie intake (12).

To our knowledge, there have been no genetic linkage studies in animal models investigating macronutrient-specific food intake. Furthermore, only a few investigations have examined quantitative trait loci (QTL) contributing to energy intake, i.e., studies of feed efficiency in chickens (49) and of food intake in mice (28). In particular Moody et al. (28), in a cross between a high heat-loss selection line and C57BL/6J mice, did not detect any QTL for energy intake in regions where heat-loss QTL were identified. Additionally, West et al. (51, 52) measured total calories in studies of dietary obesity and found only weak evidence of an association between cumulative energy intake and adiposity in various mouse strains and crosses. Thus, despite the significant contribution of energy balance to the development of obesity, no ge-

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netic loci for energy intake in mouse have been reported (33).

To uncover genetic loci involved in the preferential consumption of dietary fat or carbohydrate, we have developed a mouse model system. Macronutrient diet selection was evaluated in 13 mouse strains, using a paradigm in which separate sources of carbohydrate, fat, and protein were simultaneously available (42). The results revealed significant variation in preferential fat consumption across strains, ranging from 26% to 83% of total energy, suggesting a complex genetic trait. Of the strains surveyed, most self-selected ≥50% of energy as fat with some strains consuming an exceptionally high amount of fat, i.e., 75–80% in C57BL/6J (B6) and AKR/J mice (42). The CAST/EiJ (CAST) strain was noted for its low fat intake and preferential consumption of the carbohydrate diet. On the basis of their markedly different patterns of fat and carbohydrate consumption and nonoverlapping phenotypic distributions (42), the inbred B6 and CAST strains were selected to produce an F_2 intercross population for QTL analysis. The present study has identified loci influencing self-selected macronutrient diet intake (fat vs. carbohydrate) and total kilocalories and thus provides new evidence for the genetic determination of these traits. It has also presented the unique opportunity for comparing these food intake loci with QTL identified for body weight and adiposity traits in the same mapping population.

METHODS

Animals. Adult male and female C57BL/6J and CAST/EiJ mice were purchased from the Jackson Laboratory (Bar Harbor, ME). Mice were bred and reared in polycarbonate cages, on sterilized corncob bedding (The Andersons, Maumee, OH) at 23–24°C in rooms with a 14:10-h light/dark cycle. Mice were given ad libitum access to tap water and food (no. 5001 chow; LabDiet, Richmond, IN) containing by energy: 28% protein, 12% fat, and 60% carbohydrate. The F_1 mice were bred locally by reciprocal crosses using mice from the Jackson Laboratory parental lines. Reciprocal F_1 crosses then were used to generate a total of 591 male F_2 mice in 10 experimental cohorts that were phenotyped from 31 January 2001 to 5 March 2002. Thus four types of F_2 offspring were produced: (B6 × CAST) F_1 × (B6 × CAST) F_1; (CAST × B6) F_1 × (CAST × B6) F_1; (B6 × CAST) F_1 × (CAST × B6) F_1; and (CAST × B6) F_1 × (B6 × CAST) F_1. Nulliparous dams were used for producing all F_2 mice with the exception of 9 litters in the last cohort that were produced by dams with a parity of 2. Only progeny from litters containing 4–10 pups were used for breeding and phenotyping. Pups were weaned at 24–26 days of age and housed with siblings in same-sex groups of 1–4. At 5 wk of age, male mice were weighed and housed individually until body weight returned to baseline. Male mice from the parental strains as well as F_1 progeny were bred and phenotyped concurrently as controls in each cohort. There was no effect of cohort on F/P preference [$F(9, 492) = 1.12$, $P = 0.35$]. All animal protocols were approved by the Institutional Animal Care and Use Committee of Pennington Biomedical Research Center.

Phenotyping. At 7–10 wk of age, mice were placed individually in stainless steel hanging mouse cages 7 × 10 × 7 (width × depth × height, in inches) with wire mesh floors.

Table 1. *Composition of experimental diets*

2-Choice	Carbohydrate/Protein	Fat/Protein
Corn starch	49.62	0.00
Powdered sugar	21.24	0.00
Casein	19.88	32.77
DL-methionine	0.29	0.49
Vegetable shortening	0.00	51.93
AIN-76A vitamin mix*	1.00	1.53
AIN-76A mineral mix*	3.20	5.33
Choline chloride	0.18	0.31
Cellulose (alphacel)	4.92	7.62
Energy density, kcal/g	3.61	5.96

Ingredients are expressed as percent by weight. *The vitamin and mineral mixes contain 97% and 12% sucrose, respectively.

Polyvinylchloride nesting tubes (1.5 in. diameter, 3.5 in. length) were provided to reduce time spent on the wire bottom. Mice were maintained on a photoperiod of 12:12, at an ambient temperature of 26–27°C. Until the experimental diets were initiated, mice continued to receive no. 5001 chow (LabDiet) ad libitum and tap water was available from a bottle attached to the front of each cage. After a 7-day period of adaptation to these new housing conditions, the phenotyping protocol for macronutrient diet self-selection was begun, when the mice were 58–80 days of age. Body weights were measured to the nearest 0.1 g at the beginning and end of the 10-day phenotyping period. Food was removed from the cage at the beginning of the light cycle. Mice were euthanized by an overdose of isoflurane gas inhalation; blood was collected by cardiac puncture, and glucose was measured with a glucose meter (Bayer, Elkhart, IN). Bilateral epididymal (EPI) and retroperitoneal (RP) fat pads were dissected free and weighed. Spleens were collected for DNA isolation.

Diet self-selection protocol. Fat, carbohydrate, and total calorie intake were assessed by employing a two-choice macronutrient diet paradigm in singly housed adult male mice. The mice were provided for 10 days a choice between the fat/protein (F/P) or carbohydrate/protein (C/P) diets (Table 1). The composition of the F/P and C/P diets was equivalent for protein (22% of energy), and the balance of calories for each contributed by either fat or carbohydrate (78%). Including protein in both diet choices rather than separately prevented the long-known problems associated with the aversive taste of milk casein in rodent diet studies (32), e.g., inadequate protein intake and negative weight balance. The F/P diet contained a mixture of vegetable shortening and casein which at room temperature presented in the form of a cookie-dough consistency. The C/P diet was a dry, powdery mixture of finely milled (corn starch and powdered sugar) and small granular particles (casein). Each of the diets were presented in custom 2 oz. glass jars (Unifab, Kalamazoo, MI). Each jar was covered with a stainless steel lid containing a hole that measured 7/8 in. in diameter. A stainless steel disc was placed under the lid on top of the diet; the disc contains six circular openings (each 7/16 in. diameter) to allow food access while minimizing spillage. To ensure freshness, the F/P diet jar was replaced every other day; on alternate days, the lid was removed and fresh diet was added. The C/P jar was topped off with fresh diet daily. Jars and spilled diet (collected on disposable cardboard pads placed beneath the hanging wire cages) were weighed daily to the nearest 0.1 g using the same balance (PG-S; Mettler-Toledo, Hightstown, NJ). Measurements were made at the same time of day, in the middle of the light period. It is worth noting that spillage of the F/P

diet was nonexistent with the equipment used, thus minimizing measurement error for this phenotype. Spillage of the C/P diet was separated from feces and collected into the weigh container with a brush.

Definition of the phenotypes. Strictly defined, the animals' choices were based on their responses to specific diet formulations containing a combination of either F/P or C/P. The proportion of calories consumed from the F/P diet equals the numerical value of one minus the proportion of intake from the C/P diet. This proportion reflects the preference for fat relative to carbohydrate and is independent of factors affecting total kcal such as body weight. For simplification throughout the paper, this proportion is referred to as "F/P preference", with the obvious caveat that the inverse of this F/P proportion represents the C/P proportion or "C/P preference."

Genotyping. DNA was prepared from spleens of F_2 mice using a phenol/chloroform extraction. A group of 84 F_2 mice were identified whose F/P preference fell in the upper or lower 11% of the phenotypic distribution of the trait in a preliminary sample ($n = 373$). An additional group of 81 F_2 mice from the extremes of the distribution for the sum (10 days) of total calorie intake were also selected from the total population ($n = 502$). This sample of 165 mice included all animals from the upper and lower 10% tails of the phenotypic trait distributions for total fat and carbohydrate intake. These mice were genotyped at 98 microsatellite markers (Research Genetics, Birmingham, AL) polymorphic between B6 and CAST, with an average spacing between markers of ~20-cM intervals throughout the genome, not including the Y chromosome. PCR products were separated using 4% NuSieve (BioWhittaker Molecular Applications, Rockland, ME) agarose gel electrophoresis and visualized with ethidium bromide. To discover putative QTL linked to F/P preference, mean phenotypes were compared among mice with B6/B6, CAST/CAST, and B6/CAST alleles at each marker using analysis of variance; in this manner, six chromosomes (Chrs) with markers showing a significant ($P < 0.005$) genotype effect on F/P preference were identified for further evaluation. In these regions, some markers were added for higher resolution, and the entire F_2 population was then genotyped.

Mapping and sequencing of pancreatic colipase (Clps). Based on the known intron-exon structure of the human colipase gene (22), a mouse primer pair was designed [5′-TCCTTGTTCTTCTGCTTG-3′ (forward primer) and 5′-TCTGCTCTTACACTGCATAC-3′ (reverse primer)] and used to amplify intron 1. The initial amplification was performed with a proof-reading long-range KlenTaq polymerase (KlenTaq; Clontech) that generated 1,811-bp and 1,581-bp fragments from B6 and CAST, respectively. Sequencing of the two amplicons allowed for the design of additional primer pairs to span the polymorphic region that could be amplified using conventional *Taq* DNA polymerase: 5′-GCCAAGCACATGATGCCTGTGTAT-3′ (forward primer) and 5′-TCTGCTCTTACACTGCATAC-3′ (reverse primer). These primer pairs amplified 1,197-bp and 970-bp DNA fragments from B6 and CAST, respectively. This length polymorphism was used to genotype the F_2 population. Amplification of the coding region of the *Clps* gene was achieved by RT-PCR using the primers 5′-GTCTGAACTTCCAGCTTCCATC-3′ (forward primer) and 5′-TGCCAACAGCTGGCTGGCTCAG-3′ (reverse primer). All nucleotide sequence data have been deposited into GenBank (accession nos. AF414676–AF414679).

Statistical analyses. The means and standard errors of traits were determined for the parental strains, as well as for the F_1 and F_2 populations. Strain effects were evaluated by defining contrasts to test for differences between B6 and CAST, and between the F_1 population and the average of its two parental strains. Effects of reciprocal F_2 crosses on phenotypes were determined by analysis of variance. A repeated measures design ANOVA was used to analyze the effects of strain and time on F/P preference and total kilocalories over successive days. When a main effect was observed, individual comparisons were evaluated using Tukey's protected *t*-test. The percent genetic variance (V_G) was based on the difference between phenotypic (total) variance in the F_2 (V_T) and nongenetic (environmental) variance (V_E) calculated from phenotypic variances in the parental strains and F_1 (24) as

$$V_E = \tfrac{1}{4}\, V_{P1} + \tfrac{1}{4}\, V_{P1} + \tfrac{1}{2}\, V_{F1}$$

$$\%V_G = (V_T - V_E/V_T) \times 100$$

Genome scans for main effect QTL were performed using the method of Sen and Churchill (41). This analysis is equivalent to the interval mapping procedure of Lander and Botstein (18) but uses a different computational algorithm. Logarithm of the odds ratio (LOD) scores were computed at 2-cM increments along the entire genome, and significance was assessed by permutation analysis (7). Significant QTL were those that met or exceeded the 95% genome-wide threshold. Confidence intervals for the QTL locations were computed by finding regions where the LOD curve is within 1.5 units of its peak value. In addition to QTL with main effects, we attempted to identify pairs of QTL that might make significant contributions to the phenotypic variance through epistatic interactions. Thus simultaneous genome scans for all pairs of loci were carried out (41), employing a search strategy described previously (45). Briefly, the genome scan searches through all pairs of loci fitting a full two-way ANOVA model with an interaction term. A LOD score contrasting the full model to a null model (with no genetic effects) is computed for each pair, and genome-wide significance is established by permutation testing. A secondary test for the significance of the interaction term is computed only for those pairs that pass the genome-wide screening. The interaction test is carried out using a stringent nominal significance level (0.005), and only those locus pairs passing both tests are deemed to be interacting. In this study, no significant interactions were identified. To assess the combined effects of all QTL on a trait, we carried out a multiple regression analysis including all significant QTL. The percent of total variance explained for each trait is based on this model. Single locus vs. two locus models were compared by fitting single and two QTL models to the data to obtain the LOD score (41). A large increase in LOD under the two QTL model would indicate the presence of a second QTL. The changes observed in this study were moderate, hinting at a second QTL but failing to provide convincing evidence. The software package used in this study, Pseudomarker release version 9.1, is available at **http://www.jax.org/research/churchill**.

RESULTS

Description of phenotypes in parental strains, F_1, and F_2 populations. The mean and standard error for each trait in the parental strains and in the F_1 and F_2 hybrid offspring are shown in Table 2. The mean F/P preference and F/P kcal intake were ~2.5-fold higher in B6 than in CAST mice, whose C/P kcal intake was higher than B6, thus confirming and extending previous observations (42). Mean F/P preference and F/P kcal in F_1 mice were intermediate between the two

Table 2. *Phenotypic data for parental strains and F_1 and F_2 hybrids*

	C57BL/6J (n = 35)	CAST/EiJ (n = 38)	F_1 (n = 47)	F_2 (n = 502)
F/P preference[a]	0.526 ± 0.027†	0.228 ± 0.021	0.358 ± 0.020	0.398 ± 0.008
F/P kcal[a]	64 ± 3†	26 ± 2	50 ± 3	54 ± 1
C/P kcal[a, b]	58 ± 3†	91 ± 3	89 ± 3†	83 ± 1
Total kcal[a, b]	123 ± 2*	117 ± 2	139 ± 2†	136 ± 1
Baseline BW, g[a, b]	22.0 ± 0.2†	14.8 ± 0.2	22.1 ± 0.3†	20.8 ± 0.1
BW gain, g[b]	0.9 ± 0.1	1.2 ± 0.1	−0.3 ± 0.1†	1.3 ± 0.1
RP fat wt, g[a, b]	0.074 ± 0.004*	0.086 ± 0.004	0.056 ± 0.003†	0.076 ± 0.002
EPI fat wt, g[b]	0.399 ± 0.015	0.389 ± 0.015	0.351 ± 0.017*	0.440 ± 0.010
Glucose, mg/dl[a]	261 ± 10†	212 ± 14	263 ± 10*	275 ± 4

Values are means ± SE. [a]Difference between B6 and CAST. [b]Difference between F_1 population and the average of its two parental strains. *$P < 0.0$ 5. †$P < 0.005$. Mice were presented simultaneously with the carbohydrate/protein (C/P) and fat/protein (F/P) diets for 10 days. F/P preference was calculated as cumulative kilocalorie (kcal) intake from the F/P diet divided by cumulative kcal intake from the sum of F/P plus C/P kcal. BW, body weight; RP, retroperitoneal; EPI, epididymal.

parental strains. Heterosis, i.e., the mean of the F_1 population as less or more than the average of their two parental lines (24), was observed for all traits except F/P preference and F/P kcal intake. Extreme heterosis was observed for total kcal, weight gain, RP, and EPI. The F/P preference of the 502 B6 × CAST F_2 progeny ranged from 0.02 to 0.94 (Fig. 1*A*) and averaged 0.40 ± 0.01. The frequency distribution of F/P preference in F_2 mice was skewed slightly left, thus the data were log-transformed before linkage analysis.

As shown in Table 2, the cumulative calorie intake over 10 days was F_1 > B6 = CAST. When adjusted for body weight, total calorie intake was F_1 = CAST > B6, due to the smaller body size of CAST mice. Accordingly, there was a significant correlation between total calorie intake and baseline body weight in F_1 mice, but not in the parental strains. The absolute calorie intake of F_2 mice was distributed over a higher phenotypic range (86–192 kcal) than that of either B6 (101–144 kcal) or CAST (85–141 kcal) mice (Fig. 1*B*). B6 and CAST mice gained weight over the 10 days of phenotyping. Surprisingly, in this self-selection diet paradigm, CAST mice were fatter than B6, as indicated by absolute RP weight; considering the strain difference in body weight, EPI was proportionately greater in CAST. In contrast, F_1 mice lost weight over the same period (Table 2), indicating an effect of negative heterosis (8). F_1 mice had smaller RP and EPI fat pads than either B6 or CAST mice. That F_1 mice lost weight and were leaner, despite consuming more calories than their parental strains, suggests a lower metabolic efficiency.

Analysis of reciprocal crosses. F/P preference, F/P kcal, total kcal, and baseline body weight were significantly different between the two F_1 crosses, but C/P kcal intake was similar (Table 3). Specifically, (B6 × CAST)F_1 mice weighed more at baseline, ate more calories, and selected more fat than (CAST × B6)F_1 mice, suggesting the influence of loci on the nonautosomal chromosomes or of maternal effects in these strains. These reciprocal cross differences correspond to X chromosome linkage for baseline body weight and F/P kcal intake, for which higher phenotypic values were associated with the B6 allele at *DXMit22*. Nevertheless, sex chromosome linkage as a mechanism underlying these differences cannot be distinguished from possible maternal effects or imprinting (24). Among the F_2 reciprocal crosses, there were several traits that differed. F_2 male mice that originated from a (B6 × CAST)F_1 sire had greater F/P kcal intake, total

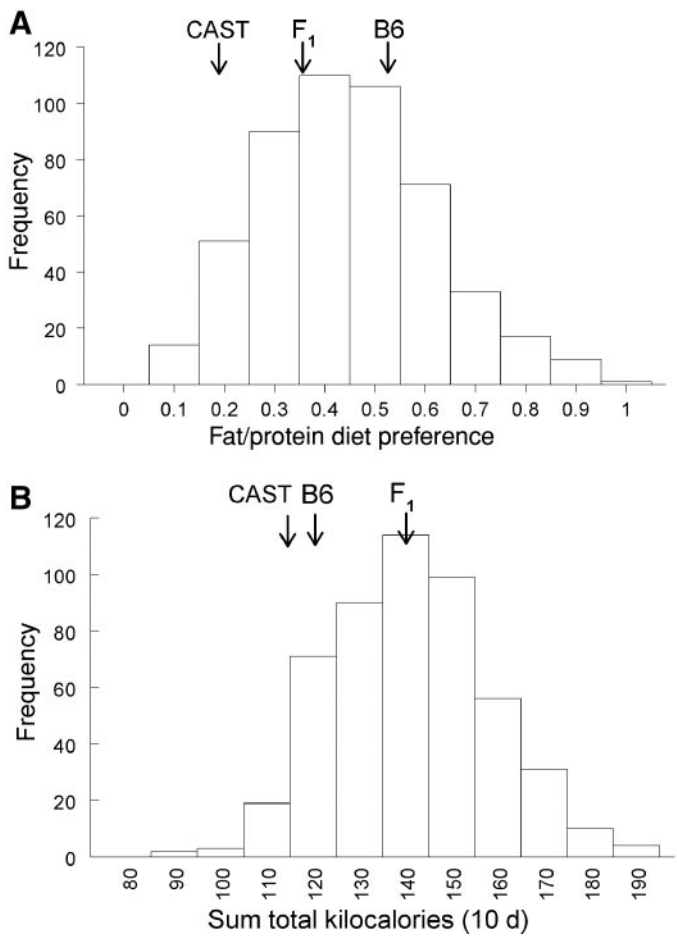

Fig. 1. Distribution of fat/protein (F/P) preference (proportion of energy from F/P diet) (*A*) and total kilocalorie intake (10-day sum) (*B*) in F_2 progeny (n = 502) of a B6 × CAST intercross. Arrows indicate mean F/P preference in the B6 (n = 35), CAST (n = 38), and F_1 (n = 47) populations. Each bin includes values up to and not including the indicated value; e.g., F/P proportions of 0.4 to 0.49 are contained in the bin labeled "0.5," and kcal values of 130 to 139 are included in the bin labeled "140."

Table 3. *Effect of reciprocal cross type (for F_1 and F_2 hybrids) on phenotypic traits*

	F_1		F_2			
Type of Cross	(B6 × CAST) F_1 (n = 25)	(CAST × B6) F_1 (n = 22)	(BC) × (BC) F_2 (n = 154)	(CB) × (BC) F_2 (n = 118)	(BC) × (CB) F_2 (n = 126)	(CB) × (CB) F_2 (n = 104)
F/P preference[a, d]	0.415 ± 0.023	0.294 ± 0.027	0.394 ± 0.013	0.421 ± 0.014	0.338 ± 0.017	0.389 ± 0.018
F/P kcal[a, b, d]	61 ± 4	38 ± 3	53 ± 2	58 ± 2	51 ± 2	51 ± 2
C/P kcal	85 ± 3	93 ± 4	84 ± 2	82 ± 2	84 ± 3	81 ± 2
Total kcal[a, b]	146 ± 3	130 ± 2	137 ± 1	140 ± 2	135 ± 2	132 ± 1
Baseline BW, g[a, c, d]	24.0 ± 1.1	20.0 ± 1.3	20.5 ± 0.2	21.4 ± 0.2	20.8 ± 0.2	20.4 ± 0.2
BW gain, g[b]	−0.2 ± 0.2	−0.4 ± 0.2	1.4 ± 0.1	1.4 ± 0.2	1.2 ± 0.1	1.0 ± 2.0
RP fat wt, g[b]	0.061 ± 0.005	0.051 ± 0.004	0.079 ± 0.003	0.083 ± 0.005	0.078 ± 0.005	0.063 ± 0.003
EPI fat wt, g	0.378 ± 0.028	0.319 ± 0.018	0.441 ± 0.017	0.466 ± 0.023	0.454 ± 0.022	0.393 ± 0.20
Glucose, mg/dl[c]	263 ± 15	263 ± 12	265 ± 8	281 ± 10	296 ± 8	260 ± 8

Values are means ± SE. The F_1 and F_2 were generated by reciprocal crosses using both strains and genders. [a]Effect of F_1 cross type; [b]effect of F_2 sire; [c]F_2 dam by F_2 sire interaction; $P < 0.05$. [d]Significant linkage on chromosome X in total F_2 population. F/P preference = sum of 10-day kcal intake from the fat/protein (F/P) diet divided by the sum of 10-day total kcal intake.

kcal intake, body weight gain, and RP fat weight (Table 3). These results may be interpreted as an effect of the Y chromosome. There were no effects of F_2 dam on any of the traits. However, there was evidence of F_2 sire × dam interactions for baseline body weight and glucose (Table 3).

Fat/protein preference across days. The daily F/P preference in B6, CAST, F_1, and F_2 mice is illustrated in Fig. 2*B*. F/P preference was established from the first day of diet presentation and was not affected by the increased kcal intake on *day 1* (Fig. 2*A*). Relative to *day 1*, F/P preference on *days 2–10* remained unchanged in B6, F_1, and F_2 mice. In contrast, the F/P preference of CAST mice declined over the 10-day phenotyping period [strain by time: $F(3, 618) = 2.48$, $P < 0.0001$], providing evidence for conditioning of food choice in this carbohydrate-preferring strain.

Kilocalorie intake across days. The time course of calorie intake over the 10-day phenotyping period was examined, and evidence of initial hyperphagia was found (Fig. 2*A*), i.e., all strain groups consumed a significantly higher percent of calories on *day 1*, compared with their average daily calorie intake on *days 2–10*: B6 (28%), CAST (20%), F_1 (17%), and F_2 (14%) mice. A compensatory response to this increased energy intake was demonstrated by the gradual decline in calorie intake observed on *days 2–5* (Fig. 2*A*), as indicated by a main effect of time [$F(9, 618) = 30.02$, $P < 0.0001$]. Overall, the F_1 and F_2 hybrid mice consumed more total daily calories than the parental strains [$F(3, 618) = 22.71$, $P < 0.0001$].

Correlations among traits in F_2 mice. F/P and C/P kcal intakes were negatively correlated (−0.78) (see Table 4), and this observation corresponds to contrasting effects of the parental strain alleles in the QTL analysis, e.g., increased intake of one diet vs. decreased intake of the other. A significant correlation between total kcal intake and C/P kcal was found (0.58), but there was no association between total kcal and F/P kcal or F/P preference, suggesting that in this model increased energy intake cannot be explained by consumption of the energy-dense fat diet. Total kcal was significantly correlated with baseline body weight (0.41) and weight gain (0.49), as well as RP (0.40) and EPI (0.31) fat pad weights. With respect to macronutrient composition, there were no relationships among weight gain and macronutrient diet intake or F/P preference, with the exception of C/P kcal. C/P kcal was also correlated with fat pad weights. Blood glucose level showed moderate associations with body weight and adiposity but was weakly correlated with C/P kcal

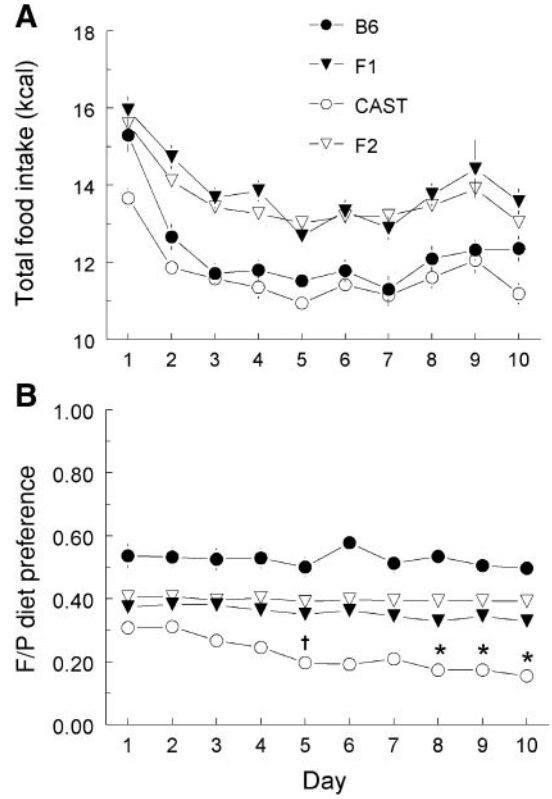

Fig. 2. Daily calorie intake (*A*) and F/P preference (proportion of energy consumed from the F/P diet) (*B*) in B6, CAST, F_1, and F_2 mice self-selecting from a choice between the F/P and carbohydrate/protein (C/P) diets across the 10-day phenotyping period. The F/P preference of CAST mice declined over the 10-day phenotyping period [strain by time: $F(3, 618) = 2.48$, $P < 0.0001$], and this effect was confirmed by pairwise comparisons: *day 1* vs. *days 5*, *8*, *9*, and *10* (†$P < 0.05$ and *$P < 0.01$, Tukey-Kramer adjustment).

Table 4. *Correlations among traits in the F_2 population*

	F/P kcal	C/P kcal	Total kcal	Baseline BW	BW Gain	RP Fat wt	EPI Fat wt	Glucose
F/P preference	0.95[c]	−0.91[c]	−0.21	−0.01	−0.06	−0.11[a]	−0.06	−0.10[a]
F/P kcal		−0.78[c]	0.08	0.11[a]	−0.09	0.01	0.04	−0.05
C/P kcal			0.57[c]	0.17[c]	0.24[c]	0.25[c]	0.16[b]	0.17[c]
Total kcal				0.41[c]	0.49[c]	0.40[c]	0.31[c]	0.21[c]
Baseline BW					−0.11[a]	0.44[c]	0.46[c]	0.28[c]
BW gain						0.62[c]	0.57[c]	0.28[c]
RP fat wt							0.80[c]	0.31[c]
EPI fat wt								0.28[c]

F/P preference was calculated as the 10-day sum of kcal intake from the F/P diet divided by the 10-day sum of F/P plus C/P kcal. [a]$P < 0.05$. [b]$P < 0.001$. [c]$P < 0.0001$.

and total calorie intake, indicating these traits are not key predictors of glucose in this model.

QTL analysis: macronutrient and total calorie intake. Results of the complete genome scan for macronutrient selection and energy intake QTL are summarized in Table 5. Linkage analyses for macronutrient intake and total kcal are reported with and without adjustment for baseline body weight by regression; therefore, two sets of LOD scores are presented. QTL analysis identified six significant loci for macronutrient intake: three for fat intake (Fig. 3) on Chrs 8 (*Mnif1*), 18 (*Mnif2*), and X (*Mnif3*); and three for

Table 5. *Estimated locations and effects of QTLs for macronutrient and kilocalorie intake, body weight, adiposity, and glucose*

Locus name		Trait	Chr	Position, cM[a]	1.5 LOD CI	Allele[b]	LOD	LOD[c]	% Var[d]
Kilocalorie intake 3	*Kcal3*	Total kcal	2	72	57–90	B	3.9	NS	3.5
		Baseline BW	2	82	74–92	B	7.8		6.9
		BW gain	2	74	61–108	B	5.6	4.8	5.0(4.3)
		RP fat	2	90	72–108	B	5.2	NS	4.7
		Glucose	2	82	60–108	B	3.2	NS	2.9
Macronutrient intake (fat) 1	*Mnif1*	F/P kcal	8	22	10–30	B	7.9	8.0	7.0(7.1)
		F/P preference	8	22	10–30	B	5.8	5.9	5.2(5.3)
		C/P kcal	8	22	0–31	C	3.4	3.7	3.1(3.3)
		Baseline BW	8	48	36–58	B	4.8		4.3
		Glucose	8	46	41–72	B	4.8	3.1	4.3(2.8)
Macronutrient intake (carbohydrate) 2	*Mnic2*	C/P kcal	6	46	36–64	C	4.1	3.4	3.7(3.1)
		RP fat	6	26	8–56	C	6.4	6.8	5.7(6.0)
			6	42	8–56	C	6.4	5.6	5.7(5.0)
		EPI fat	6	44	0–62	C	2.9	NS	2.6
Macronutrient intake (carbohydrate) 1	*Mnic1*	C/P kcal	17	10	3–24	C	6.0	6.7	5.4(6.0)
		F/P kcal	17	10	0–28	B	3.1	3.0	2.8(2.8)
		F/P preference	17	10	0–24	B	3.8	4.9	3.4(4.4)
Kilocalorie intake 2	*Kcal2*	Total kcal	17	16	8–37	C	NS	4.9	(4.4)
		RP fat	17	18	0–25	C	NS	4.4	(4.0)
		EPI fat	17	22	5–57	C	NS	3.5	(3.1)
Macronutrient intake (fat) 2	*Mnif2*	F/P kcal	18	24	10–58	C	5.4	6.0	4.8(5.4)
		F/P preference	18	46	4–58	C	3.1	3.0	2.8(2.8)
Kilocalorie intake 1	*Kcal1*	Total kcal	18	20	10–26	C	5.2	7.7	4.6(6.8)
		BW gain	18	18	6–36	C	3.5	3.8	3.1(3.4)
		Glucose	18	48	42–58	B	4.4	3.9	4.0(3.5)
Macronutrient intake (fat) 3	*Mnif3*	F/P kcal	X	18	10–58	B	5.1	4.0	4.6(3.6)
		F/P preference	X	36	8–60	B	3.5	4.1	3.1(3.7)
		Baseline BW	X	18	12–26	B	12.1		10.5
			X	38	9–48	B	10.1		8.8
Macronutrient intake (carbohydrate) 3	*Mnic3*	C/P kcal	X	40	14–61	C	NS	4.1	(3.7)
		Glucose	X	64	44–73	C	NS	5.7	(5.1)

Significant and suggestive LOD threshold levels are 3.5 and 2.6, respectively, as determined by permutation analysis; NS, nonsignificant; CI, confidence interval. [a]Position of peak adjusted LOD score; genetic loci are positioned based on the consensus linkage map (MGI Database). [b]Allele linked to an increase in phenotypic score; B = C57BL/6J; C = CAST/Ei. [c]Adjusted for baseline body weight by regression. [d]Percentage of total variance explained by the QTL (values in parentheses represent variances for weight adjusted data).

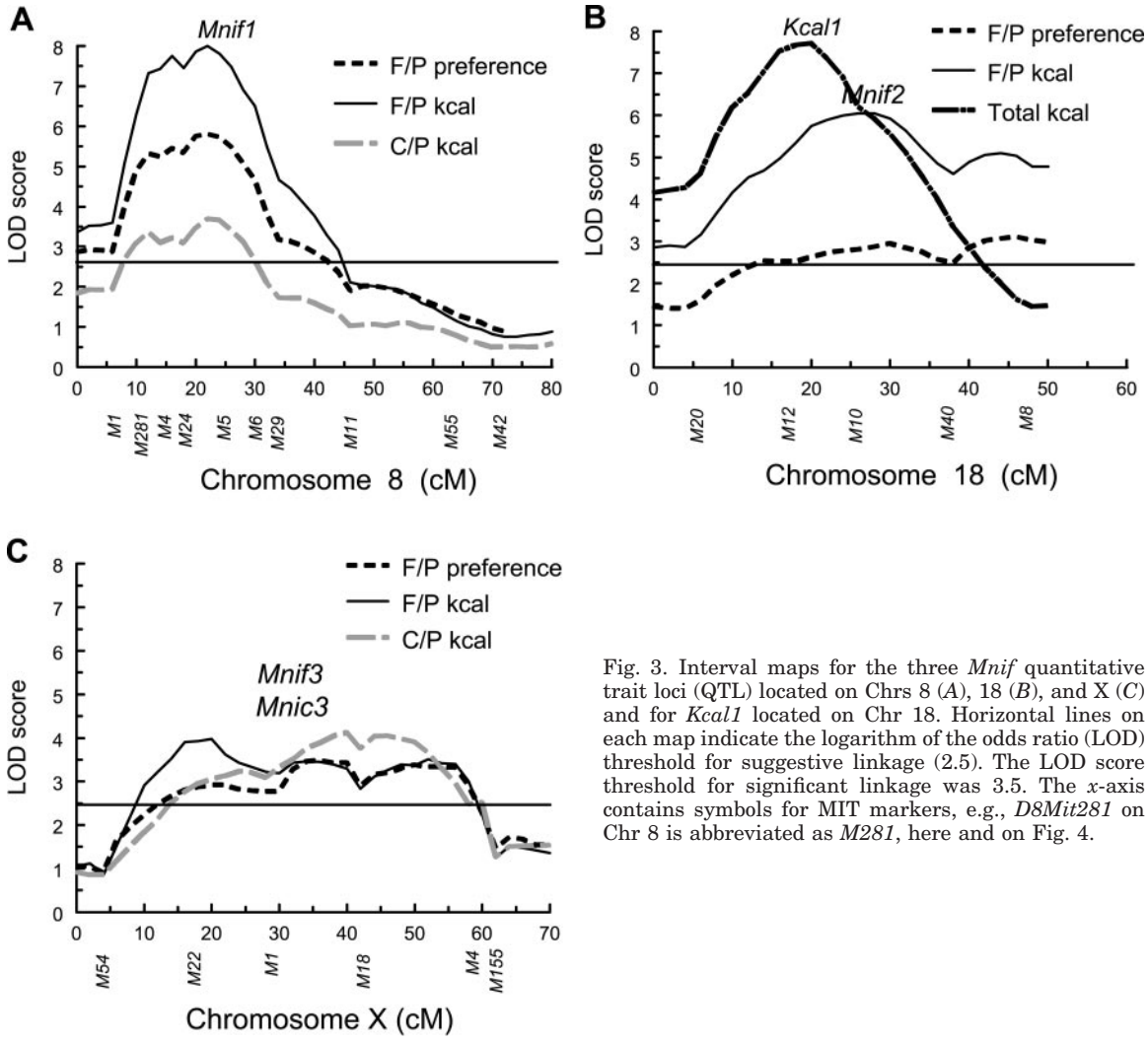

Fig. 3. Interval maps for the three *Mnif* quantitative trait loci (QTL) located on Chrs 8 (*A*), 18 (*B*), and X (*C*) and for *Kcal1* located on Chr 18. Horizontal lines on each map indicate the logarithm of the odds ratio (LOD) threshold for suggestive linkage (2.5). The LOD score threshold for significant linkage was 3.5. The *x*-axis contains symbols for MIT markers, e.g., *D8Mit281* on Chr 8 is abbreviated as *M281*, here and on Fig. 4.

carbohydrate intake (Fig. 4) on Chrs 17 (*Mnic1*), 6 (*Mnic2*), and X (*Mnic3*). All these QTL were statistically significant, independent of body weight, with the exception of *Mnic3*. Each of these genetic loci controlled 4–7% of the total variance. Two of these regions colocalized with significant QTL for kcal intake, corrected for body weight, on Chrs 17 (*Kcal1*) and 18 (*Kcal2*), although the LOD curves for this trait appear to be different in character than those for macronutrient intake. A body weight-dependent locus for calorie intake was detected on distal Chr 2 (*Kcal3*; LOD plot not shown); i.e., linkage disappeared when baseline body weight was included in the model. *Mnif1* and *Mnic1* showed only suggestive linkage for carbohydrate and fat intake respectively. With the exception of *Mnic2*, all of the macronutrient loci were also associated with F/P preference. However, in all cases, the LOD score was lower for F/P preference than for macronutrient intake (fat or carbohydrate), and no unique loci were found that were linked exclusively to F/P preference.

An increase in both F/P kcal and F/P preference was linked to the B6 allele at *Mnif1* and *Mnif3* on Chrs 8 and X, respectively, but with the CAST allele at *Mnif2* on Chr 18. Although *Mnif1* showed the strongest linkage for F/P kcal intake (LOD = 8.0), traits for both F/P preference and C/P intake also mapped on this chromosome with similar profiles but lower LOD scores. The peak LOD value for *Mnif1* mapped between *D8Mit24* and *D8Mit5* and accounted for 7% of the total variance in fat intake. The 1.5 LOD support interval spans ~13 cM (12–35 cM). The *Mnif2* locus encompasses a broad interval that peaks in close proximity to *D18Mit10*. The *Mnif3* locus on Chr X, controlling F/P preference and F/P kcal, is a broad and multimodal QTL that likely contains two or more loci, although there is no strong statistical support for this conclusion when comparing the two-locus to single-locus LODs (see METHODS). This complex QTL may resolve into separate components in future analyses using congenic and subcongenic strains.

An increase in C/P kcal intake was linked to the CAST allele at all three carbohydrate loci: *Mnic1*, *Mnic2*, and *Mnic3*, an effect consistent with the parental strain phenotype. *Mnic1* exerted a significant effect on carbohydrate intake (LOD = 6.7), with lesser effects

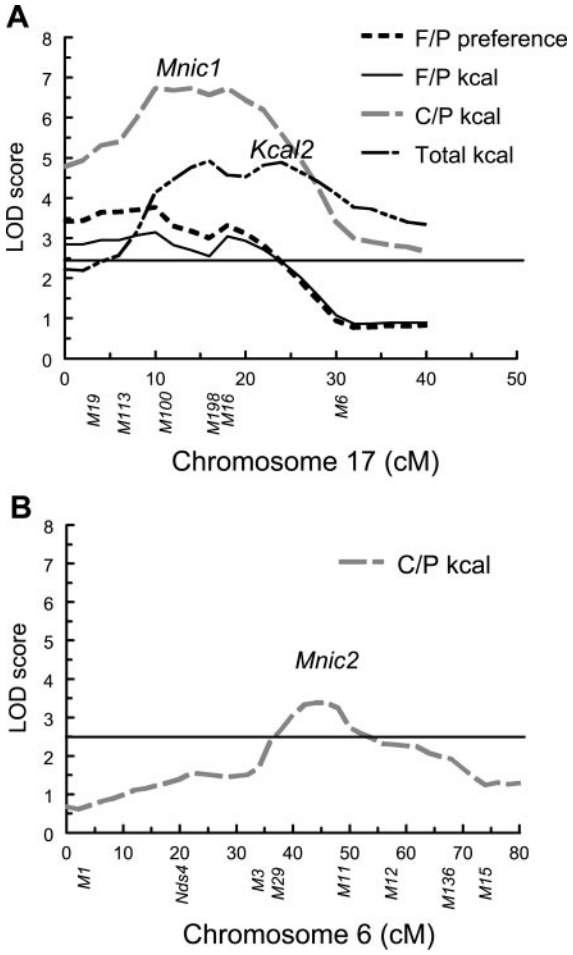

Fig. 4. Interval maps for the two *Mnic* QTL located on Chrs 17 (*A*) and 6 (*B*), and for *Kcal2* located on Chr 17. Horizontal lines on each map indicate the LOD threshold for suggestive linkage (2.5). The LOD score threshold for significant linkage was 3.5.

on F/P preference and F/P kcal intake (Fig. 4). The 1.5 LOD confidence interval for *Mnic1* spans ~16 cM (7–23 cM). A second locus with a significant effect on carbohydrate intake (*Mnic2*) was found on Chr 6 (Fig. 4). *Mnic2* showed a fairly distinct peak in the interval between *D6Mit29* and *D6Mit11* at ~45 cM. *Mnic3* was present only when body weight was used as a covariate.

The combined effect of loci on Chrs 8, 17, 18, and X for F/P kcal intake accounts for an estimated 19% of the total variance and 61% of the genetic variance. For C/P kcal, four loci on Chrs 6, 8, 17, and X accounted for an estimated 16% of total variance, and the proportion of genetic variance explained was 31%. Total genetic variance was calculated as the difference between total variance in the F_2 population and the estimate of environmental variance, based on the phenotypic variances of the parental and F_1 strains, using the proportion recommended by Mather and Jinks (24).

The CAST allele at both *Kcal1* (LOD = 7.7) and *Kcal2* (LOD = 4.9) (Figs. 3–4) was linked to increased calorie intake over 10 days. The LOD curves for energy intake at these loci were different in character than those of the colocalized QTL *Mnif2* and *Mnic1*, suggesting that different loci may be involved. Without body weight as a covariate, *Kcal2* was absent and an additional locus for kcal intake appeared on distal Chr 2 (*Kcal3*). The dependence of *Kcal3* on body weight might be explained by the existence of a body weight QTL (maximum LOD 7.8 cM) found in the same region of Chr 2 (75–90 cM) in our mapping population (see Table 5).

In view of the pronounced hyperphagia on *day 1*, the data were examined for QTL unique to this day, but none were found. However, C/P kcal showed an absence of linkage on *day 1* vs. *days 2–10* at *Mnif1* (*D8Mit24*), *Mnic1* (*D17Mit100*), and *Mnic2* (*D6Mit11*). By contrast, no changes in linkage by day were found for fat intake. For example, the absence of linkage for C/P kcal on *day 1* at *D8Mit24* was concomitant with significant linkage for F/P kcal at that locus. The observation that delayed linkage affects C/P kcal, and not F/P kcal, argues against a general effect of *day 1* hyperphagia on intake of both diets. Additionally, the allelic effect of *Kcal2* was absent on *day 1* [*D17Mit100*, $F(2, 499) = 0.061$, $P = 0.94$], whereas subsequent days (*days 2–10*) showed significant allelic variation for this trait. No delayed allelic effects for calorie intake were detected on Chr 18 where *Kcal1* resides.

QTL analysis: body weight, adiposity, and glucose. QTL for baseline body weight were detected on Chrs 2, 8, and X (Table 5). The QTL on Chr X had a large effect, with a peak LOD score of 12.1, but was very broad. QTL for weight gain found on Chrs 2 and 18 colocalized with *Kcal3* and *Kcal2*, respectively, where the LOD curves for weight gain appeared similar in character to those for total kcal. Evidence for significant QTL influencing fat pad weight was found on Chrs 6 and 17. QTL for RP fat coincided with C/P kcal on Chr 6 (*Mnic2*) and with total kcal on Chr 17 (*Kcal2*). Additional body weight and adiposity QTL were identified on chromosomes other than those containing macronutrient and kcal QTL: RP fat on Chr 15 at 16 cM, as well as baseline body weight, body weight gain, EPI and RP fat on Chr 16 at ~40 cM (data not shown). The locus for RP fat found on proximal Chr 15 is located near the dietary obesity QTL identified previously in this cross (53); other dietary obesity QTL have been identified in this region using different models (for review, see Refs. 5 and 34). Significant linkage for glucose was found on Chrs 2, 8, 18, and X. Additional body weight, adiposity, or glucose QTL may exist in regions that escaped detection due to the selective genotyping employed (see METHODS).

Analysis of a candidate gene. Mnic1 is contained within a LOD support interval which spans ~20 cM on Chr 17. By searching the Mouse Genome Database, possible candidates in this region were identified, including pancreatic colipase (*clps*), a gene with apparent relevance to dietary stimuli (21). To confirm the map position of *Clps* relative to the *Mnic1*, a 230-bp length polymorphism in the intron sequence between B6 and CAST was identified and used. *Clps* mapped between *D17Mit198* and *D17Mit16* in our F_2 population, which

is well within the 1.5 LOD support interval of this QTL. Amplification of the complete coding region of the gene by RT-PCR revealed no sequence differences, with the exception of two silent nucleotide polymorphisms, neither of which would be predicted to alter the amino acid sequence of colipase.

DISCUSSION

This is the first study identifying genetic linkage for the self-selected intake of fat, carbohydrate, and total kilocalories in which measurements of actual food intake were employed. The results provide clear evidence for the genetic determination of these food intake traits. In male F_2 progeny of a B6 × CAST intercross, six macronutrient intake loci were identified: three for fat intake on Chrs 8 (*Mnif1*), 18 (*Mnif2*), and X (*Mnif3*), and three for carbohydrate intake on Chrs 17 (*Mnic1*), 6 (*Mnic2*), and X (*Mnic3*). Two of these (*Mnif2* and *Mnic1*) colocalized with QTL for kilocalorie intake, *Kcal1* and *Kcal2*. Without body weight as a covariate, another kcal locus was detected on distal Chr 2 (*Kcal3*). These macronutrient and kcal QTL, with the exception of loci on Chrs 8 and X, encompassed chromosomal regions influencing body weight gain and adiposity in this F_2 population.

QTL for macronutrient intake. Of the six significant QTL influencing macronutrient intake, the strongest was *Mnif1* for F/P kcal (LOD = 8.0). Two genes with apparent biological relevance to food intake traits are located fairly close to this region: *Lpl* encoding lipoprotein lipase (16) and *Ucp* for the cold-inducible mitochondrial uncoupling protein 1 (15). However, their map positions of 33 and 38 cM, respectively, are somewhat distal to the peak LOD for *Mnif1*. The strongest QTL influencing C/P kcal was *Mnic1*. A relevant candidate gene for *Mnic1* on Chr 17 is *Clps* encoding pancreatic colipase (50). Pancreatic colipase is a cofactor that in the lumen of the small intestine activates pancreatic triglyceride lipase (PTL), an enzyme essential for the absorption of long-chain fatty acids. Increased dietary fat intake leads to increased mRNA levels of both PTL and colipase (for review, see Ref. 21). On tryptic digestion, colipase yields the pentapeptide enterostatin, which by exogenous administration has been shown to suppress high-fat diet consumption in rats (30). In our intercross, *Clps* maps within the 1.5 LOD support interval for *Mnic1*. However, we found no amino acid polymorphism in pancreatic colipase between B6 and CAST, suggesting that a difference in amino acid sequence cannot account for this QTL. Differences in gene expression or in RNA stability of this gene could exist as a result of upstream regulation or downstream effects, and this prospect is currently under investigation. Alterations in fat metabolism due to differences in colipase expression or activity could affect macronutrient intake, based on the observation of an inverse relationship between pancreatic colipase activity and fat intake in a diet self-selection paradigm (30). Interestingly, in the absence of diet choice, mice deficient in procolipase (*Clps* −/−) were hyperphagic on a high-fat diet in apparent attempt to compensate for calories lost due to fat malabsorption (10).

QTL for kilocalorie intake. Evidence for two QTL influencing total calorie intake, independent of body weight, was found: *Kcal1* and *Kcal2*. For both loci, increased energy intake was associated with inheritance of the CAST allele. *Kcal1* maps in the region of the glucocorticoid receptor (GR) gene *Nr3c1* (13). The GR protein is a transcription factor activated by glucocorticoids that are involved in a variety of physiological processes including the mobilization of energy in peripheral tissues and the stimulation of feeding in the central nervous system (48). Moreover, evidence from a variety of models supports a key role of glucocorticoids, in conjunction with insulin, in regulating long-term energy balance (11). The colocalization of *Kcal2* with *Mnic1* suggests that these two QTL may share a common genetic mechanism on Chr 17, e.g., a gene controlling short-term energy intake may be acting through a pathway related to carbohydrate utilization (44). Consistent with this hypothesis, a significant correlation was found between total calories and C/P kcal intake in F_2 mice (Table 4). In addition, a body weight-dependent QTL for total kcal (*Kcal3*) was found on distal Chr 2. In this region, QTL influencing body weight (6), heat loss (28), and obesity (20, 26, 46) have also been described. When designing linkage studies, one consideration is that the parental strains selected for an intercross show the greatest difference possible in trait expression. Remarkably, there was no difference in total kcal intake between the B6 and CAST strains, despite the 33% higher body weight of B6 mice (Table 2). However, when adjusted for body weight, kcal intake was significantly higher in the smaller CAST mice, suggesting a higher level of energy expenditure. Consistent with this supposition, CAST mice have shown significantly higher levels of spontaneous behavior compared with C57BL/6ByJ (which is genetically very similar to C57BL/6J) in measures that included locomotion, jumping, and grooming (19). Also, in B6 mice, a lower basal heat loss has been shown (28). We hypothesize that in our B6 × CAST intercross, differences in physical activity level as well as metabolic rate are contributing to the variation in energy intake (29).

Absence of interaction among QTL. Our statistical analyses did not support significant interactions among the chromosomal loci linked to self-selected macronutrient diet intake. This result could be interpreted as support for the existence of separate mechanisms controlling the consumption of fat and carbohydrate. Furthermore, the observation that *Mnif1*, *Mnif2*, and *Mnif3* showed significant linkage to both F/P kcal intake and F/P preference while *Mnic2* was linked only to C/P kcal intake seems to indicate independent contributions to the food intake traits. However, it is more likely that linkage for F/P preference and F/P kcal intake failed to reach threshold criterion for significance at *Mnic2* and the same may be true for C/P kcal intake at *Mnif2*. The similar LOD profiles for

these macronutrient intake traits is likely due to their intercorrelations.

QTL with temporal changes. Some of the QTL linked to C/P kcal intake, i.e., *Mnif1*, *Mnic2*, and *Mnic1*, showed an absence of allelic variation on *day 1* (data not shown), suggesting a physiological change as a consequence of hyperphagia or one that relates to a candidate mechanism. Specifically, a greater consumption of the C/P diet on *day 1* by mice homozygous for the B6 allele ameliorated the significant genotypic variation observed on *days 2–10* at these loci. The reason for this temporal change in linkage is not clear but a candidate mechanism such as transient expression of a neuropeptide or neurotransmitter during initial diet exposure may be responsible. For example, when hypothalamic expression of neuropeptide Y and agouti-related protein was examined at intervals following exposure to high-fat feeding, a reduction in mRNA was observed after 2 days, but not at later time points (54). These early onset changes in neuropeptide expression corresponded to increased food consumption and illustrate the importance of considering time-dependent effects of candidate genes in nutrient- or energy-related phenotypes.

QTL for body weight, adiposity, and glucose. Much of the observed weight gain in this model likely represents increased adiposity as indicated by the correlations between weight gain and fat pad weights, although it is possible that some growth may have occurred during the 10-day phenotyping period when mice were 8–11 wk of age. In addition, two weight gain QTL detected on Chrs 2 and 16 encompassed QTL for fat pad weight. On distal Chr 2, QTL for baseline body weight, weight gain, kcal intake, RP, and glucose all colocalized to the same region. The LOD profile for baseline body weight and weight gain QTL was very similar to that of the kcal intake QTL. At these loci, the same allele was associated with an increase in both kcal intake and body weight, i.e., the B6 allele at *Kcal3* (Chr 2) and the CAST allele at *Kcal2* (Chr 18). Numerous weight and adiposity QTL have been identified previously on distal Chr 2 (5, 34), a region that also corresponds to the location of a heat loss QTL, *Hlq2* (28). *Kcal3* is the first QTL for energy intake to be mapped to this chromosomal region, and its presence emphasizes further the importance of this site in regulating energy balance.

Do macronutrient intake QTL overlap loci for dietary obesity? Several QTL for body weight gain and adiposity were found in regions identified through selective genotyping of mice with extreme macronutrient and kcal intake phenotypes (Table 5). QTL for RP and EPI fat were found in close proximity to *Mnic1*, *Mnic2*, and *Kcal2* and a weight gain locus colocalized with *Kcal3*. These results are consistent with the observation that C/P kcal and total kcal were correlated with both weight gain and fat pad weight in F_2 mice (Table 4). Only one obesity locus coincided with QTL for F/P kcal, i.e., linkage for body weight gain localized near *Mnif2*, but showed a LOD profile very similar to that for *Kcal1*. Although the common location of QTL for food intake and dietary obesity phenotypes suggests that these traits may be driven by polymorphisms in the same genes, it is not possible with an intercross analysis to distinguish pleiotropy from tight linkage.

The male B6 mouse is sensitive to dietary obesity (51) and has shown a preference for eating fat in the self-selection paradigms tested thus far (42), whereas obesity-resistant CAST mice (26) prefer carbohydrate (42). The possibility was considered that genetic linkage analysis for macronutrient diet selection might detect QTL for dietary obesity mapped previously in this cross (9, 26, 34, 53). However, our results did not show linkage for fat or carbohydrate intake in chromosomal regions where dietary obesity QTL have been located in B6 × CAST intercross populations fed a 30% fat diet, i.e., one locus linked to adiposity on Chr 15 (53), as well as three loci (*Mob5*, *Mob6*, *Mob7*) for adiposity on Chr 2 and one (*Mob8*) on Chr 9 (26). In addition, our macronutrient loci were not associated with regions linked to weight gain on Chrs 2 and 8, or with percent body fat on Chr 9, identified in a C57BL/6J-*hg*/*hg* × CAST/EiJ cross fed a chow diet (9). Nonetheless, loci for obesity and weight gain on Chr 2 described in two earlier reports (9, 26) fell within the confidence limits of our *Kcal3*, a weight-dependent locus that was also linked to weight gain and adiposity in our mapping population. The lack of commonality between macronutrient intake QTL in the present study and dietary obesity QTL in other studies using this intercross is not unexpected considering the differences in environmental factors. For example, all prior investigations of dietary obesity QTL have fed mice a single diet of fixed macronutrient composition. The present study is unique in that only protein intake was fixed, with each mouse composing their total intake from the choice between a F/P and a C/P diet, thus making it possible to examine the effects of wide-ranging macronutrient intakes on genetic linkage for body weight and adiposity. Consequently, some of the body weight and adiposity QTL in the present study may also reflect patterns of macronutrient intake rather than variation in nutrient partitioning alone. Notably, in this self-selection paradigm, it appears that carbohydrate, rather than fat consumption, may be more important in determining obesity in this F_2 population. Further differences in environmental factors are that mice were fed the experimental diets for a short period of time compared with other protocols, and the phenotypic data were collected at 8–11 wk of age, which is younger than other studies of dietary obesity in this mouse cross, e.g., 5–17 wk (53) and ~4 mo (26).

With respect to the parental strains, it is remarkable that in this model inbred CAST mice gained more weight than B6 mice, and weight gain was correlated with F/P preference ($r = 0.35$, $P < 0.05$) and F/P kcal ($r = 0.40$, $P < 0.05$). Moreover, in the CAST strain, significant correlations were observed between F/P kcal and adiposity, with or without adjustment for baseline body weight (EPI, $r = 0.49$ and 0.50, respectively $P < 0.005$; RP, $r = 0.51$ and 0.52, respectively, $P < 0.005$). In the B6 strain, only EPI/baseline body

weight showed a significant correlation with weight gain (0.35, $P < 0.05$). These observations are in contrast to the lean body phenotypes described previously for this strain in dietary obesity models employing a single, composite diet of moderate fat content (26, 53), and further emphasize the role of environmental factors in these traits.

Human QTL for macronutrient intake. A recent scan of the human genome has revealed QTL for energy and macronutrient intake that are based on data for dietary phenotypes obtained through methods of self-report (C. Bouchard, personal communication). A search of the human/mouse homology database (**http://www.ncbi.nlm.nih.gov/HomoloGene/**) revealed that none of the QTL identified in these human linkage analyses corresponded to the mouse QTL for macronutrient or energy intake described in the present report.

Physiological framework for candidate genes. The mechanisms underlying phenotypic strain differences in food selection and intake remain unclear but may involve genetically determined components in any system that receives, integrates, or organizes the animal's response to food. These neural pathways include: *1*) input or sensory systems through which the nervous system receives nutritional and/or metabolic information, including gustatory, gastrointestinal, and hepatic signals; *2*) integrative systems for processing both internal and external information, and *3*) output systems including locomotor and oropharyngeal controls (3). Therefore, a number of physiological systems could be involved in controlling the total calories and relative amounts of macronutrients ingested.

With respect to these processes, mice in the current study may have learned to associate the perceived flavor of the mixed macronutrient diets, i.e., the combination of taste, smell, and texture cues, with their postingestive, metabolic, or energetic consequences through a form of classic conditioning (40, 47). There is also evidence for innate or unlearned flavor preferences or aversions for sugars, proteins, and fats (see Ref. 40 for review). Additionally, gastrointestinal stimuli alone could be sufficient to condition nutrient learning (23) based on evidence that gastrointestinal and hepatic chemosensors respond to the macronutrient composition of ingested food through specific sensory mechanisms (35). Genetic variation in pathways involved in carbohydrate or fat metabolism could condition the avoidance or preferential consumption of macronutrient-rich diets. For instance, blocking the utilization of glucose or fatty acids with antimetabolic drugs activates separate neural pathways and stimulates feeding (37) in a nutrient-specific manner (38). Therefore, genes encoding proteins that are key regulators of fat oxidation, glucose metabolism, and energy expenditure are likely candidates for modifying the self-selected intake of diets varying in nutrient composition. Genetic variation in neural or neurochemical systems may also influence ingestive behavior phenotypes. In summary, the genetic loci revealed in this study will stimulate an examination of both new and existing mechanisms of food intake regulation.

Does the phenotype depend on the nutrient source or the paradigm? Any of the orosensory or metabolic effects of nutrients contained in the experimental diets used in this study, alone or in combination, could be responsible for the identified QTL. Specifically, the macronutrient intake traits reported here reflect the selection of fat and carbohydrate from two composite diets containing: *1*) equivalent amounts of milk protein providing 22% of kcal, *2*) vegetable shortening as the fat source, *3*) carbohydrate in the form of corn starch and powdered sugar, and *4*) supplemental vitamins, minerals, and cellulose. The sensory contrast between the F/P and the C/P diet formulations is large, allowing for the possibility that diet selection in this paradigm is based on individual sensory characteristics of diets such as taste, smell, or texture (4), or on postingestive effects. Thus the conclusion that mice in this study selected a diet solely on the basis of its generic macronutrient content, i.e., fat or carbohydrate, must be interpreted with caution. The possibility that textural differences in the two diets may have influenced the animals' self-selection was examined in our laboratory by employing liquid, i.e., suspendible high- and low-fat, isocaloric diet mixtures. We found that B6 mice self-selected a higher proportion of energy from the liquid high-fat diet than CAST mice did in a 20-day study (Smith Richards et al., unpublished data), indicating that this phenotypic strain difference generalizes from semisolid to liquid-based diets. We also considered the possibility that sucrose contained in the C/P diet, although a small proportion, could influence phenotypic responses. However, in the present study, no linkage was found for *Sac*, a genetic locus on Chr 4 with a major effect on sweetener preference (1) that contains the candidate gene *Tas1r3* encoding the T1R3 receptor (2, 17, 25, 27, 39). Other unidentified factors could have contributed to the phenotypic responses, and it is likely that a different diet paradigm would discover additional loci related to food consumption traits.

In summary, we have shown that self-selected macronutrient diet intakes, i.e., fat, carbohydrate, and total kilocalories, are inherited as polygenic traits, and we have identified a number of QTL influencing these phenotypes. Phenotypic screening of individual congenic segments is now in process along with the comprehensive identification of candidate transcripts using database search methods, microarray analyses of gene expression, and sequence identification of coding variants within macronutrient QTL.

Perspectives

The results of this study provide new insight into the genetic basis of naturally occurring variation in nutrient intake phenotypes. The identified chromosomal loci determining macronutrient and energy intake in the mouse will more than likely lead to candidate genes involved in human macronutrient selection, an area with particular relevance to the study of obesity, diabetes, and cardiovascular disease. Ongoing studies will allow for the identification and evaluation of candidate

genes, pathways, and systems involved in the voluntary intake of carbohydrate and fat, thereby enhancing our understanding of the mechanisms regulating food intake.

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Preliminary data from this study have been published in abstract form (43).

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