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Genetic determinants of atherosclerosis, obesity, and energy balance in consomic mice

Sabrina H. Spiezio · Lynn M. Amon · Timothy S. McMillen · Cynthia M. Vick · Barbara A. Houston · Mark Caldwell · Kayoko Ogimoto · Gregory J. Morton · Elizabeth A. Kirk · Michael W. Schwartz · Joseph H. Nadeau · Renée C. LeBoeuf

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Abstract Metabolic diseases such as obesity and atherosclerosis result from complex interactions between environmental factors and genetic variants. A panel of chromosome substitution strains (CSSs) was developed to characterize genetic and dietary factors contributing to metabolic diseases and other biological traits and biomedical conditions. Our goal here was to identify quantitative trait loci (QTLs) contributing to obesity, energy expenditure, and atherosclerosis. Parental strains C57BL/6 and A/J together with a panel of 21 CSSs derived from these progenitors were subjected to chronic feeding of rodent chow and atherosclerotic (females) or diabetogenic (males) test diets, and evaluated for a variety of metabolic phenotypes including several traits unique to this report, namely fat pad weights, energy balance, and atherosclerosis. A total of 297 QTLs across 35 traits were discovered, two of which provided significant protection from atherosclerosis, and several dozen QTLs modulated body weight, body composition, and circulating lipid levels in females and males. While several QTLs confirmed previous reports, most QTLs were novel. Finally, we applied the CSS quantitative genetic approach to energy balance, and identified three novel QTLs controlling energy expenditure and one QTL modulating food intake. Overall, we identified many new QTLs and phenotyped several novel traits in this mouse model of diet-induced metabolic diseases.

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S. H. Spiezio
Institute for Systems Biology, 401 North Terry Ave, Seattle, WA 98109, USA

L. M. Amon
Seattle Biomedical Research Institute, 307 Westlake Avenue North, Seattle, WA 98109, USA

T. S. McMillen · C. M. Vick · B. A. Houston · M. Caldwell · K. Ogimoto · G. J. Morton · M. W. Schwartz · R. C. LeBoeuf
Division of Metabolism, Endocrinology, Nutrition, and Diabetes and Obesity Center of Excellence, Department of Medicine, University of Washington, Seattle, WA 98109, USA

E. A. Kirk
Bastyr University, 14500 Juanita Drive, Kenmore, WA 98028, USA

J. H. Nadeau
Pacific Northwest Research Institute, 720 Broadway, Seattle, WA 98122, USA

R. C. LeBoeuf (✉)
Department of Medicine, University of Washington, 815 Mercer Street, Seattle, WA 98109, USA
e-mail: leboeuf@u.washington.edu

Introduction

Environmental factors and genetic variants acting alone and in combination influence two interrelated and highly prevalent metabolic diseases, obesity, and atherosclerosis (Drong et al. 2012; Lusis 2000). Obesity, particularly when coupled with insulin resistance and dyslipidemia, is a significant risk factor for vascular disease, but mechanisms driving this risk remain unclear (Murea et al. 2012). Many genetic studies have attempted to clarify the relationship between obesity and atherosclerosis, but they show that single-gene variants individually and collectively account for only a small part of the genetic variation controlling these disorders (Stefan and Nicholls 2004; Weiss et al. 2012). Thus, continued efforts to characterize gene–gene

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and gene–environment interactions and to identify specific genes remain important endeavors.

To simplify gene identification, animal models have been used because better control of environmental exposures and genetic background allows the effect of particular dietary nutrients on disease induction, progression, and severity to be studied. Also, gene discovery as well as gene–gene and gene–environment interactions can be identified efficiently. Toward these ends, a complete panel of mouse chromosome substitution strains (CSSs) was developed (Singer et al. 2004) starting with two parental strains known to differ markedly in their predisposition to diet-induced obesity (Surwit et al. 1995), atherosclerosis (Paigen et al. 1987b), iron metabolism (Ajioka et al. 2007), and many other traits (Mouse Phenome Database, The Jackson Laboratory, Bar Harbor, ME). This CSS panel consists of 21 inbred strains of mice, each with a single A/J-derived chromosome (Chr) that was introgressed into the C57BL/6J (B6) genome by multiple backcrosses and selection (B6.Chr$^{A/J}$). (A mitochondrial CSS was also generated but was not included in the present study.) This CSS panel is available (The Jackson Laboratory) and has been surveyed previously for hundreds of traits including circulating levels of sterol and amino acids, anxiety (Singer et al. 2004), hemostasis and thrombosis (Hoover-Plow et al. 2006), iron metabolism (Ajioka et al. 2007), pubertal timing (Krewson et al. 2004), acute lung injury (Prows et al. 2007), diet-induced obesity, and many others (Burrage et al. 2010; Hoover-Plow et al. 2006; Nadeau et al. 2012; Singer et al. 2004). In each case, quantitative trait loci (QTL) were identified that controlled significant variation in these traits. Importantly, deep congenic analyses yielded remarkably small genetic intervals, with an average of four genes per interval, and strong candidate genes controlling several complex traits including resistance to diet-induced obesity and glucose homeostasis (Buchner et al. 2008; Millward et al. 2009; Yazbek et al. 2010). These data, coupled with the observation that the CSS surveys identified robust QTLs that were not detected in intercrosses (Burrage et al. 2010), establish the value of this CSS panel in the identification of genes and their functional characterization complex diseases. Furthermore, gene–gene interactions that are emerging as key elements in disease risk, onset, progression, and severity are readily detected in CSSs (Buchner et al. 2008; Shao et al. 2008). The utilization of CSSs has become more widespread based on progenitor strains from genetically diverse subspecies (Gregorova et al. 2008; Takada et al. 2008).

Here, we expand the characterization of the B6.Chr$^{A/J}$ CSSs to include novel phenotypes associated with metabolic syndrome including individual fat pad weights, energy balance, and atherosclerosis. In particular, we identified two novel QTLs associated with protection against atherosclerosis. We identified several dozen QTLs modulating body weight and composition, and circulating lipid levels in females and males. The genetics of energy balance was examined for the first time and three QTLs controlling energy expenditure and one QTL modulating food intake were found. Overall, new and robust QTLs were identified, providing evidence and mouse resources for gene identification and functional studies in metabolic diseases.

Materials and methods

Mice

C57BL/6J and A/J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Breeding pairs for each CSS were obtained from the Case Western Reserve University Animal Resource Center (Cleveland, OH). All animals were maintained in the animal care facility of the University of Washington in a temperature-controlled room (25 °C) with fixed 12-h light/dark cycle. They had free access to food and water but food was removed 4 h in the morning before collection of blood samples from the retro-orbital plexus or at necropsy. Blood was collected in tubes containing 1 mM EDTA, and plasma was stored at −80 °C until analysis. Mice were housed 3–5 mice per cage unless otherwise noted. All experiments were approved by the University of Washington Institutional Animal Care and Use Committee according to the principles of laboratory animal care.

With time, spontaneous mutations that arise at each generation lead to genetic divergence among strains that were originally identical. To minimize the impact of drift, the Jackson Laboratory developed the Genetic Health Stability Program, which involves periodically replacing breeder colonies with mice derived from an archive of frozen embryos (jaxmice.jax.org/geneticheath/stability.html). In this way, the number of generations from the ancestral population is greatly reduced and genetic drift minimized. To protect from subline divergence, CSSs were shared with the Jackson Laboratory for preservation and distribution as a community resource. These CSSs are now maintained as part of Jackson Laboratory's Genetic Health Stability Program.

Experimental design

Mice were maintained on pelleted rodent chow (Wayne Rodent BLOX 8604, Teklad, Madison, WI, USA) (CHOW) until 6–8 weeks of age, at which time they were randomly assigned to one of two treatment groups. For males, diets were CHOW or a high-fat/high-sucrose

(HFHS) diet (#S1850; BioServe Frenchtown, NJ). CHOW contained 4 % fat, 24 % protein and 4.5 % crude fiber. The HFHS diet contained 35.5 % fat (primarily lard), 20 % protein, and 36.2 % carbohydrate (primarily sucrose) as described previously (Schreyer et al. 2002; Surwit et al. 1988). For females, diets were CHOW or an atherogenic diet (ATH) (#TD02028; Harlan Teklad, Madison WI) containing 21.2 % fat (by weight; as milk fat), 1.25 % added cholesterol, and 0.5 % cholic acid (McMillen et al. 2005).

At 6–7 weeks of age, blood was obtained for plasma analytes before the treatment started, and diet feeding initiated for the duration of 14 (females) and 16 (males) weeks. Body weights were obtained at the start and end of the feeding studies. Upon necropsy, body length (snout ventricle length [SVL]) and tissue weights were measured. The Adiposity Index (AI) was calculated as: total fat pad weights/body weight × 100) (West et al. 1994b). At the end of the studies, mice were bled and killed, and plasma was prepared and frozen at −80 °C until analysis. Prior to removal of tissues, mice were perfused with sterile PBS (pH 7.4) via the left ventricle following severing of the hepatic artery. Tissues were removed, weighed, and aliquoted into 10 % neutral-buffered formalin or snap frozen and stored at −80 °C.

For body composition and indirect calorimetry measurements, separate groups of CSS and C57BL/6 male mice were maintained on the HFHS diet for 6 weeks. These mice were housed individually starting at 4 weeks of diet feeding, and body composition and calorimetry were measured during week 5. This short-term feeding regime was undertaken for two reasons. First, this approach increases the likelihood of detecting a change in energy expenditure (EE) that contributed to, rather than being a consequence of, a change of body weight. Second, short-term feeding resulted in groups of mice with similar body weight and composition, allowing a simple normalization of EE by lean body mass (Kaiyala et al. 2010).

Atherosclerosis quantification

Atherosclerosis was evaluated by analyzing serial sections at the aortic root essentially as described (Kunjathoor et al. 1996). In brief, the upper sections of the hearts were fixed overnight in 10 % neutral-buffered formalin and embedded in paraffin the following day. Aortic root lesion area was quantified, beginning at the termination of the aortic valve and spanning 400 µm of the ascending aorta. Every other section (5 µm thick) through the aortic root was taken for analysis. A subset of 5 sections from each animal spanning the region was stained with Movat's stain (Movat 1955) to quantify lesion area (NIH ImageJ software).

Body composition

Analyses of lean and fat mass content was performed on conscious immobilized male mice using quantitative magnetic resonance (EchoMRI 3-in-1 machine whole body composition analyzer; Echo MRI, LLC., Houston, TX) (Taicher et al. 2003; Tinsley et al. 2004). Mouse body weight and composition were assessed immediately before and after the indirect calorimetry measures.

Energy balance

Food intake was measured in two ways: (1) over 4–6 days for HFHS-fed mice that were individually housed, where weighed food was provided daily, and each day excess food was collected, and the difference of food provided versus food not consumed was used to calculate food intake, and (2) using a continuous (24-h) monitoring system (Feed-Scale System; Columbus Instruments) during indirect calorimetry measurements.

Evaluation of energy metabolism was done by indirect calorimetry [measurement of oxygen consumption (VO_2) and carbon dioxide production (VCO_2)] as described (Gelling et al. 2008; Sarruf et al. 2010). Total Energy Expenditure (TEE) is a conversion from VO_2, which is done by Columbus software using the standard Lusk formula (TEE = (3.815 + 1.232 * RQ) * VO_2 in l/h where RQ is the ratio of VCO_2 to VO_2) (Elia and Livesey 1992). Heat was calculated using the Lusk formula (McLean and Tobin 1987). Ambulatory activity was measured by the infrared beam breaks using an Opto-Varimetrix-3 sensor system (Columbus Instruments, Columbus, OH). Consecutive adjacent infrared beam breaks were scored on individual mice as an activity count that was recorded each hour for 24–36 h.

Analytical protocols

Blood glucose levels were measured following a 4 h fast in the morning using blood obtained from a tail nick with a portable glucose measuring device (Accu-Chek Advantage®). Plasma total cholesterol and triglyceride levels were determined using colorimetric kits as described previously (Pamir et al. 2009).

Statistical analyses

Data are reported as mean ± SEM, and statistical significance was established at $p < 0.05$. Student t test and non-parametric ANOVA (Bonferroni correction to account for multiple hypotheses testing with 21 CSSs) analyses were performed. Multiple comparisons were performed as appropriate, focusing on genotype and diet status. Results

were considered significant if the corrected significance level was $p < 0.05$. This implies an expected rate of only 0.05 false positives per trait across the entire panel. ANCOVA analyses were done using packages available in R language (http://www.r-project.org/).

Results

CSS mice fed low fat (control) and high-fat (test) diets were used here to extend phenotypic information previously gathered for these strains fed other types of test diets (Burrage et al. 2010; Singer et al. 2004). Because we wanted to present a comprehensive set of data for metabolic phenotypes across these strains, we added atherosclerosis-related traits for female mice and novel obesity-related traits for male mice (Supplementary Tables 1–4). Significant differences between the CSSs and the host B6 strain, evaluated by a statistical algorithm that accounts for testing multiple hypotheses, were taken as evidence for at least one QTL on the substituted chromosome (Singer et al. 2004). Also, in assigning QTLs to CSSs throughout this report, we accepted QTLs for which CSSs showed significance from B6 and differed by at least 15 % in absolute values. These parameters were chosen in order to account for potential non-genetic variation caused, for example, by mouse coprophagic behavior and residual lipase activities in mouse plasma (Dallinga-Thie et al. 2007), as well as a desire to identify robust QTL that could be followed with panels of congenic strains (Buchner et al. 2008; Yazbek et al. 2010).

Atherosclerosis and plasma lipids are modulated by genetic variants on A/J-derived chromosomes among female mice

For cases of inbred mice lacking genetic modifications, special diets containing cholesterol and bile acids ("ATH diet") are required to induce lesion formation (Nishina et al. 1990). Female mice are more susceptible to lesion formation than males and C57BL/6J (B6) mice, but not A/J are susceptible to diet-induced atherogenesis (Nishina et al. 1993; Paigen et al. 1987a; Wang et al. 2006). Thus, we studied female mice fed an ATH diet to initiate aortic root lesion formation.

Following ATH diet feeding for 14 weeks, aortic root lesion sizes ranged from 82 to 13,500 μm^2 (Fig. 1a; Supplemental Table 1). Among these strains, two CSSs carried QTLs that conferred significant protection from lesion development (CSS-3, 82 ± 55 μm^2, $n = 4$ and CSS-19, 426 ± 100 μm^2, $n = 4$) as compared to B6 ($3{,}026 \pm 369$ μm^2, $n = 5$; $p < 0.0001$ between B6 and both CSSs) (Table 1). The genetic effects resulting from introgression of chromosomes 3 or 19 were strong considering that the B6 strain, along with another strain studied by others (C58/J), is the most susceptible to lesion formation across more than 40 inbred strains tested (Mouse Phenome Database; http://www.jax.org/phenome). In addition, several CSSs showed strong trends for conferring greater lesion development than the B6 parent (e.g., CSS-13 at $10{,}903 \pm 1667$ μm^2, $p = 0.056$ ($n = 5$) and females from the strain CSS-Y at 6962 ± 947 μm^2, $p = 0.081$ ($n = 5$)). These data support previous findings that multiple genes affect atherosclerosis risk (Chen et al. 2007; Lusis et al. 2004). Further, the A/J parent is known to be resistant to lesion formation (Paigen et al. 1987b, 1985). Thus, the paradoxical finding of CSS strains with lesions larger than B6 suggests that the A/J strain carries alleles that confer resistance as well as susceptibility to lesion formation. In the absence of selection for contrasting phenotypes, most strains will harbor combinations of these genetic variants.

Because dyslipidemia is a risk factor for atherosclerosis in mice and humans (Hewing and Fisher 2012), we evaluated plasma lipid levels in ATH fed (Fig. 1b and c; Supplemental Table 1) and CHOW fed (Supplemental Table 2) mice. For CHOW fed mice, plasma total cholesterol (TC) and triglyceride (TG) levels ranged from 55 to 87 and 13 to 60 mg/dl, respectively. Four QTLs were identified for TC levels (for CSSs-1, 4, 5, 13) (Table 1). For TG levels, 20 QTLs were identified and nearly all showed CSS TG levels greater than B6. With ATH diet feeding, TC levels increased for all strains and ranged from 135 to 335 mg/dl. In contrast, TG levels decreased with values ranging from 6 to 28 mg/dl. Eight QTLs were identified for ATH diet TC levels and 8 for TGs, some of which were distinct from TC QTLs (Table 1).

Multiple QTLs were also identified for body weight and length, and relative liver weights (Table 1; compare data in Supplemental Tables 1 and 2). The QTL on Chr. 1 for body weight (BW) is particularly interesting as it shows a consistent pattern of yielding values greater than B6 among both females and males fed CHOW or high-fat diets (Table 2). Overall, 2 and 8 QTLs for BW were found for female mice fed CHOW and ATH diets, respectively. For CHOW-fed females, 13 QTLs were found for body length (SVL) and these were associated with reduced lengths as compared to B6. This suggests that multiple QTL control SVL and that gene effects result in phenotypes seen for the A/J phenotype. We identified 12 QTLs for SVL for ATH diet-fed mice with 7 of these associated with shorter lengths than B6. For relative liver (LV) weights (LV/BW), 1 and 8 QTLs were identified for CHOW and ATH fed mice, respectively.

Interestingly, atherosclerosis lesion sizes were not correlated with plasma lipid or body size parameters (bottom

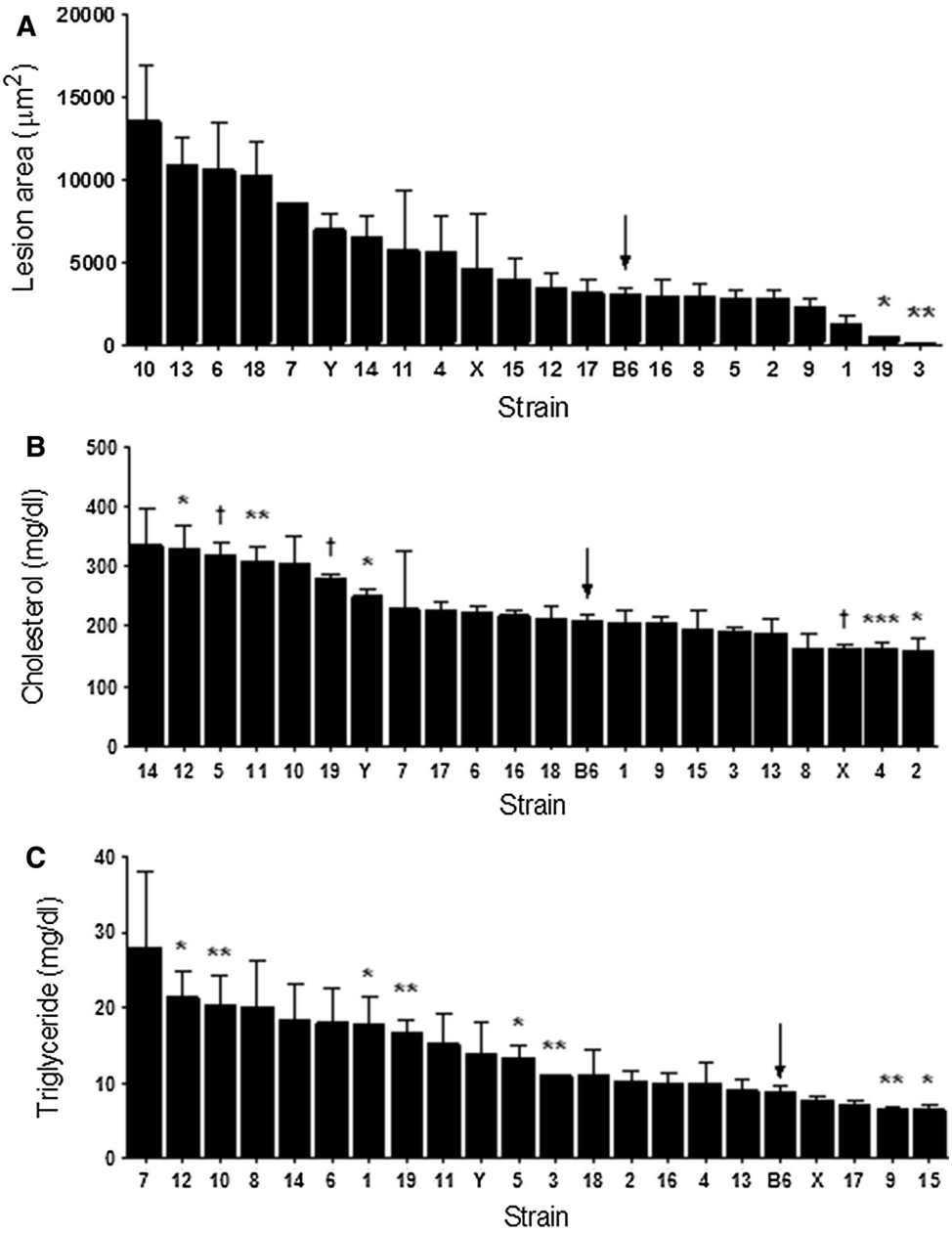

Fig. 1 Atherosclerosis and lipid traits for female mice fed the atherogenic (ATH) diet for 14 weeks. *Values* are presented for 21 chromosome substitution strains (CSSs) and parental strain, C57BL/6 (B6) (*arrow*). Each CSS ("Strain") is labeled with its chromosome number or as '*X*' or '*Y*' chromosomes. **a** Hearts were collected and processed as described in the text for the aortic root lesion area (μm^2) quantification. **b** Plasma triglyceride and **c** total cholesterol levels are presented (mg/dl). Plasma was collected and processed as outlined in the text. Absolute values for mice fed the ATH and CHOW diets are given in Supplemental Tables 1 and 2. *Values* are presented as mean ± SEM, $n = 3$–14; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, $^{\dagger}p < 0.0004$ from B6. Bonferroni corrections were applied to account for multiple hypotheses testing with 21 CSSs

row, Table 3) suggesting that atherosclerosis QTLs are independent of TC and TG. However, this would be revealed more formally during the development of congenic strains. Correlations (r) were found between TC levels and liver weight parameters for CHOW fed (e.g., for LV Mass: $r = 0.71$, $p < 0.001$) and ATH fed ($r = 0.85$, $p < 0.001$) mice. Body weight also correlated with liver weight for CHOW fed mice ($r = 0.58$, $p < 0.01$). For ATH fed mice, body weight and length were correlated ($r = 0.54$, $p < 0.001$). The only correlation observed for plasma TG levels was with mouse length (SVL) for CHOW fed mice ($r = 0.46$, $p < 0.05$).

Overall, significant atherosclerosis QTLs were observed among female CSSs, and two strains (Chrs-3, -19) can be used to identify genetic factors controlling resistance to lesion formation. The decrease in atherosclerosis conferred by these QTL cannot be attributed to changes of either body weight or plasma lipid levels.

Metabolic phenotypes in male mice are modulated by A/J-derived chromosomes

We evaluated 12 and 14 phenotypes for male mice fed CHOW or the HFHS diet, respectively, including body

Table 1 Quantitative trait loci (QTL) for multiple phenotypic traits among female chromosome substitution strains (CSSs) fed rodent chow (CHOW) or the atherogenic (ATH) diet for 14 weeks

Diet	Trait	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y	# QTL
CHOW	BW																						2
	SVL																						13
	L/BW																						1
	TG																						20
	TC																						4
ATH	BW																						8
	SVL																						12
	L/BW																						8
	TG																						8
	TC																						8
	Lesion																						2
#QTL/Chr		5	5	7	5	7	3	2	1	5	3	5	4	5	3	4	3	3	2	8	3	3	

CSSs are listed with their chromosome number (1 through 19) or as 'X' and 'Y'. QTL are shown with shaded boxes. Dark colors indicate trait values greater than B6 and light colors indicate trait values less than B6. The total number (#) of QTL for each trait is given in the far right column and the total number of QTL per chromosome (Chr) is shown in the bottom row

Trait abbreviations are *BW* body weight, *SVL* snout to vent length, *L* liver, *TG* total triglyceride, *TC* total cholesterol, and *Lesion* aortic root atherosclerosis lesion area

weight and length, tissue weights, plasma glucose and lipid levels, and energy balance traits. Several of our clinical traits are unique (adiposity index, absolute plasma TC levels, energy balance) as compared to two previous reports in which plasma sterol levels and diet-induced obesity traits were also evaluated across this CSS panel (Burrage et al. 2010; Singer et al. 2004). An important concept here is that QTLs found to be in agreement among the studies are likely to be robust, providing key starting points for deep congenic analysis (Yazbek et al. 2011, 2010).

Multiple QTLs were identified for metabolic and body composition traits for CHOW and HFHS mice (Table 2). For CHOW fed mice, 8 body weight QTLs were identified with 3 resulting in heavier mice than B6 (Fig. 2a; Supplemental Table 3). Twelve QTLs were identified related to body composition based on AI (Table 2; Fig. 2b) and additional QTLs were found that control individual fat pad weights (Table 2). Three QTLs (seen for CSSs-1, 5, 6) resulted in increased body fat and 9 QTLs (seen for CSSs-4, 7, 10, 12, 13, 15, 17, 19, Y) were identified for reduced body fat. These data suggest that the genetic control of basal body size and composition is complex, involving multiple genes and that A/J alleles contribute to both greater and lesser body fat content.

With high-fat feeding, A/J mice are known to be smaller and leaner than B6 mice (Surwit et al. 1995). But, of 12 QTLs for body weight, 5 manifested greater body weights than B6 (Table 2; Fig. 2a; Supplemental Table 4). Ten body length (SVL) QTLs were found, 8 of which were associated with greater lengths than B6 (Table 2; Supplemental Table 4). We identified 10 QTLs with overall body fat lower than B6 as assessed by AI (for CSSs-4, 9, 10, 11, 12, 13, 15, 16, 17, *X*). In addition, CSS-3, -8, and -14 had lower body weight and lower multiple fat pad weights than B6, suggesting that genes on at least 13 chromosomes regulate physiology toward reduced adiposity. Of interest is that CSS-9 had a reduced AI as compared to B6 but was heavier than B6 due to increases in liver mass as well as inguinal and retroperitoneal fat pads, demonstrating the physiological complexity of the body weight trait. CSS-2 was unique with values for body weight, fat pad weights, and AI greater than B6. Also of note is that 13 QTLs were identified for abdominal fat pad weight and in all cases, weights were lower than for B6. This was not so for other fat pads for which one or more CSSs had fat pads heavier than B6. Thus, genetic regulation among the individual fat pads is likely to be distinct (Taylor et al. 2001), and A/J carries alleles permissive for both increased and decreased fat depot sizes.

Six QTLs for glucose (Table 2; Fig. 2c), 6 QTLs for TG, and 14 QTLs for TC were identified for CHOW-fed mice (Table 2). Blood glucose levels were reproducible within most CSSs (Fig. 2c) even though values were obtained on different days due to staggering of each strain due to breeding constrictions. QTL effects reduced glucose

Table 2 Quantitative trait loci (QTL) for multiple traits among male CSS fed CHOW or HFHS diet for 16 weeks

Diet	Trait	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	X	Y	#QTL
Chow	BW																						8
	SVL																						3
	L/BW																						3
	ABD																						10
	ING																						15
	RET																						10
	BAT																						9
	AI																						12
	Gluc																						6
	TG																						6
	TC																						14
HFHS	BW																						12
	SVL																						10
	L/BW																						10
	ABD																						13
	ING																						12
	RET																						5
	BAT																						11
	AI																						11
	Gluc																						9
	TG																						10
	TC																						8
	Food																						1
	EE																						3
#QTL/Chr		11	10	9	14	7	11	12	9	10	10	11	12	21	4	13	6	12	2	10	8	9	

CSSs are listed with their chromosome number (1 through 19) or as 'X' and 'Y'. QTL are shown with shaded boxes. Dark colors indicate trait values greater than B6 and light colors indicate trait values less than B6. The total number (#) of QTL for each trait is given in the far right column and the # QTL per chromosome (Chr) is shown in the bottom row

Trait abbreviations are *BW* body weight, *SVL* snout to vent length, *L* liver, *ABD* abdominal fat, *ING* inguinal fat, *RET* retroperitoneal fat, *BAT* brown adipose tissue, *AI* adiposity index, *Gluc* blood glucose, *TG* total triglyceride, *TC* total cholesterol, *Food* food intake, and *EE* energy expenditure

and lipid levels as compared to B6, except for CSS-6 for which glucose levels were higher than for B6 mice. For HFHS diet-fed mice, plasma glucose and lipid levels were modulated by 8–10 QTLs per trait (Table 2). Interestingly, the 8 QTLs for TC were again associated with reduced levels as compared to B6 (Supplemental Table 4), as found for CHOW fed mice. For TG levels, 2 of the 10 QTLs resulted in reduced levels as compared to B6 and for glucose, 6 of 9 QTLs showed values lower than B6. In summary, each of these plasma traits shows multigenic control.

Overall, QTLs from every chromosome tested were identified as associated with one or more traits of body weight and composition, and levels of plasma glucose and lipids. These strains provide important resources for further examination of genes involved in body weight and composition. Indeed, studies of CSSs-6 and -17 have already provided a wealth of genetic information concerning control of body weight and glucose homeostasis (DeSantis et al. 2013; Millward et al. 2009; Yazbek et al. 2010)

Correlations among metabolic and body composition traits suggest common genetic controls within each diet group

Relationships among the traits were examined using correlation analyses (Table 4). Among CHOW-fed males, body weight was significantly correlated with mouse length and individual tissue weights, and individual fat pad weights correlated significantly with each other, but not

Table 3 Pearson correlation coefficients (r) between traits for female mice fed rodent chow (CHOW) or the atherogenic (ATH) diet

BW	0.27	0.58**	0.17	0.35	0.11	0.36	–
0.54**	SVL	0	0.17	0.55**	0.46*	0.14	–
0	0.17	LV MASS	0.71***	0	0.40	0.71***	–
0.43*	0.37	0.93***	LV/BW	0.25	0.38	0.54**	–
–	–	–	–	Glucose	0.33	0.11	–
0.17	0.37	0.35	0.36	–	TG	0.15	–
0.13	0	0.85***	0.82***	–	0.36	TC	–
0.14	0.19	0.19	0.23	–	0.36	0.20	Lesion size

The upper right section shows r values for CHOW and the lower left quadrant is for the ATH diet

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

with liver weight. Plasma glucose levels were weakly correlated with BAT and abdominal depot weights, but TG levels showed no significant correlations. Plasma TC levels correlated most strongly with adipose tissue weights ($r = 0.66$–0.71, $p < 0.001$) and less so with body weight ($r = 0.56$, $p < 0.01$) and SVL ($r = 0.51$, $p < 0.05$). Thus, genetic factors are not necessarily acting in concert to control tissue weights and lipid levels for mice in the basal state.

Several of these relationships were significantly altered with high-fat feeding. For HFHS diet-fed male mice, body weight was again correlated with body length as well as with liver and fat pad weights. In contrast to CHOW fed mice, liver weight was highly correlated with weights of three fat pads (inguinal, retroperitoneal and brown adipose tissue, and plasma TC levels ($r = 0.77$, $p < 0.001$)). These results are consistent with known roles for liver in fat synthesis, storage, and lipoprotein production. Surprisingly, HFHS abdominal fat pad weights correlated more weakly with body weight and length ($r = 0.61$, $p < 0.01$) than for CHOW animals and had weak correlations with other fat pad weights, suggesting that among these CSSs, abdominal fat has a different relationship and potential role than other fat pads in establishing body size and lipid levels. Glucose and TG levels were significantly correlated ($r = 0.71$, $p < 0.001$) and this is consistent with the HFHS diet being diabetogenic in nature.

Diet responsiveness varied among strains

A/J mice are considered to be hyporesponsive, and B6 are hyperresponsive to high-fat diet feeding in terms of body weight and composition (Surwit et al. 1995). To examine diet responsiveness as a quantitative trait across CSSs and parental lines, we determined the percent difference in mean values for each of twelve traits for mice fed CHOW versus HFHS diets (using data from Supplemental Tables 3 and 4).

In our study, A/J was the least responsive strain in terms of body weight gain (15 % increase). This is interesting because although multiple genes contribute to body weight (e.g., Table 2), a complete set of A/J alleles is required in concert to confer hyporesponsiveness to feeding of the high-fat diet. CSS-3 was the only CSS strain that showed markedly reduced body weight gain (22 %) comparable to A/J (Fig. 2a), suggesting that this chromosome harbors key gene(s) controlling diet hyporesponsiveness to body weight. Of interest was that CSS-2 and -9 exhibited marked increases in relative body weight with HFHS diet feeding (70–82 %) as compared to B6 (56 %) (Fig. 2a).

In order to better recognize patterns of trait responsiveness among the CSSs, we performed hierarchical clustering across all traits for the 21 CSSs and two parental lines (Fig. 3). Strains fell into three primary diet responsiveness groups. Group 1 (CSSs-4, -Y, -7 and -10) was characterized by relatively large increases in body weight and length as well as increases in fat pad weights for HFHS versus CHOW fed mice. In contrast, Group 2 strains showed lower relative changes in body weight and fat depots. Two sub-groups within Group 2 were further distinguished by their differences in relative changes in glucose and plasma TC levels (CSSs-13, -12, -14, -8, -16, -5, -*X* and B6; CSSs-1, -6, -18, and A/J). Group 3 showed mixed extents of changes in adipose depots and were distinguished largely by relative increases in liver and body weights, with lesser relative changes in plasma glucose levels as compared to Group 2. Overall, the greatest distinguishing characteristics for responsiveness were for changes in tissue weights and glucose levels.

A/J-derived chromosomes modulate energy balance

To better understand the metabolic basis of body weight differences among the CSSs and to explore energy balance parameters as QTL traits, we applied indirect calorimetry, activity, and food intake assessments to several CSSs. We

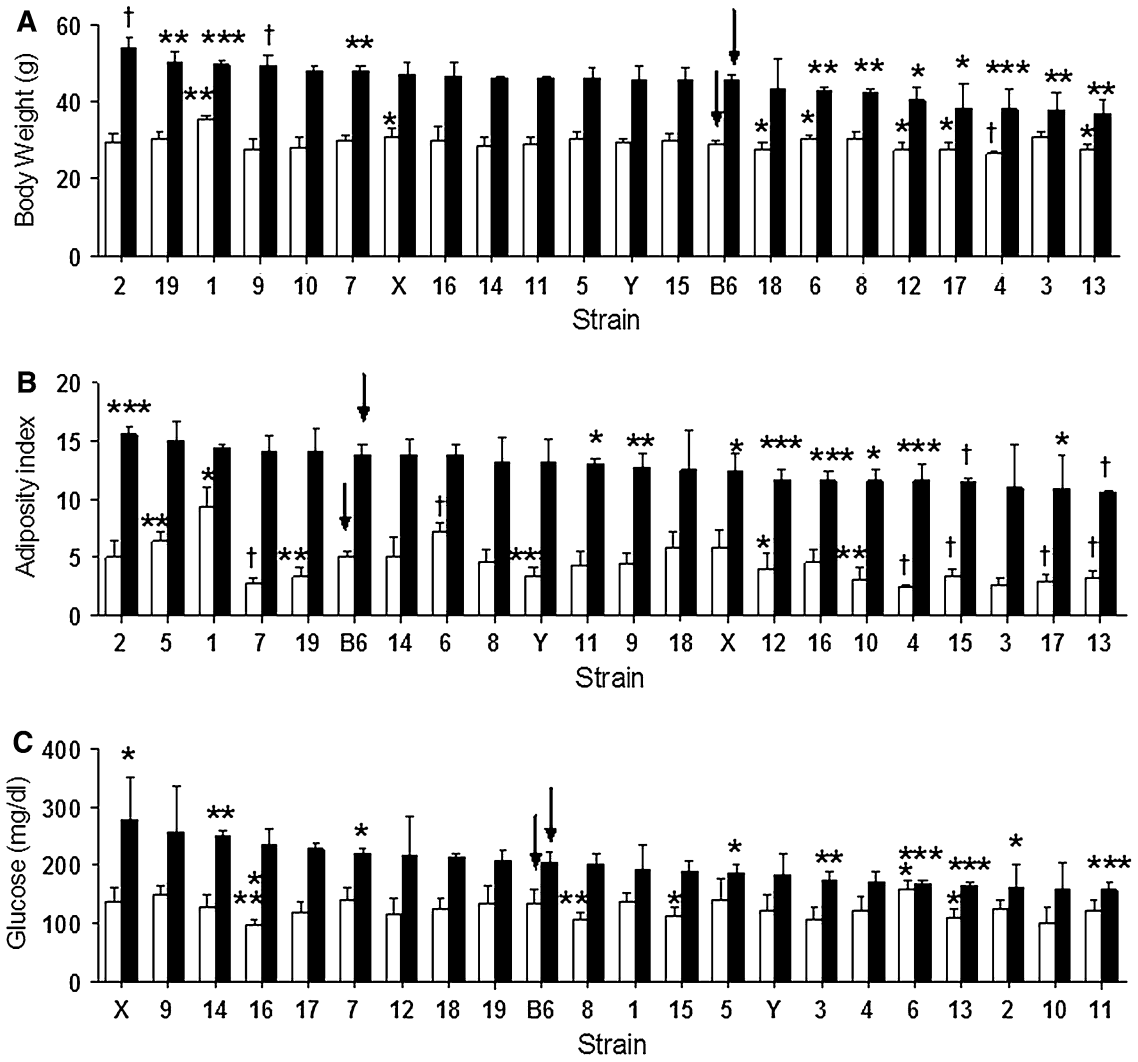

Fig. 2 Body weight, adiposity index, and plasma glucose traits for male mice fed test diets for 16 weeks. *Values* are presented for 21 chromosome substitution strains (CSSs) and parental strain, C57BL/6 (B6) (*arrow*). Each CSS ("Strain") is labeled with its chromosome number or as '*X*' or '*Y*' chromosomes. Trait values are for mice fed CHOW (*open bars*) or high-fat/high-sucrose (HFHS) (*filled bars*) diets. **a** Body weights were taken at the end of the study. **b** Adiposity index reflects overall body fat content and was calculated as: AI = total fat pad weights/body weight × 100. **c** Blood glucose levels were taken at the end of the study following a 4 h fast in the morning. Absolute values for mice fed the CHOW and HFHS diets are given in Supplemental Tables 3 and 4. Please note that although many CSSs show trait values significantly different than B6 (*asterisk*), not all are included as QTL (Table 2). As discussed in the text, in addition to statistical differences between CSSs and B6, to qualify as a QTL, trait values for CSSs must differ by greater than 15 % of B6 trait values in order to identify robust phenotypes needed for eventual congenic strain development (*see text*). *Values* are presented as mean ± SEM, $n = 12$; *Values* are presented as mean ± SEM, $n = 3$–14; $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$, † $p < 0.0004$ from B6. Bonferroni corrections were applied to account for multiple hypotheses testing with 21 CSSs

focused on three CSSs for which HFHS diet-fed body weight and fat mass were higher (CSS-2) and lower (CSSs-4 and -13) than found for B6 at 16 weeks of feeding the HFHS diet. We chose to feed mice the HFHS diet for only 5 weeks in order to test for early onset differences in energy balance parameters. Even at this time point, significant differences were found in body weights and compositions among strains. CSS-2 showed significantly greater relative body fat mass and less lean mass than B6 (Table 5). CSS-13 weighed significantly less than B6, and showed trends ($p < 0.06$) for reduced relative fat and increased lean masses than B6. No significant differences in body weight or composition were seen at this time point between B6 and CSS-4.

Food intake was assessed both manually (not shown) and during indirect calorimetry measurements (Table 5). In both cases, food intakes were comparable among B6, CSS-2, and CSS-4 mice. CSS-13 mice showed significantly higher food intake than B6 mice. Taking into account body weights for these mice, CSS-13 takes in ~30 % more food

Table 4 Pearson correlation coefficients (r) between traits for male mice CHOW or HFHS diet

BW	0.77***	0.80***	0.72***	0.68***	0.74***	0.67***	0.14	0.29	0	0.56**
0.62**	SVL	0.59**	0.50*	0.52*	0.53**	0.49*	0.17	0.24	0.12	0.51*
0.80***	0.34	LV MASS	0.36	0.38	0.40	0.32	0.47*	0.27	0.11	0.40
0.61**	0.61**	0.24	ABD	0.93***	0.98***	0.91***	0.44*	0.43*	0.11	0.68***
0.85***	0.38	0.90***	0.46*	ING	0.96***	0.86***	0.36	0.24	0.37	0.70***
0.83***	0.51*	0.73***	0.50*	0.74***	RET	0.90***	0.39	0.33	0.14	0.66***
0.84***	0.29	0.74***	0.42*	0.77***	0.71***	BAT	0.44*	0.43*	0.12	0.71***
0.54**	0.11	0.93***	0	0.74***	0.52*	0.55**	LV/BW	0	0.12	0.12
0.34	0.44*	0.31	0.24	0.12	0.12	0	0.26	Glucose	0	0.29
−0.33	−0.60**	0.24	−0.32	0.21	0	0	0.12	−0.71***	TG	0.28
0.74***	0.46*	0.77***	0.50*	0.74***	0.60**	0.51*	0.69**	0.53**	−0.60**	TC

The upper right section shows r values for CHOW and the lower left quadrant is for the HFHS diet

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

per gram body weight than B6. These data suggest that CSS-13 exhibits poorer absorption of food and/or greater energy expenditure than B6 to maintain the reduced body weight found for these mice at 5 weeks and even 16 weeks of HFHS diet feeding (Supplemental Table 4). We refer to this QTL as "Food1".

Indirect calorimetry and home activity measurements were used to characterize energy expenditure phenotypes among the strains. Tests were performed for each of the three CSSs simultaneously with sets of age and treatment-matched B6 mice (Table 5). Unadjusted EE during the light and dark cycles (TEE) was significantly higher for B6 than CSS-2, and a strong trend of higher TEE was found for B6 versus CSS-4 ($p < 0.052$). Differences were not significant between TEE values for B6 and CSS-13. Upon normalizing EE for lean mass, no significant differences were found among the genotypes (data not shown).

Since EE is in part a function of body size and it has been shown that simple ratios of EE to body mass or lean mass can yield confounded outcomes (Kaiyala et al. 2010; Speakman 2013), we used regression analysis to better incorporate effects of total body weight on energy expenditure across mouse groups. After adjustment for total body mass using ANCOVA, B6 mice exhibited significantly higher EE than CSS-2 (by 1.08 ± 0.16 kcal/day, $p < 0.008$) and CSS-4 (by 0.65 ± 0.20, $p < 0.03$), suggesting that CSS-2 and CSS-4 mice are hypometabolic as compared to B6 mice. This was also reflected in the unadjusted heat component presented per hour for dark and light cycles for CSS-2 mice, although values did not reach significance for CSS-4 ($p = 0.052$) (Table 5). TEE is composed of an activity and resting metabolic rate (RMR) quotient (van Klinken et al. 2013). Since ambulatory activity was comparable between B6 and each of these CSSs, reduced RMR was primarily responsible for the reduced EE traits. CSSs-2 and -4 provide the first QTL associated with energy expenditure in mice and humans, and we refer to them as "EE1" and "EE2".

CSS-13 mice were also likely to have increased RMR as compared to B6 as they were smaller than B6 but ate more food and showed significantly reduced home cage activity. To explore EE for CSS-13 in more detail, another cohort of age matched male mice (8 weeks of age) maintained on normal rodent chow were examined for EE traits over 6 days. After adjustments for total body mass and room temperature using ANCOVA analyses (there was no correlation between covariates of room temperature and total body mass; (Larson-Hall 2010)), CSS-13 mice exhibited significantly higher EE than B6 (by 0.88 ± 0.14 kcal/day, $p < 0.0.005$ at 25 °C and by 0.81 ± 0.20 kcal/day, $p < 0.02$ at 30 °C), suggesting that CSS-13 mice are hypermetabolic as compared to B6 mice. Thus, we assign a QTL to chromosome 13, referred to as "EE3".

Discussion

Three main findings emerged from this study. First, two QTLs conferring protection from atherosclerosis were identified as well as two provisional QTLs for which lesion development exceeded values for B6. Second, new QTLs conferring pro- and anti-obesity phenotypes were identified including a robust Chr. 2 QTL for marked obesity. Third, for the first time, energy balance QTLs (Food1, EE1, EE2, and EE3) were identified. Energy balance measurements may prove useful for assigning A/J alleles to specific biological functions controlling body weight and composition.

Overall, we report a total of 297 QTLs involving 35 traits, for an average of 8.5 QTLs per trait. These results highlight a general property of CSSs, namely the ability to detect a large number of QTLs. Several large surveys have been conducted in four panels of mouse and rat CSSs with

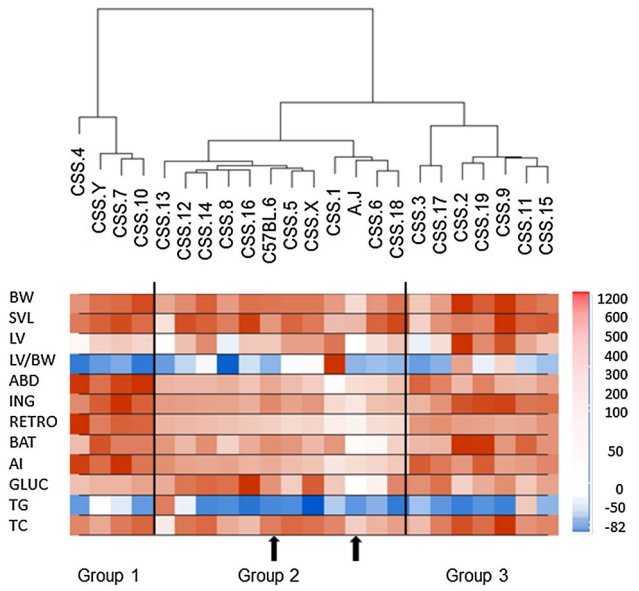

Fig. 3 Hierarchical clustering and trait heatmap among male CSS and parental strains for responsiveness to diet between CHOW and HFHS diets. Diet responsiveness is defined as percent change in mean values for each trait between CHOW and HFHS diets. This was calculated for 12 traits for each of 23 strains. The *upper* dendrogram network is based on squared Euclidean distances between strain groups using the parameter of diet responsiveness across all traits. The dendrogram lists the CSS mouse strains and A/J and C57BL/6 (*arrows*). The *lower* heatmap shows diet responsiveness in terms of *colors* which replace absolute values for percent change from CHOW to HFHS diets. *Each row* represents changes within a trait (listed to the *left* of the heatmap) and ranges of response are given in the *color key* to the *right* of the heatmap. Trait *color* representations are ordered across the 23 strains corresponding to the dendrogram. The preponderance of changes in trait values are *positive* as reflected by the mostly '*red*' coloring across each trait row in the heatmap, with '*blue*' coloring indicating *negative* values (−82 to 1,200 %). Designations for *Groups 1*, *2*, and *3* delineate mouse groups in the dendrogram and are discussed in the text. Trait abbreviations are *BW* body weight, *SVL* snout-vent length, *LV* liver weight, *LV/BW* liver weight/body weight, *ABD* abdominal fat pad weight, *ING* inguinal fat pad weight, *RETRO* retroperitoneal fat pad weight, *BAT* brown adipose tissue weight, *AI* adiposity index (see Fig. 2), *GLUC* blood glucose concentration, *TG* plasma total triglyceride concentration, and *TC* plasma total cholesterol concentration

similar results (Shao et al. 2008; Singer et al. 2004; Spiezio et al. 2012). Singer et al. surveyed a total of 53 traits for serum levels of sterols and amino acids, diet-induced obesity and anxiety and found 150 QTLs. Shao et al. surveyed a total of 90 traits for blood, bone and metabolic traits, finding 342 QTLs. Together, these studies found an average of ~3 QTLs per trait for the B6.Chr$^{A/J}$ CSSs panel (Shao et al. 2008; Singer et al. 2004). These surveys represent the minimum number of QTLs because a significant difference between a CSS and its host strain implicates at least one QTL on the substituted chromosome. Typically many more QTLs are found (Buchner et al. 2008; Shao et al. 2008; Singer et al. 2004; Yazbek et al. 2011). Indeed, Yazbek et al. reported 14 QTLs affecting three traits in a single 30 Mb segment of mouse Chromosome 6 (Yazbek et al. 2011). Other mouse resources usually find many fewer QTLs, despite great genetic heterogeneity in the resource population. Thus, CSSs readily detect many QTLs whose locations on substituted chromosomes can be rigorously resolved with panels of congenic strains that are readily made by using a CSS as the progenitor strain.

Pro- and anti-atherosclerosis QTLs

This is the first time that this CSS panel has been assayed for atherosclerosis. This remains an important endeavor as an understanding of genes involved in atherosclerosis is still incomplete. For instance, dozens of QTLs have been described for mouse atherosclerosis (Bennett et al. 2012; Chen et al. 2007; Hsu and Smith 2013; Kuhel et al. 2002) but few specific genes have been identified (Wang et al. 2004, 2005; Yang et al. 2010). One issue is the poor resolution of linkage analyses across backcrossed and intercrossed family structures. Association studies have not yet been reported (Thaisz et al. 2012). Another issue is that in some cases, atherosclerosis QTL have been seen under conditions of extreme hyperlipidemia instigated by using parental strains carrying mutations in lipid transport genes (Dansky et al. 2002; Smith et al. 2006; Teupser et al. 2006; Yang et al. 2010). Finally, emphasis has been placed on atherosclerosis susceptibility and little information is available on genes encoding resistance to lesion formation (Nadeau and Topol 2006). Here, we identified two QTL providing robust resistance to diet-induced lesion formation located on chromosomes 3 and 19. There are known lipid QTLs on these chromosomes (e.g. for HDL(Ackert-Bicknell et al. 2013)) and further congenic strain development from CSSs-3 and -19 may reveal if the ATH QTLs are due to such plasma lipid genetic factors.

CSS-3 is particularly interesting as there is another report of an atherosclerosis QTL on Chr. 3 identified in an intercross between hyperlipidemic mice carrying the low density lipoprotein receptor deficiency (LDLR−/−) for which FVB/NJ contributed a resistance allele as compared to the other parent, B6 (Teupser et al. 2006). CSS-3 is also distinguished as causing traits that recapitulate human familial combined hyperlipidemia, thought to be driven by a mutation in the thioredoxin interacting protein gene (*txnip*) (Castellani et al. 1998). CSS-19 harbors an atherosclerosis QTL as found in an intercross between FVB and B6, this time carrying apolipoprotein E deficiency (apoE−/−) (Dansky et al. 2002). In this case, the FVB allele promoted atherosclerosis. For both chromosomes, QTL controlling high density lipoprotein (HDL) levels have been identified and it would be worthwhile to follow this trait during congenic studies of atherosclerosis for

Table 5 Energy balance traits for C57BL/6 (B6) and CSS-2, CSS-4, and CSS-13 mice

Trait	B6(2)	CSS-2	B6(4)	CSS-4	B6(13)	CSS-13
BW (g)	28.0 ± 0.4	27.2 ± 0.7	26.7 ± 0.5	25.8 ± 0.6	27.3 ± 0.3	23.7 ± 0.4†
Fat mass (%)	23.4 ± 1.8	29.0 ± 1.8*	21.9 ± 1.1	19.5 ± 1.7	20.1 ± 1.5	16.5 ± 0.6
Lean mass (%)	72.1 ± 1.7	66.9 ± 1.6*	73.6 ± 1.0	75.5 ± 1.5	75.6 ± 1.6	79.3 ± 0.7
Food intake (g)	3.15 ± 0.5	3.19 ± 0.1	3.38 ± 0.41	3.15 ± 0.4	3.05 ± 0.2	3.9 ± 0.2**
TEE D	97.7 ± 3.2	70.2 ± 1.8**	103.1 ± 2.0	96.9 ± 2.5	99.5 ± 2.0	94.1 ± 1.7
TEE L	79.9 ± 2.0	86.4 ± 1.3**	83.9 ± 1.5	79.7 ± 1.5	79.8 ± 1.8	78.1 ± 1.0
VO_2 D (ml/kg/h)	4,858 ± 138	4,783 ± 112	5,258 ± 80	4,992 ± 125	4,831 ± 105	4,637 ± 458
VO_2 L (ml/kg/h)	3,972 ± 79	3,878 ± 54	4,280 ± 76	4,108 ± 81	3,872 ± 97	3,773 ± 372
Heat D (Kcal/h)	0.462 ± 0.015	0.415 ± 0.008**	0.491 ± 0.010	0.459 ± 0.011	0.473 ± 0.010	0.446 ± 0.008
Heat L (kcal/h)	0.378 ± 0.009	0.336 ± 0.009**	0.396 ± 0.007	0.374 ± 0.008	0.377 ± 0.009	0.367 ± 0.005
Amb D	24,707 ± 2,307	25,113 ± 1,666	22,477 ± 1,432	22,148 ± 2,395	24,347 ± 1,528	16,957 ± 869***
Amb L	5,068 ± 495	4,724 ± 434	5,444 ± 1,226	4,100 ± 390	4,840 ± 538	4,556 ± 453

B6(2), B6(4), and B6(13) refer to separate sets of control B6 mice studied in conjunction with CSS-2, -4, and -13, respectively. Male mice were fed with the HFHS diet for 5 weeks prior to conducting indirect calorimetry and ambulatory activity (cage beam breaks) measurements. Food intake was performed during indirect calorimetry analyses. Traits were evaluated as described in the text. Energy expenditure values were averaged over the course of 12-h periods during the *Dark* (D) or *Light* (L) phases

Trait abbreviations are *BW* body weight, *TEE* total energy expenditure, *TEE* [$VO_2 \times 3.941$] + [$VCO_2 \times 1.11$]), VO_2 oxygen consumption as a function of lean body weight, and *Amb* ambulatory activity. *Values* are presented as means ± STD; $n = 8$. Significance is presented as between B6 and CSSs as * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; † $p < 0.0001$

these CSSs. These results support the concept that atherosclerosis is a complex disease and that multiple genes are crucial for determining the final outcome of the disease state. The CSSs-3 and -19 will provide important resources to identifying atheroprotective genes.

Comparative studies and robust QTL for obesity traits

Two previous studies report plasma sterol levels and diet-induced obesity across this CSS panel (Burrage et al. 2010; Singer et al. 2004). We compared our results to these studies in order to identify QTL sufficiently robust to be evident even though we used different diet formations, assay protocols, and husbandry conditions. Such QTLs are most likely to provide key starting points for further deep congenic analyses.

For circulating cholesterol levels, results herein and Singer et al. (Singer et al. 2004) showed an approximately 30 % variation in TC levels among the strains. They identified 8 QTLs and almost all chromosomes (not CSS-16 and CSS-17) were detected among our 14 QTLs. CSSs-5 and -13 were not included in the previous panel (Singer et al. 2004). CSSs-4, 11, 12, X, and Y were clearly replicated between studies and provide particularly useful strains for genetic studies of plasma lipids.

Using diets with low and high sucrose contents, Singer et al. (2004) found 16 body weight QTLs of which we replicated 7, all with body weights less than B6. In addition, we identified 5 CSSs with body weights significantly greater than B6. Overall, CSSs-3, 4, 6, 8, 12, 13, and 17 provide resources to identify anti-obesity genes. Indeed, CSS-6 and -17 have already proven useful for gene identification (Buchner et al. 2008; Millward et al. 2009; Yazbek et al. 2010).

A second study of the CSS panel utilized different diet formulations and durations of feeding than our study (Burrage et al. 2010). Nonetheless, comparing body weight outcomes, significant correlations were found between our HFHS diet-fed males and each of their replicate data sets ($r = 0.643$–0.713; $p < 0.03$). Overall, our current report compares well with previous studies and at least 12 QTLs were found to provide robust and consistent outcomes suitable for future congenic studies.

A/J alleles provide anti- and pro-obesity phenotypes

For CHOW fed mice, A/J showed greater fat pad weights and overall adiposity than C57BL/6 mice as seen previously (Rebuffe-Scrive et al. 1993; Surwit et al. 1995). Three CSSs had QTLs mirroring the A/J response: CSS-1, -5, and -6 and, depending on which fat pad, 9–15 QTLs were found for fat pads smaller than C57BL/6. Due to these multiple QTL, control of fat pad weight even in the 'basal' CHOW fed state is extremely complex.

It is known that A/J mice are more resistant to body weight gain when fed diets high in fat than C57BL/6 mice (Surwit et al. 1995). This occurred here as, compared to CHOW fed mice, A/J mice fed the HFHS diet gained 13 % body weight while C57BL/6 mice gained 35 %. Interestingly, none of the CSSs showed the remarkable weight gain

resistance as A/J. This suggests that multiple A/J chromosomes are required for full manifestation of resistance to diet-induced obesity (DIO). In fact, multiple A/J chromosomes conferred greater body weights and fat contents as compared to C57BL/6. In particular, CSS-2 and -9 showed increased body weight and increased fat pad weight for two or more fat depots. Such paradoxical allelic affects have been seen in another study of obesity in an F2 cross between lean SWR versus fat AKR mice for which SWR alleles contributed to fatness (West et al. 1994a). Again, control of body fat and body fat distribution is complex and likely influenced by additive effects, epistasis, modifier genes, and mode of gene inheritance. Performing subcongenic analyses of CSS-2 will be particularly helpful in identifying genes promoting DIO. For the study of genes protective toward DIO, strains CSS-3 and -13 will be particularly informative due to reduced diet responsiveness and smaller size, respectively, for these strains.

We tested the hypothesis that basal body composition predicted overall diet responsiveness. Body weights and AI for CHOW fed mice correlated significantly to corresponding values for mice fed the HFHS diet ($r = 0.400$ for body weight and $r = 0.578$ for AI; $p < 0.05$, $n = 19$ strains).

Energy balance QTLs

Energy homeostasis is the process by which stable body weight is maintained by matching energy intake to energy expenditure (EE) (Guyenet and Schwartz 2012; Schwartz 2006). Distinct traits of energy balance include feeding behavior, energy expenditure, and substrate utilization. By identifying QTLs associated with these traits, our goal was to identify new sub-phenotypic QTLs that may simplify our understanding of diet-induced obesity. Several energy balance QTLs were identified including food intake on Chromosome 13 (Food1), two QTLs for hypometabolism on Chr. 2 (EE1) and Chr. 4 (EE2), and one for hypermetabolism on Chr. 13 (EE3). To our knowledge, only one other report has explored the genetics of energy balance traits (Mathes et al. 2011) although QTLs were not identified as was done herein. Because of the wide diversity in traits among genetically distinct traits, additional genetic studies could be used to identify genes and gene pathways controlling energy balance and thereby, obesity.

Several key points can be derived from these findings. First, QTLs modulating energy homeostasis can be identified among sets of mice lacking major gene targeted mutations, supporting the robustness of indirect calorimetry measurements. Second, the QTLs identified here can now be explored further in terms of gene identification using deep congenic analysis as done previously for obesity (Yazbek et al. 2010). Third, these studies open the door to utilizing other genetic mouse resources for systems genetic approaches of energy balance such as the Collaborative Cross and Hybrid Mouse Diversity Panel (Bennett et al. 2012; Welsh et al. 2012). Fourth, distinct sets of genes contribute to body weight and composition as evidenced by the differing chromosomes contributing to these traits.

Finally, data show that energy balance is in part, under genetic control and thus, in studies of genetically modified mice, control strains should be of the same genetic background in order to clearly delineate contributions from experimental groups.

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