THE GENETIC CONTRIBUTION TO INGESTIVE PROCESSES: AN INBRED MOUSE STRAIN SURVEY

by

Sarah R. Lewis-Levy

A dissertation submitted to the Graduate Faculty in Psychology/Neuropsychology sub-program in partial fulfillment of the requirements for the degree of Doctor of Philosophy, The Graduate Center of the City University of New York

2009

This manuscript has been read and accepted for the
Graduate Faculty in Psychology in satisfaction of the
dissertation requirement for the degree of Doctor of Philosophy.

Date	Richard J. Bodnar, Ph.D.
Date	Dr. Maureen O'connor

Richard J. Bodnar, Ph.D.

Susan D. Croll, Ph.D.

Benjamin Kest, Ph.D.

Supervision Committee

THE CITY UNIVERSITY OF NEW YORK

Abstract

THE GENETIC CONTRIBUTION TO INGESTIVE PROCESSES: AN INBRED MOUSE STRAIN SURVEY

by

Sarah R. Lewis-Levy

Adviser: Dr. Richard J. Bodnar

Genetic variance in the consumption of sweet and bitter tastants as well as fat intake has been established through the use of inbred mouse strains. The use of limited ranges of strains, limited sets of concentrations, a failure to examine genetic variance for glucoprivic- or lipoprivic-induced intake formed the basis of this dissertation. The aims investigated whether 11 inbred (A/J, AKR/J, BALB/c/J, CBA/J, C3H/He/J, C57BL/6/J, C57BL/10/J, DBA/2/J, SWR/J and 129P3/J) and one outbred (CD-1) mouse strains differed in their intake of a wide concentration range of sucrose (0.0001-20%) or fat (Intralipid: 0.00001-5%) and intake (1-4 h) following the anti-metabolic glucose analogue, 2-deoxy-D-glucose (2DG: 200-800 mg/kg) or the free fatty acid oxidation inhibitor, mercaptoacetate (MA: 5-100 mg/kg).

Sucrose intake was assessed across 9 concentrations using two-bottle tests controlling for concentration order, bottle positions, and kilocalorie intake consumed as sucrose. Strain specific differences were observed for the amounts and percentages of

sucrose intake as well as for compensatory decreases in chow intake as sucrose concentrations increased. Sucrose intake correlated with previous data for saccharin intake and variants of the *Tas1r3* taste receptor gene, particularly at lower concentrations.

To assess whether strain differences in fat intake were similar to those of sucrose intake, a highly similar methodology examined intake of Intralipid across 9 concentrations in the same strains. There were also clear and consistent strain differences across a wide range of measures for Intralipid intake. Interestingly, there were marked positive correlations in strain-specific intakes of sucrose and Intralipid.

Glucoprivic intakes were evaluated following systemic 2DG. Strain specific differences were observed in 2DG-induced feeding across different doses. A parallel study evaluating strain-specific lipoprivic intake used MA. Strain specific differences were also observed in MA-induced feeding. Correlations were generally not observed between lipoprivation and glucoprivation, lipoprivation and fat intake, and glucoprivation and sucrose intake, suggesting different mechanisms of action for observed genetic variance.

Together, the results of the four aims for this dissertation demonstrate a strong and distinct role for genetic variance in these four types of homeostatic ingestive responses that have implications for elucidating the genetic and environmental factors that may contribute to obesity.

Table of Contents

List of Tables

List of Figures:

I. Rationale for Specific Aims:

Systematic analyses of mouse strain differences have been used to understand the genetic control of many aspects of ingestive behavior. Reviews of the heritability of the consumption of sweet tasting carbohydrate sources and high fat diets (Reed et al.,1997; West & York, 1998) not only indicate the presence of genetic variance in ingestive responses, but present evidence for the identification of strains with divergent sensitivities for subsequent quantitative trait loci (QTL) analyses. QTL analyses are used to localize chromosomal regions, and ultimately genes, critically involved in such differences. In addition to studies examining food and water intake per se and/or macronutrient choice, two major behavioral approaches using preference tests between a given ingestive stimulus and a control (e.g., water) are commonly employed to assess this genetic variance, especially for hedonically-driven ingestive stimuli. The first approach involves comparing two or a small number of inbred mouse strains to make specific comparisons between "sensitive" and "insensitive" strains for a particular ingestive response. Many of these types of studies are among the older studies in the literature, although this approach is also used in many more recent genetic QTL studies to define gene loci. A second, more recent approach uses large numbers of inbred strains. This latter form of a strain survey insures reliability, allows the assessment of heritability estimates, and facilitates the identification of a number of strains with highly divergent responses in order to optimize the success of subsequent QTL mapping. Both of these types of studies have been successful at demonstrating strain differences in food and water intake as well as for a wide array of ingestive stimuli such as salts, bitter tastants, saccharin, sugars, ethanol, glutamate/umami and fats.

Inbred Mouse Strains: When assessing the genetic contribution to any phenotype, comparisons of isogenic inbred strains are the most widely used, and are in practical terms, singularly utilitarian. A given inbred strain of mice, by virtue of repeated brother-sister mating (such as those bred for at least 20 generations and distributed by Jackson Laboratories in Bar Harbor, ME), are homozygous at every allele and genetically identical to one another. Yet each inbred strain possesses a unique genotype, and comparisons among strains can further isolate genotype differences as a function of behavioral differences. Furthermore, genetic models using inbred mice can be potentially more instructive than using transgenic mouse models because they are not subject to possible confounding variables associated with mutations, such as developmental, compensatory, and multiple behavioral effects (pleoitropy) of the mutated gene (see review: Mogil & Grisel, 1998). Therefore, this dissertation utilized eleven inbred mouse strains (A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL6/J, C57BL10/J, DBA/2J, SJL/J, SWR/J, 129P3/J) that includes those strains most frequently cited in prior studies examining the genetics of ingestive behaviors. In addition to allowing for replicability and comparisons to previous findings, use of these major groups of mouse strains provides the benefits of easy accessibility and recourse to the extensive descriptive data concerning their scientific history, use in research, and behavioral characteristics (with particular interest in behaviors related to ingestive responses, the purpose of this dissertation). These data, which include descriptions of characteristics of each inbred mouse strain bred by the Jackson Laboratory, has been collected and collated, and is available at the Jackson Laboratory website (**www.jax.org/jaxmice**) and its links to the Mouse Phenome Database (**www.jax.org/phenome**) as well as the Mouse Genome

Informatics (www.informatics.jax.org). Further, the inclusion in this dissertation of a commonly-used outbred wild- type strain (CD-1) allows for direct comparisons of ingestive responses to inbred strains. In contrast to inbred strains, outbred mice are genetically heterogeneous from one another, and therefore any variance in their behavioral responses cannot be attributed to genetic factors. Thus, the use of an outbred strain introduces an additional control when investigating the role of the genetic contribution to ingestive responses, and allows for the understanding of global changes in increased or decreased behavioral sensitivity as a function of inbreeding per se.

Ingestive Stimuli: In addition to the inherent nutritive and caloric value of specific ingestive stimuli, substances such as sugars and fats also possess hedonic features that contribute to the high degree of preference for these macronutrients in animals. The hedonic component of ingestion involves both orosensory stimulation as well as post-ingestive consequences (e.g., Sclafani, 2006b). The orosensory mechanism involves taste and texture receptors in the oral cavity, whereas post-ingestive mechanisms include the digestive consequences once food reaches the gastrointestinal tract. Most studies examining genetic variance in ingestive responses employed a preference test approach, to infer differences in responses to hedonic stimuli across murine strains. However, animals will also behaviorally respond to overall (e.g., deprivation) as well as specific lack of availability of specific macronutrients, (i.e. glucose or lipids) from utilization by increasing food intake, and thereby reveal much about their respective roles in the regulation of food intake. Thus, in addition to over-consumption of sugars and fats because of hedonic attractiveness, animals can be motivated to increase overall food intake in response to such homeostatic challenges as glucoprivation and lipoprivation.

By temporarily depleting the body's resources of glucose and lipids respectively through the administration of 2-deoxy-D-glucose (2DG: e.g., Wick et al., 1957) and mercaptoacetate (MA: e.g., Bauche et al., 1981), an animal will necessarily compensate for this challenge by increasing intake (e.g., Langhans and Scharrer, 1987; Scharrer and Langhans, 1986; Smith and Epstein, 1969; Smith and Root, 1969) to re-establish homeostasis of its bio-system. Davidson et al. (1997) proposed a relationship between intake and privation, with selective sensitivity to sucrose and fat following the induction of glucoprivation and lipoprivation respectively. Additionally, investigating ingestion as a consequence of homeostatic challenges allows for the analysis of the metabolic features of food intake without the additional behavioral orosensory elements involved in preference studies. Therefore, the present series of studies extends the analysis of genetic variance in ingestive responses to the analyses of hedonically-driven stimuli including sugar (sucrose) and fat (Intralipid) sources, as well as to homeostatic mechanisms in which temporary nutrient-specific privation of glucose (2DG) and lipids (mercaptoacetate) was achieved. This allowed for the assessment of potential differences in genetic variance as functions of palatable intake (sucrose vs. Intralipid), homeostatic state (glucoprivation vs. lipoprivation), glucose manipulation (sucrose access vs. 2DG-induced glucoprivation), and lipid manipulation (Intralipid access vs. mercaptoacetate-induced lipoprivation).

Hypotheses and overall controls:The four studies comprising this dissertation addressed four specific aims.

Specific Aim 1: The first specific aim was designed to examine whether genetic variance exists in gluco-sensing mechanisms by surveying the ingestive responses of

eleven inbred mouse strains and one outbred strain in their respective abilities to detect and consume nine different concentrations of sucrose (0.0001, 0.001, 0.01, 0.1, 1.0, 2.5, 5, 10, 20%) as compared to water intake in two-bottle preference tests over 24 hour intervals with chow intake assessed as well, while position preference and orders of sucrose presentation were controlled. Based on previous studies with more limited numbers of strains and sucrose concentrations (see Background section), it was hypothesized that divergent responses will manifest themselves among the inbred and outbred strains in terms of the magnitude of sucrose intake consumed at particular concentrations, the sensitivity to different sucrose concentrations (as measured by significant differences from corresponding water intake), and in the changes in kilocalorie intake of sucrose relative to chow across concentrations. If found, it would demonstrate a role for genetic variance in sucrose intake.

Specific Aim 2: To assess commonalities or differences in genetic variance between sugar and fat intake, the second specific aim examined whether genetic variance exists in lipo-sensing mechanisms by surveying the ingestive responses of the same eleven inbred and outbred mouse strains to detect and consume nine different concentrations of Intralipid (0.00001, 0.0001, 0.001, 0.01, 0.1, 0.5, 1, 2, 5%) as compared to water intake in two-bottle preference tests over 24 h intervals in a protocol identical to that described in the first specific aim. It was hypothesized that divergent responses will again manifest themselves among the inbred and outbred strains in terms of the magnitude of Intralipid intake consumed at particular concentrations, the sensitivity to different Intralipid concentrations (as measured by significant differences from corresponding water intake), and changes in kilocalorie intake of Intralipid relative to

chow across concentrations. If found, it would demonstrate a role for genetic variance in intake of liquefied fat. The use of highly-similar protocols for analyses of genetic variance of sugar and fat intake allowed for further statistical comparisons to assess whether the contribution of genetic variance to these ingestive responses was similar or divergent as a function of the macronutrient.

Specific Aim 3: Whereas the first two aims examined spontaneous intake of sugars and fats, the third and fourth studies examined whether genetic variance occurred in the increased chow intake following homeostatic challenges specific to glucose and lipid availability. Thus, the third specific aim of this dissertation was to examine ingestive responses of eleven inbred mouse strains and one outbred strain in their ability to increase food intake across a time course (1-4 hours) following the induction of glucoprivation by systemic administration of the anti-metabolic glucose analogue, 2DG, at doses of 200, 400, 600 and 800 mg/kg relative to vehicle control with dose order counterbalanced (ascending-descending) within and between strains. It was hypothesized that divergent responses will manifest themselves among the inbred and outbred strains in terms of the magnitude and sensitivity of chow intake to glucoprivation induced by systemic 2DG. The use of protocols evaluating genetic variance of sucrose intake on the one hand and glucoprivic responses on the other hand allowed for analytic comparisons to assess whether the contribution of genetic variance to these ingestive responses was similar or divergent as a function of glucose state (access versus privation), and can demonstrate similar or dissimilar genetic mediation for this form of ingestive behavior.

Specific Aim 4: The fourth specific aim of the dissertation was to survey the same strains for their ability to increase food intake across a time course (1-4 hours) following

induction of lipoprivation by systemic administration of the free fatty acid oxidation inhibitor, mercaptoacetate at doses of 5, 35, 70 and 100 mg/kg relative to vehicle treatment in a protocol otherwise identical to Specific Aim 3. It was hypothesized that divergent responses will manifest themselves among the inbred and outbred strains in terms of the magnitude and sensitivity of chow intake to lipoprivation induced by systemic Mercaptoacetate. This protocol permitted comparisons of genetic variance of fat (Intralipid) intake with lipoprivic (Mercaptoacetate) responses, as well as comparisons of genetic variance of glucoprivic (2DG) intake with lipoprivic (Mercaptoacetate) responses. These comparisons can assess whether the contribution of genetic variance to these ingestive responses was similar or divergent, as a function of lipid state (access versus privation) or as a function of the type of privation (glucoprivation versus lipoprivation).

Potential Consequences: Correlational analyses of strain distribution patterns in intake responses among these four studies can identify possible genetic commonalities and differences. For example, if rank order differences in sucrose sensitivity among strains were very similar to strain-dependent differences in the magnitudes and sensitivities to feeding responses induced by glucoprivation, it would support a unitary mechanism in gluco-sensing responses. In contrast, if large differences in strain rank-order sensitivities were found between gluco-sensing and glucoprivic responsivity, this would support the notion that they operate via very different genetic mechanisms of action. Similar analysis would apply to fats. In conclusion, for both sugars and fats, the demonstration of genetic correlation of two heritable traits (i.e. sensing ingestive responses and privation-induced ingestive responses) among isogenic strains can be used

as evidence of the existence of pleiotropic genes with a common influence on both traits (Hegmann & Possidente, 1981). Furthermore, positive correlations between inbred mouse strain surveys of gluco-sensing and lipo-sensing responses with two-bottle preference tests can reveal a common genetic mechanism for this form of spontaneous intake. This would support Davidson et al.'s (1997) proposal of "metabolic memories" leading rats to selectively respond to sucrose or peanut oil following the induction of glucoprivation or lipoprivation respectively. Finally, a correlation between glucoprivation-induced feeding and lipoprivation-induced feeding, would suggest a common genetic mechanism for these homeostatic responses.

Controls: Interpretation of studies in the area of genetic analysis of food intake is limited by the absence of important controls. The order of presentation of concentrations for different substances has been shown to be a significant variable in prior work (Harder et al., 1989). These control variables were considered in the present studies by counterbalancing the concentration order for both palatable substances (sucrose and fat) in the two-bottle preference test, as well as for the order of the dosages used to induce privation states (glucoprivation and lipoprivation). The potential carry-over and learning effects elicited by the order of presentation of concentrations and dosages were minimized by exposing half of the mice both within and across strains to an ascending concentration order and the remainder to a descending concentration order.

Another potential confounding variable was addressed by systematically switching the bottle positions of the sucrose or Intralipid solution bottle and water bottle across animals and across strains according to a left(L)-right(R)-R-L and a R-L-L-R sequence. This eliminated potential bottle position preference effects, which was another

variable of potential importance in prior work (Bachmanov et al., 2002b). Finally, along with the intakes from the two bottle preference tests, careful measurement of chow intake was simultaneously assessed to allow for the determination of strain differences in kilocalorie intake as a function of sucrose or fat concentration relative to chow. This also allowed for the analysis of compensatory control of total kilocaloric intake when given the additional opportunity to consume sugars and fats. The studies for this dissertation extended prior research by employing the above controls and by the utilization of a larger number of mouse strains (11 inbred and one outbred), across a greater range of nine different palatable concentrations (0.0001%-20% for sucrose and 0.00001%-5% for Intalipid) in the two-bottle 24-h preference tests. Use of this large number of strains and a broader range of concentrations allowed for the potential detection of a wide variety of responses and hopefully provided a more comprehensive survey of genetic variance for subsequent use in QTL analysis. These hypotheses can be viewed in terms of the body of work examining genetic variance in different forms of ingestive behavior that are presented in the next Background section.

II. Background:

Many studies have reported divergent responses in inbred strains for a variety of ingestive stimuli. To characterize the expanse of such studies, the first major section of the Background reviewed previous literature addressing genetic variance among inbred mouse strains in their ingestive responses to four quantitatively and qualitatively different types of tastes: 1a) salts, 1b) bitter tastants, 1c) ethanol, and 1d) glutamate and umami taste. The second major section of the Background reviewed previous literature addressing genetic variance among inbred mouse strains in their ingestive responses to the hedonic classes of stimuli typically eliciting the most pronounced increased intake: 2a) saccharin, 2b) sucrose, and 2c) fats. The third major section of the Background reviewed the mechanisms of action of responses that increase intake through temporary inactivation of metabolic pathways: 3a) glucoprivation and 3b) lipoprivation. This is followed by a series of hypotheses (Part 4) of the Background studies that form the rationale for the present set of proposed studies.

1A. Salts: Animals often voluntarily consume more salt than is required for their growth and survival (Stricker, 1990). Although salt may be have a palatable or aversive taste depending on the concentration, it is thought to be detected in the mouth by taste receptor cells, whose precise identity remains speculative and controversial (Chandrashekar et al., 2006). One approach to understanding salt intake in non-deprived animals is comparing individual differences for this behavior. The use of inbred mouse strains for the investigation of the genetic and experiential effects of taste became an attractive model following the publication of seminal studies assessing murine strain differences for the intake of bitter tastants such as sucrose octaacetate (Lush, 1981),

strychnine (Lush, 1982), quinine (Lush, 1984) and sweet tasting substances (Lush, 1989). This model has also been used for the investigation of salt intake, and generally involves the use of two-bottle preference tests, which compare the drinking preferences for sodium chloride (NaCl) solutions to water between different inbred strains. Preferences are generally defined as significant differences in the percent of the NaCl solution intake over the total fluid intake. A relatively early study (Beauchamp & Fisher, 1993) compared salt intake in two different strains, C57BL/6J and 129/J. When tested with moderate concentrations (0.075-0.15 M) of NaCl solution in 48-hour preference tests, the 129/J mice showed significant preferences for this solution relative to water, whereas the C57BL/6J mice rejected this range of solutions by consuming significantly less of these of these solutions. Thus, the intake of saline solution was related to genetic variance in these two inbred mouse strains, and this effect was more pronounced in mice previously exposed to the NaCl solutions. Whereas C57BL/6J mice which had prior NaCl experience displayed even more marked avoidance responses, intake of the NaCl solution failed to differ in naïve relative to experienced 129/J mice. Post-ingestive consequences of saline consumption were provided as a partial explanation for the differences in salt preference between these two strains (Beauchamp & Fisher, 1993).

Three other studies extended the investigation of the influence of experiential factors on salt intake, using two inbred strains: C57BL/6J and 129X1/SvJ. Tordoff and Bachmanov (2002) evaluated test duration effects on salt preferences, and found that the exposure of the strains to 2-4 days of repeated testing enhanced their salt preferences, and therefore recommended this as a more sensitive approach to reduce neophobia when using two-bottle preference tests. Tordoff and Bachmanov (2003a) examined whether

choice, as defined by the number of bottles available, influenced salt preferences in these two strains. They found that the use of three or more bottles, in which two or more contained saline solutions, enhanced salt preferences. This effect was monotonically related to the number of NaCl spouts available. It should be noted that the latter two studies (Tordoff & Bachmanov, 2002; 2003a) dispute the previously-reported finding (Beauchamp & Fisher, 1993) of a greater salt preference in 129/J mice relative to C57BL/6J mice. Tordoff and Bachmanov showed a higher NaCl preference for the C57BL/6J strain, when compared to the 129X1/SvJ strain. Further, the enhanced preferences following exposure of 2-4 days of testing (Tordoff & Bachmanov 2002) in both strains differed from the previous finding (Beauchamp & Fisher, 1993) of no influence of prior exposure on the 129/J mice. These differences might be attributed to the fact that different substrains of 129 mice (129X1/SvJ versus 129/J) were studied. In our present series of studies, we used the same strains of inbred mice across all four paradigms. Additionally, because different sodium chloride concentrations were used in the earlier (Beauchamp and Fisher, 1993) and later (Tordoff and Bachmanov, 2002; 2003a) studies, the present series of studies tested the same concentrations of sucrose or Intralipid, or the same doses of 2DG and mercaptoacetate across each of the eleven strains.

Finally, a third experiential variable analyzed was the influence of different maintenance diets on saline preferences (Tordoff et al., 2002). Larger differences between the C57BL/6J and 129X1/SvJ mice preference scores for a 75-mMol/L sodium chloride solution were observed when the strains were maintained on purified diets than standard cereal-based diets (commonly referred to as “chow”). Because the maintenance

diet can have strain-dependent effects on taste solution preferences, the present studies standardized lab chow across all paradigms to allow for direct comparisons.

The above referenced studies utilized only two inbred strains (one supposedly sensitive, and the other sub-sensitive) to compare salt intake responses. These findings were extended by other studies that increased the number of strains under evaluation, allowing for a wider range of responses. Evaluation of five mouse strains (NZB/B1NJ, SM/J, 129/J, C57BL/6ByJ and CBA/J) chosen for expected variability in traits and progenitors of several sets of recombinant inbred strains, were exposed to a range of sodium chloride solutions (Bachmanov et al., 1998b). NZB/B1NJ mice demonstrated greater NaCl preference than intermediate 129/J, SM/J and C57BL/6ByJ strains which were followed by the lowest responders, the CBA/J strain. Whereas both the NZB/B1NJ and 129/J mice displayed strong preferences at low concentrations, only the NZB/B1NJ group showed persistent preferences at the highest concentrations, suggesting that separate genes underlie the strain differences in acceptance of dilute and concentrated NaCl solutions. This observation demonstrates the benefit of employing a wide range of testing concentrations. Presentation order significantly affected NaCl solution intake and degree of preferences in all strains. Ascending order of presentation of the NaCl concentrations yielded greater preferences for lower concentrations, and descending order resulted in greater avoidance of lower solution concentrations. This finding agrees with other reports of prior exposure influencing taste preferences (Harder et al., 1989; Tordoff & Bachmanov 2002). Therefore, order of presentation of either sucrose or Intralipid concentrations on the one hand, or 2DG or meracaptoacetate doses on the other hand might also constitute a significant factor, and provides a rationale as to why order effects

(ascending and descending series) were counterbalanced within and across strains across all four paradigms.

A subsequent evaluation of 28 mouse strains (Bachmanov et al., 2002a) confirmed that NZB/B1NJ mice most avidly consumed high concentrations of NaCl, but not for potassium chloride (KCl) or calcium chloride ($CaCl_2$) solutions. At lower sodium chloride concentrations, CAST/EiJ mice showed the strongest preferences, whereas CBA/J, C3H/HeJ and AKR/J mice showed the strongest avoidance of NaCl. By greatly increasing the number of strains under analysis, this study allowed for the identification of a mouse strain (CAST/EiJ) that showed greater preferences for lower NaCl concentrations than in the previous study (Bachmanov et al., 1998), demonstrating the significant benefit of large strain surveys.

1B. Bitter Tastants: Because of the well-established relationship between bitterness and toxicity, it is important to survival for free ranging animals, such as mice, to be able to recognize and reject bitter tasting stimuli (Stricker, 1990). One of the earliest studies investigating strain differences in response to bitter substances (Warren & Lewis, 1970) employed a two bottle preference test of water and increasing concentrations of a solution of sucrose octa-acetate (SOA), a bitter sugar derivative. The degree of acceptance of SOA versus water was compared for outbred wild type mice to four inbred mouse strains (C57Bl6, C57L, C3Hf/He and CFW) in order to assess the heritability of this response. The CFW strain showed the greatest degree of avoidance for the SOA solution compared to the other inbred strains and outbred mice. Through the use of QTL analysis, it was observed that the dominant allele, *Soa,* was responsible for the disparity in the detection and avoidance of the SOA solution. Thus, mice

homozygous for the allele, *Soa,* relative to their heterozygous counterparts displayed greater aversion to a bitter substance. Although this study controlled for bottle positions, it did not control for the order of the SOA solution presentation, which has since been shown to be a salient variable for taste preference responses (Bachmanov et al., 1998; Harder et al., 1989; Tordoff & Bachmanov 2002). Lush (1981) extended these results, testing 31 inbred mouse strains and two outbred strains for their responses to drinking a single SOA concentration (0.1mM). Because the SWR/J strain was the only strain to show an aversion to this low concentration, two allelic forms, Soa^a for avoidance and Soa^b for indifference were proposed to explain the genetic differences between the mice that showed an aversion to SOA and those that did not. A "demitaster" category of mice was subsequently described (Harder et al., 1992) when 14 inbred strains were analyzed in two-bottle preference tests for intake of a milder 0.1 SOA concentration as compared to a more concentrated 1mM SOA solution. The intermediate phenotype included eleven strains that avoided the 1mM but not the 0.1mM SOA solution, and thereby possessed a third allele (Soa^c) representing this intermediate response to SOA (Harder et al., 1992). These results emphasize the importance of including a range of different concentrations and multiple strains when employing two-bottle preference tests.

The physiological source of these differences in sensitivity may exist anywhere in the taste transduction pathway between the taste receptors and central nervous system. To indentify them, electrophysiological recordings of taste responses were assessed in two gustatory nerves, the chorda typmpani and glossopharangeal nerves, for taster (SWR/J), non taster (C57BL/6J) and congenic cross (SW.B6-Soa^b) strains. SWR/J taster mice displayed strong SOA avoidance, and potent neural responses in the chorda tympani

and glossopharyngeal nerves, whereas non-taster SW.B6 mice displayed behavioral indifference and weak neural responses to SOA (Inoue et al., 2001). This suggests that the effect of the *Soa* genotype on SOA avoidance is demonstrated by differential peripheral taste receptor cell responsivity transduced via the chorda typmpani and glossopharangeal nerves. Bitter taste is mediated by a family of approximately 30 highly divergent G-protein-coupled receptors in the T1R2 group (Chandrashekar et al., 2006; Nelson & Hoon; 2001).

In addition to characterizing the response of inbred mice to SOA, Lush (1982) found that bitter tasting strychnine solutions yielded a similar pattern of responses to SOA, such that out of 27 inbred strains, only the SWR/J strain showed a strong aversion to dilute solutions (100 μM) of this bitter substance. Therefore, responsiveness to strychnine was proposed to be mediated by the *Soa* gene as well. Subsequent assessment of yet another bitter substance, quinine, (Lush, 1984) involving 29 strains consuming a 0.8 mM (and other 0.4-1.6 mM) solution, indicated that whereas SWR, 129/Sv and C57BL/6By mice displayed powerful aversions (2-4% consumption) over this range and would be categorized as tasters, A2G, DBA/2 and BALB/cBy strains displayed a more moderate quinine aversion (30-43%) in a two-bottle taste test, and were thereby categorized as non-tasters. Because of this differential sensitivity, the genetic difference between tasters and non-tasters of quinine was therefore proposed to be distinct from the *Soa* gene and attributed to a specific gene determining quinine detection, the *Qui* gene. Although characterized separately as two distinct genes, the progeny of a backcross involving both *Soa* and *Qui* provided evidence of an interaction between these genes (Lush, 1984). Capeless and co-workers (1992) subsequently confirmed the genetic loci

of the *Soa* gene to be on mouse chromosome 6, which is the same genetic loci as the praline-rich protein (PROP) loci, *Prp.* However, *Soa* and *Prp* genes appeared to be distinct because insertion of *Prp* transgenes from taster mice failed to alter SOA avoidance in non-taster mice (Harder et al., 2000). A correlation between quinine and PROP avoidance was described (Harder & Whitney, 1998) such that C57BL/6J mice strongly avoided both 0.1mM quinine and 1mM PROP solutions in two-bottle preference tests, whereas C3H/HeJ mice were indifferent to both, suggesting a common though indirect genetic basis for these two responses and the *Soa* and *Qui* genes.

1C. Ethanol: The identification of ethanol-related phenotypes in animal models has been related to human alcoholism through the use of inbred mouse strains and preference studies. In evaluating environmental factors that influence ethanol consumption, Tordoff and Bachmanov (2003b) provided between two and six bottles of alcohol (10%) or water, and demonstrated that alcohol intake was positively related to the number of alcohol bottles available, and inversely related to the number of water bottles available for C57BL/6J and 129X1/SvJ mice. These results (Tordoff & Bachmanov, 2003b) are consistent with findings of increased intake for other taste solutions (saccharin, citric acid, quinine and NaCl) when two bottles of each solution were presented along with a water bottle (Tordoff & Bachmanov, 2003a). Another study (Finn et al., 2005) evaluated the effects of fluid restriction on alcohol intake levels and found greater consumption of ethanol (5%, 7%, and 10%) in C57BL/6J inbred mice known previously to be alcohol-preferring, as well as heterogeneous WSC mice who previously failed to exhibit strong alcohol preferences.

Genetic variation in alcohol intake in inbred mouse strains revealed distinct phenotypes of high-preferring and low-preferring strains for alcohol with C57BL/6J mice consuming significantly more ethanol at 10-30% concentrations over 2-4 hours than DBA/2J mice (Rhodes et al., 2005). A typical 24-hour two-bottle preference test was not employed because it is difficult to determine when the animals drink to the point of intoxication (as measured by blood ethanol concentrations), and because the drinking is episodic across a 24-hour period. Bachmanov and co-workers (1996a) found that C57BL/6J mice displayed higher ethanol preferences than 129/J mice in a pattern similar to that observed for sucrose and citric acid. Indeed, correlations of ethanol and sucrose consumption were found among mice of the F2 generations of these two strains, and the genetically-determined component of these correlations was stronger than the component related to environmental factors. This observation is consistent with the hypothesis that higher ethanol intake is related to the higher hedonic attractiveness of its sweet taste component. The sweet taste of ethanol may be mediated by sweet taste receptors of the T1R group (Chandrashekar et al., 2006; Nelson et al., 2001; Zhao et al., 2003).

QTL analysis of ethanol preference at 3 and 10% concentrations in C57BL/6By and 129P3/J F_2 hybrids identified three loci. Ethanol intake at the high concentration was related to a locus on distal chromosome 4 and another on proximal chromosome 7, whereas a third, male-specific locus on chromosome 8 affected ethanol preference at the low, but not the high concentration. Additional linkages on chromosomes 2, 9, 12, 13, 17 and 18 were also detected (Bachmanov et al., 2002c).

1D. Glutamate/Umami: The mechanisms underlying taste preferences for glutamate and umami-type stimuli and the basis for their ability to enhance palatability

remain largely unknown, though relatively consistent with findings of sweet solutions. Using two extreme-responding C57BL/6J and 129/J strains identified using sweet and salty (e.g. Bachmanov et al., 1996a; Beauchamp & Fisher, 1993) taste solutions, monosodium glutamate (MSG 0.1-1000mM) intake was assessed in two-bottle preference tests (Bachmanov et al., 2000b). C57BL/6J mice detected MSG at lower concentrations, preferred MSG across a greater range of concentrations, and consumed greater amounts of MSG at high concentrations than 129/J mice. This pattern of strain differences for MSG intake were opposite to those observed for NaCl acceptance, suggesting that MSG taste responsiveness is unrelated to the detection of saltiness. Despite similar findings of strain differences in MSG and sweet tasting substances, several differences in responsiveness for these taste stimuli occurred in this study (Bachmanov et al., 2000b). First, prior experience with 300 mM solution of MSG, but not with saccharin, enhanced the subsequent expression of MSG acceptance, indicating a role for postingestive factors influencing MSG intake. F_2 generations of C57BL/6By and 129/J mice, bred for sucrose and saccharine preferences, failed to reveal corresponding changes in MSG preferences. Finally, the modes of inheritance were different for MSG preference, which appeared to be inherited as a recessive allele rather than an additive or partial dominant allele for sweeteners. These observations all suggested a unique genetic mechanism for umami taste responsiveness (Bachmanov et al., 2000b).

Electrophysiological recordings of the chorda typmani and glossopharyngeal nerves in C57BL/6By and 129/J mice for 10-1000 mM MSG solutions also revealed differences from those elicited by sweet tasting stimuli (Inoue et al., 2004a) such that increased ingestive responses were associated with increased electrophysiological

recordings for sucrose and saccharin (Inoue et al., 2004b), whereas increased ingestive responses to umami taste in C57BL/6J mice were accompanied by either unchanged or decreased neural responses in the chorda tympani or glossopharyngeal nerves (Inoue et al., 2004a). Both sweet and umami tastes are detected in the tongue via the T1R receptor group, a small family of 3 G-protein coupled receptors. However, whereas the T1R2+T1R3 heterodimer forms the sweet taste receptor, the heterodimeric T1R1+T1R3 sensor is specific to the umami flavor (Chandrashekar et al., 2006; Zhao et al., 2003).

Since the studies for this dissertation were designed to evaluate genetic variation in the intake of sugars and fats, this second Background section provides evidence from the literature of genetic variance for the sweet-tasting substances, saccharin (2a) and sucrose (2b) followed by fat intake (2c).

2A. Saccharin: Just as the avoidance of bitter substances can be important for an animal's survival, the preference for sweet substances can signal nutrient-rich foods necessary for adequate growth and development (Stricker, 1990). Genetic differences in the degree of preference for sweet tasting stimuli such as saccharin have been consistently observed (e.g., Blizard et al., 1999; Capeless & Whitney, 1995; Fuller, 1974; Inoue et al., 2004b; Lush, 1989; Nachman, 1959; Pelz et al., 1973; Reed et al., 2004; Tordoff et al., 2002). Saccharin is experimentally useful because of its palatable taste without caloric consequences, thereby isolating the taste variable. Nachman (1959) demonstrated the heritability of saccharin preference, finding that F_1 and F_2 generation progeny of saccharin-preferring rats displayed comparable saccharin preferences to the parents, whereas water-preferring parents and their progeny failed to display saccharin preferences. Strong preferences for a 0.1% saccharin solution relative to water were

subsequently observed in BALB/cJ, C57BL/6J and I^S/Bi, but not 101Bag/R1 mice (Pelz et al., 1973). Correspondingly, C57BL6/J mice displayed greater intake of this same 0.1% saccharin concentration than DBA/2J mice. This preference was more likely related to the incentive value of saccharin rather than the detection threshold, given that the degree of preference was reduced for DBA/2J mice at higher saccharin concentrations (Fuller, 1974). Also, enhanced saccharin intake induced by food deprivation occurred in both strains, but to a lesser degree in the DBA/2J mice. Lush (1989) then analyzed the response of 26 inbred mouse strains for the intake of a 1.6 mM saccharin solution, and demonstrated a pattern of strong preferences in A/2G, C57BL/6Ty C57BL/10 and SWR strains (73-93%) relative to AKR, CBA/Ca, C3H/He, DBA/2Ty and 129/SV strains (53-59%). Capeless and Whitney (1995) evaluated four ascending saccharin concentrations (0.03-0.1 mM) in five inbred mouse strains, and found that only the C57BL/6J strain showed a preference for all saccharin concentrations, whereas the BALB/cJ, C3H/HeJ, 129/J, and DBA/2J mice only responded to the high concentrations, effects tempered by the lack of control for the order of presentation of the saccharin solutions, which can influence preference scores (Harder et al., 1989).

Maintenance diets of the mice (Tordoff et al., 2002) altered preference differences between the C57Bl/6J and 129x1/SvJ strains for a 2 mM saccharin solution, with greater effects when they were fed standard cereal-based diets ("chow") rather than purified diets. Heritability estimates reveal how much of the variability for saccharin preference is attributable to genetic differences among the mice tested, with 81-94% of the total variability observed in initial preference for a 0.1% saccharin solution (Pelz et al., 1973),

and 78% of the genetic variation associated with consumption of a 0.1% saccharin concentration in one outbred and seven inbred strains (Ramirez and Fuller, 1976).

Extreme-responding strains, identified for saccharin intake in these previously-described two bottle preference tests served as progenitors for quantitative trait loci (QTL) analysis and subsequently, for the identification of trait-relevant genes. Four studies (Blizard et al., 1999; Fuller, 1974; Inoue et al., 2004b; Lush, 1989) utilized QTL techniques to evaluate saccharin intake by crossing sensitive responding C57BL mice with subs-sensitive DBA/2J or 129P3/J mice. These revealed that both the saccharin (*Sac*) preference locus and the sweet taste receptor gene, *Tas1r3*, were localized to distal chromosome 4. Indeed, Fuller (1974) initially described the *Sac* gene in elucidating differences between preference responses of C57BL/6Jsrains and DBA/2J strains for a 0.1% saccharin solution. Lush (1989) concluded that since the variation in preference among strains for any one sweet substance is highly correlated with the variation in preference for the other sweet substances, this *Sac* gene is responsible for detecting sweetness in general, and is not specific to saccharin only. This finding was further validated by Nelson and Hoon (2001), who demonstrated that the sweet taste receptor recognized sweet tasting molecules as diverse as saccharin, sucrose, dulcin and aceseulfame-K. Blizard and co-workers (1999) mapped the *Sac* gene to distal chromosome 4, whereas Inoue and co-workers (2004b) linked the *Sac* gene to the *Tas1r3* gene, which encodes the T1R3 receptor protein. When the T1R3 receptor binds to the T1R2 protein, the resultant T1R2+3 heterodimer is responsible for sweetness perception in the taste buds of the oral cavity (Chandrashekar et al. 2006; Nelson & Hoon, 2001; Zhao et al., 2003). Finally, sequence variants of the previously identified *Tas1r3* taste

receptor gene which codes for the protein associated with this preference, and their relevance to saccharin intake, were demonstrated by genotyping these polymorphisms in 30 inbred strains and comparing their allelic frequencies for these variants with their saccharin preference. This study concluded that the mouse *Sac* locus is identical to the *Tas1r3* gene (Reed et al., 2004).

2B. Sucrose: In contrast to saccharin, a non-nutritive sweetener, sucrose consumption requires a more complex integration of peripheral sensory, central nervous system and post-ingestive events because it provides a rich source of calories. Nevertheless, genetic variance among inbred mouse strains has been observed for sucrose intake in a similar fashion to saccharin, (e.g., Bachmanov et al., 1997; Blizard et al., 1999; Inoue et al., 2004b; Lush, 1989; Ramirez & Fuller, 1976; Stockton & Whitney, 1974). Because different mouse strains displayed varying degrees of preference for saccharin (Pelz et al., 1973), investigators were interested in assessing whether a similar pattern of preferences would emerge for sucrose (Stockton & Whitney, 1974). Therefore, two-bottle preference tests were used to evaluate sugar (sucrose and glucose) consumption in five mouse strains (three inbred strains C57BL/6J, I^s/Bi and 101Bag/Ri and two F1 generations of the latter two strains) of both sexes. These strains, used in the initial saccharin study (Pelz et al., 1973) were tested across five concentrations (0.005-1 M), presented in an ascending order. Similar to saccharin preference, C57BL6/J mice displayed greater intake of all five sucrose concentrations compared to 101BAg/R1 mice. This suggested that these extreme-responding inbred strains differ at a single peripheral mechanism involved in responsiveness to taste cues. A significant sex by concentration

interaction was observed with females drinking less sugar solutions at the two lowest concentrations, and more at the higher concentrations.

Bachmanov and co-workers (1997) confirmed the prior findings of highest intake in C57BL6/J male mice for a single 4% sucrose solution, as compared to 129P3/J mice. This greater sensitivity of C57BL/6J mice to sweetened solutions like saccharin and sucrose relative to 129P3/J mice was subsequently extended to a wide range of sweeteners including maltose, acesulfame-K, sucralose and SC-45647 as well as to the amino acids, D-phenylalanine, D-tryptophan, L-proline and glycine (Bachmanov et al., 2001c). A larger strain survey of 26 mouse strains evaluated for sucrose (50 mM) intake found that strong sucrose preferences, just like strong saccharin preferences were greater in A/2G, C57BL/6Ty, C57BL/10J and SWR/J strains (73-97%) than in AKR/J, CBA/Ca, C3H/HeJ, DBA/2Ty and 129/SV strains (51-61%) (Lush, 1989).

To investigate the interaction between oral and post-oral responses, licking activity, recorded for sucrose (8 and 16%), was greater in SWR/J mice than AKR/J mice, suggesting greater orosensory flavor factors (Smith et al., 2001). Evaluation of conditioned flavor preferences induced by intragastric infusions of a 16% sucrose solution (Sclafani & Glendinning, 2005) revealed that although both C57BL/6J and 129X1/SvJ strains developed preferences for the sucrose-paired conditioned stimulus, as compared to the water-paired conditioned stimulus, C57BL/6J mice displayed stronger preferences and intake than 129X1/SvJ mice during training. Since the intragastric infusions isolated the postingestive element of intake, the higher intake of the C57BL/6J mice was proposed as being a result of a stronger orosensory response to sucrose. This increased oronsensory response was conceptualized as functioning in a positive feedback

loop, leading to greater intakes and consequently greater stimulation of postingestive nutrient detectors and then further enhancement of consumption. When the same mouse strains were given prior exposure to sucrose concentrations, the subsequent strain difference in sucrose preference (although not absolute intake) was eliminated. These findings indicate that although the 129X1/SVJ mice may have a less sensitive orosensory response to sucrose, previous experience of oral and postingestive effects of more concentrated solutions can subsequently enhance their preference for sucrose. The additional finding that strain differences were minimized even in sucrose naïve mice at higher concentrations of sucrose (8-32%) suggests that the postingestive effects of higher concentrations reduce the differences based on different orosensory sensitivities of these two strains (Sclafani, 2006a). The oral/postingestive feedback model can also explain why strain differences in sugar intake were greater than those observed with non-nutritive sweeteners, as originally noted by Bachmanov et al. (2001c).

Psychophysical studies employing operant conditioning with a gustometer determined whether intake of sucrose, glucose and glycine in 'taster' (e.g., C57BL/6J and SWR/J) and 'non-taster' (e.g., 129P3/J and DBA/2J) mouse phenotypes is due to differences in sensory sensitivity relative to hedonic responsiveness (Eylam & Spector, 2004). 'Taster' mice had lower detection thresholds for sucrose as compared to 'non-taster' mice, corresponding well with reports of increased responsiveness to low concentrations of sucrose in two-bottle intake tests. This suggests that the taster phenotype has a sensory basis and is not simply a matter of strain differences in the hedonic evaluation of low sucrose concentrations (Eylam & Spector, 2004). In addition, electrophysiological recordings of the chorda tympani gustatory nerve revealed stronger

responses to sucrose in C57BL/6J mice than in 129P3/J mice, thereby further emphasizing a sensory basis for strain differences in sucrose preference (Inoue et al., 2004b). Finally, high heritability estimates of 83% were reported for consumption of a 3% sucrose concentration in seven inbred and outbred strains (Ramirez & Fuller, 1976). Such heritability values of sucrose varied as a function of concentration, greater at higher (0.5M) than lower (0.005M) concentrations (Stockton & Whitney, 1974).

Quantitative trait loci (QTL) analyses revealed two loci (most likely the proximal *dpa* and distal *Sac*) on Chromosome 4, which accounted for over 50% of the genetic variability in sucrose intake. These were proposed to modulate intake by altering peripheral neural responses to sucrose. The proximal dpa locus appeared to affect the sensitivity or response threshold, whereas the other distal Sac locus affected the response magnitude at supra-threshold sucrose concentrations. These authors further proposed that the response threshold depended on properties of the sweet taste receptor, whereas response magnitude depended on the intensity of the intracellular signal (Bachmanov et al., 1997). Lush (1989) initially found strong correlations in strain preferences for sucrose and saccharin and concluded that the *Sac* gene is responsible for detecting both these sweet tasting substances. Subsequently, the same genetic mechanism responsible for saccharin intake was also found for sucrose (Blizard et al., 1999; Inoue et al., 2004b), indicating parallel involvement of the *Sac* and *Tas1R3* loci localized to distal chromosome 4 for sucrose intake.

Nelson and Hoon (2001) demonstrated that the although the *Sac* locus encodes for the T1R3 protein, the T1R2 and T1R3 proteins combine to function as a sweet receptor for a diverse range of sweet tasting substances. Each of the T1R2 and T1R3 subunits

contribute to the receptive range of the sweet taste receptor through the binding of sugars at the long N-terminal domain site. A single nucleotide polymorphism was shown to reduce ligand binding to the T1R3 protein (Nie et al., 2005), which coincides with the findings of reduced sweet taste sensitivity in mice with *Tas1R3* polymorphisms (Reed et al., 2004). Mice which had either the *Tas1R2* or *Tas1R3* gene deleted showed residual but reduced responses to sweeteners, whereas deletion of both genes abolished preferences and neural responses to sweeteners altogether (Zhao et al., 2003). These results indicate that both the T1R2 and the T1R3 proteins are necessary for maximum sweet taste detection, though each likely contributes differently to the process (Nie et al., 2005; Zhao et al., 2003).

It is clear that the most substantial amount of research in assessing genetic variance on hedonically-driven intake has been done with sucrose, and therefore this became the focus of our first specific aim. The following section will demonstrate a role of genetic variance in fat intake.

2C. Fats: Obesity is generally considered to be the result of a combination of environmental as well as genetic factors. Although the environmental component of dietary consumption of fats has been associated with weight gain and body fat accumulation, because fats are palatable substances, there may be a genetic basis for the preference of dietary fat and its resultant weight gain. Therefore, a range of studies used inbred mice to examine the interactions between genetic and environmental contributions to fat preference and/or obesity (Alexander et al., 2006; Bachmanov et al., 2001; 2002b; Leibowitz et al., 2005; Reed et al., 2003; Sclafani, 2007; Smith et al., 1999; 2000; West et al., 1992).

Intake of 1-100% soybean oil solutions yielded higher preferences for fat in two-bottle preference tests, and greater weight gain in C57BL/6ByJ relative to129P3/J mice. The former group gained more weight despite overall equal caloric intake to 129P3/J mice (Bachmanov et al., 2001). Given previous similarities in strain specific responses to sucrose and ethanol (Bachmanov et al., 1996a; 1996b), and given that the increased preferences and intakes were most pronounced for lower concentrations of the soybean oil, involving less postingestive caloric consequences, the increased fat preferences may be related to strain-specific chemosensory perception for fat as for sweet solutions (Bachmanov et al., 2001). Smith et al. (2001) observed lower preference thresholds in two bottle tests and higher licking activity of corn oil in SWR/J mice compared to AKR/J mice, also suggesting greater orosensory flavor factors. Recently, Sclafani (2007) replicated the findings of Bachmanov et al. (2001) of elevated fat preference in C57BL/6J mice relative to 1299P3/J and 129X1/SvJ mice, and also found that prior experience with fat solutions (Intralipid and non-nutritive olestra oil) reduced the subsequent strain differences in fat preference. The previous experience may reinforce the postingestive effects of fats, thus implicating the primary strain difference as being orosensory in nature. Interestingly, these strain specific responses to nutritive and non-nutritive oil were similar in nature to that observed when testing for sucrose and saccharin. These results, (Sclafani, 2007) along with increased preferences in C57BL/6J mice relative to 1299P3/J mice in response to intragastric infusions of 5.6% soybean oil (Sclafani & Glendinning, 2005), suggest that these two strains differ in their orosensory, but not their post-oral response to fat, as similarly observed for sugar (Sclafani, 2007).

A large (28) strain survey for water intake, chow intake and body weight (Bachmanov et al., 2002b) revealed significant strain differences and high heritability estimates for body weight (h^2 =0.87), indicating a strong genetic role for food intake and particularly weight gain. However, the genetic basis for weight gain appears to interact with the environmental factor of diet composition. The percentage of energy derived from fat in the diet is positively correlated with body fat content (see review: West & York, 1998). Several diet selection studies investigated the interaction of the fat content of diets and the genetic predisposition for obesity (Smith et al., 2000; West et al., 1992). Nine inbred mouse strains were exposed to high- and low-fat diets over seven weeks (West et al., 1992). Whereas some strains (e.g., AKR/J mice) displayed moderate intake of a high-fat diet that promoted weight gain and obesity, other strains (e.g., SWR/J mice) consumed large amounts of the high-fat diet without weight gain. Although the weight effects were largely due to variation in the dietary fat content, this variable weakly correlated with total energy intake. This indicates that hyperphagia alone was not responsible for the weight gain. Another survey of 12 inbred strains and one outbred strain (Smith et al., 2000) used a self-selection protocol in which separate carbohydrate, fat and protein diets were simultaneously available for 26-30 days. Whereas AKR/J and C57BL/6J strains self-selected the highest proportion of fat in macronutrient diet selection, which was directly related to epididymal fat, SWR/J and CAST/Ei strains consumed a great deal of fat that was inversely correlated with epididymal fat. The AKR/J and C57BL/6J obesity- prone strains were therefore considered highly sensitive to dietary obesity. In contrast, the obesity-resistant SWR/J and CAST/Ei strains were

thought to be highly sensitive to a negative feedback signal generated by increasing body fat (Smith et al., 2000).

The precise physiological mechanism underlying dietary resistance and susceptibility to obesity is largely unknown. In order to rule out the possibility that the preferences for a high fat diet found in certain strains (Smith et al., 2000) were related to the specific physical properties of the diet provided, the AKR/J and SWR/J mice were presented with high fat diets possessing differing physical, sensory and nutritive properties. These strains displayed similar effects whether the fat source was solid (vegetable shortening and lard), whether the high and low-fat diets were isocaloric liquid preparations, or whether they were in powdered form. Thus, the increase of proportional fat intake in AKR/J mice relative to SWR/J mice persisted across dietary paradigms. This was attributed to the robust and reliable fat preference of the AKR/J mice. In contrast, macronutrient preference in SWR/J mice varied based on the diet, suggesting a differential response by this strain to certain orosensory or postingestive factors (Smith et al., 1999). Physiological explanations involving endogenous hormones and peptides have also been proposed (Alexander et al., 2006; Leibowitz et al., 2005). Whereas the AKR/J and DBA/2J strains, which consumed greater amounts of fat and exhibited greater adiposity, were found to have elevated levels of leptin and insulin, the C57BL/6J strain, which showed an equal preference between protein and fat, displayed normal insulin and leptin levels (Alexander et al., 2006). In contrast, obesity-resistant SWR/J and A/J mice consumed more fat than carbohydrate, but failed to gain weight, potentially because of lower insulin levels, increased capacity of skeletal muscle to metabolize fat, enhanced paraventricular galanin, and reduced arcuate neuropeptide Y (Leibowitz et al., 2005).

Fat may be detected through a variety of mechanisms i.e., odor, texture, post-ingestive cues and more recent evidence postulates that perhaps fats may be detected by taste (Laugerette et al., 2007; Mattes, 2005). Gilbertson et al.(1998; 2005) demonstrated that taste receptor cells respond to polyunsaturated fatty acids through an inhibition of delayed rectifying Potassium channels (DRK). A greater responsiveness to the fatty acids and lower densities of DRK channels were found in obesity resistant (S5B/P1) rats as compared to obesity prone (O-M) rats. This result suggests that the obesity resistant rats are more sensitive to fat detection and can consequently reduce fat intake and avoid obesity (Gilbertson et al., 1998). Gilbertson et al., (2005) also postulated that fatty acids potentiate and magnify the taste of sweetness through inhibiting a DRK channel in the taste cell, thereby enhancing the cell's response to other tastants. This was based on the finding that the addition of fatty acids to a low (0.5mM) concentration of saccharin enhanced the preference for saccharine in two bottle preference tests, in the obesity resistant rats. Other investigators (Laugerette et al., 2005) reported that the fatty acid transporter CD36, found in lingual papillae, was responsible for the oral detection of long-chain fatty acids (LCFA) in mice. Consequently, when the CD36 gene was inactivated, the preference for LCFA was abolished. To reconcile the two different mechanisms proposed for "tasting" fats, Laugerette et al. (2007) proposed that two-lipid mediated sensory systems co-exist in the tongue. The polyunsaturated fatty acid activated DRK channels may constitute a taste modulating system which enhances the palatability of other tastants, whereas the lingual CD36 may be a fat taste sensor used to select lipid-rich foods. Although texture may be the predominant orosensory cue for

detecting dietary fat, mounting evidence also implicates a role for taste mechanisms with varying "fat taste" sensitivities, in rodents and possibly in humans (Mattes, 2005).

In terms of the genetic mechanisms underlying fat preference and adiposity, QTL analyses of inbred mouse strains were successful in identifying several candidate loci (Reed et al., 2003; Smith-Richards et al., 2002). Crosses between fat-preferring C57BL/6J and carbohydrate-preferring CAST/EiJ mice in an F2 population yielded three loci responsible for fat intake: on chromosomes 8 (*Mnif1*), 18 (*Mnif2*) and X (*Mnif3*). In addition, two significant QTL loci for cumulative kilocalorie intake adjusted for baseline body weight, were found on chromosomes 17(*Kcal1*) and 18 (*Kcal2*). Without body weight adjustment, another significant kilocalorie locus appeared on distal chromosome 2 (*Kcal3*) (Smith-Richards et al., 2002). Moreover, a genome scan of a F_2 generation intercross between the C57BL/6ByJ and 129P3/J mouse strains, known to differ in body weight, body length and adiposity, identified loci on chromosomes 2, 4, 9 and 16 responsible for body weight, body length, and adiposity, with suggestive sex-dependent linkages on chromosomes 4 and 9 (Reed et al., 2003). The above studies, taken together, reveal that obesity is a complex trait influenced by genetics as well as its interactions with sex, age and diet for the determination of body size and adiposity in mice.

The previous two background sections have reviewed studies of spontaneous intake of ingestive stimuli including sugars and fats. The third and final section of the Background will address intake in response to the homeostatic challenges provided by the induction of 3a) glucoprivation and 3b) lipoprivation. This general review of privation states is brief because the specific topic of genetic variance of inbred mouse strains in response to glucoprivation and lipoprivation, has not been yet been well investigated.

3A. Glucoprivic Responses: The anti-metabolic glucose analogue, 2DG (Wick et al., 1957), increases food intake following systemic (e.g., Booth, 1972; Smith & Epstein, 1969; Smith & Root, 1969; Thompson & Campbell, 1977) and cerebro-ventricular (Berthoud & Mogenson, 1977; Miselis & Epstein, 1975) administration in rats, monkeys and humans. This ingestive response can occur in rats in the absence of other signs of glucoprivation (Engeset & Ritter, 1980; Ritter et al., 1978) and reduced glucose oxidation (Nonavinakere & Ritter, 1983). 2DG-induced feeding in rats is stimulated by ventricular administration of fructose, but not glucose (Miller et al., 2002). 2DG-induced feeding in rats is also dependent on necessary metabolic changes (Even & Nicolaidis, 1985), circadian rhythm (Penicaud et al., 1986; Thompson et al., 1989), level of thirst (Salter & Watts, 2002; Watson & Biderman, 1982; Watson et al., 1986) and diet composition (Delprete & Scharrer, 1992;, Kanarek & Mayer, 1978). Moreover, synergistic feeding interactions are observed following combined treatment with 2DG and either fatty acid oxidation inhibition with methyl palmoxirate (Friedman & Tordoff, 1986) or ventricular insulin administration (Clegg et al., 2003). Further, glucoprivic feeding in rats is impaired following blockade of central glucoreceptors with alloxan (Murname & Ritter, 1985a; 1985b; Ritter et al., 1982; Sanders et al., 2004; Woods & McKay, 1978) or stress-induced alterations in noradrenergic function (Ritter et al., 1978; Rowland, 1992, Scheurink & Ritter, 1993, but see Rowland & Bellush, 1985). 2DG appears to produce its ingestive effects in rats through selective activation of epinephrine-containing neurons in dorsal medulla (Ritter et al., 1998), effects attenuated by either immunotoxic destruction (Hudson & Ritter, 2004; Ritter et al., 2001) or prior repeated 2DG treatment (Sanders & Ritter, 2000; 2001).

Whereas 2DG-induced feeding is clearly delineated in rats, the presence of its ingestive actions has not been universally observed in other species. Thus, 2DG-induced feeding failed to occur under similar dosing and ingestive conditions in Golden and Siberian hamsters (Angel & Taranger, 1991; Bartness & Clein, 1994; Bartness et al., 1995; Lowy & Yim, 1982; Ritter & Balch, 1978; Rowland, 1983), deermice (Rowland, 1985) and spiny mice (Czech, 1988). 2DG induces feeding in ground squirrels in only their hyperphagic, but not hypophagic phase (Nizielski et al., 1986), in lean, but not fatty Zucker rats (Tsujii & Bray, 1990), and in rats selectively bred for high, but not low saccharin intake (VanderWheele et al., 2002). 2DG-induced feeding in outbred mice is reduced by either nitric oxide synthase inhibition (Czech, 1998) or gold-thio-glucose injections (Bergen et al., 1996**)**, and 2DG reduces hypophagia induced by epinephrine in outbred mice (Villanueva et al., 1996). Further, whereas wild-type mice display normal feeding responses to 2DG, this response is absent in mice genetically deficient in dopamine (Hnasko et al., 2004), the dopamine-3 receptor gene (Benoit et al., 2003), dopamine beta-hydroxylase (Ste Marie & Palmiter, 2003) or neuropeptide Y (Sindelar et al., 2004). The latter findings underscore the importance of these genes in glucoprivic feeding, and raise the possibility that naturally-occurring differences in these and other genes may form the basis for the existence of genetic variance in glucoprivic feeding responses.

3B. Lipoprivic Responses: The free fatty acid oxidation inhibitor, MA (Bauche et al., 1981) significantly increases food intake following systemic administration (Langhans & Scharrer, 1987; Scharrer & Langhans, 1986). This lipoprivic ingestive response has been compared with 2DG-induced increases in food intake to determine if

they share similar circuitry. Both MA and 2DG administration elicit c-fos responses in the NTS, lateral parabrachial nucleus, central nucleus of the amygdala and the dorsal motor nucleus of the vagus (Ritter & Dinh, 1994), and elevate sympathoadrenal plasma levels of epinephrine and norepinephrine (Scheurink & Ritter, 1993). However, MA-induced and 2DG-induced feeding show different sensitivities to vagotomy (Ritter et al., 1990), capsaicin treatment (Ritter & Taylor, 1989), brain lesions (Calingasan & Ritter, 1993; Nisoli et al., 1996; Ritter & Hutton, 1995), peripheral responses (Ritter et al., 1995), interoceptive sensory signals (Benoit, & Davidson, 1996) and macronutrient selection (Ritter et al., 1999; Singer & Ritter, 1994; Singer & Ritter, 1996; Singer et al., 1998).

4. Conclusions of the Background: The investigation of the genetics of ingestive behaviors has burgeoned in the past three decades. In particular, the uses of inbred mouse strains and QTL analyses have become an invaluable tool in the determination of the hereditability of these behaviors. Certain genetic loci have been associated with specific feeding behaviors. Nevertheless, there are limitations in these studies. These include use of too few inbred mouse strains, too few concentrations of the ingestate being tested and lack of important controls such as counterbalancing the bottle placement for two-bottle preference tests, and the order of presentation of the concentrations. In addition, the studies reviewed above often employed different methodologies, such as different ages and genders of mice, different concentrations, measurements of molarity versus volumetric measures, and the use of different substrains for inbred lines. These issues have lead to difficulty in direct comparisons of published studies and in their replication. Additionally, even though genetic variability in sugar and

fat intake has been amply documented, no work has yet been done to evaluate genetic variability on intake, following glucoprivic and lipoprivic states, using inbred mouse strains. These considerations have established the need for undertaking the present set of studies.

III. Rationale:

The following section will address the rationale for selecting the various parameters employed in these dissertation studies. *The choice and number of strains* was based on prior work using inbred mouse strains for the study of ingestive behaviors. The strains surveyed for the present series of studies include the major groups of strains that have been used in prior research of ingestive behavior, allowing for comparisons of present results to previous studies. In addition, all these strains were obtainable from Jackson Laboratories and have extensive data collected about their characteristics. This knowledge can be helpful in anticipating expected differences in their behaviors. The number of strains selected, eleven (11), was based on the fact that previous studies, which included a large range of strains, allowed for a more accurate ability to identify even subtle differences in behavior (e.g., Lush, 1989). In addition, the identification of diverse responses in a variety of strains can provide a more accurate basis for future QTL analyses, a process that can reveal the candidate loci or genes that may be responsible for these behaviors. Finally, a wide range of inbred strains may extend the findings of many previous studies, which relied on a small number of strains when examining intake preferences, and can insure a greater level of reliability, as well as allow for the assessment of heritability estimates.

The decision to test only male mice -- despite the possible influence of gender on taste preference -- was based on the fact that the estrous phase of the cycle introduced an additional variable, because it was found to reduce intake in females (e.g., Geary & Asarian, 1999). In our studies, which utilize multiple concentrations of sucrose or Intralipid and/or multiple doses of 2DG or MA over an extended period of time,

controlling for the estrous phase would have presented a significant confounding variable.

The rationale for using *a concentration-dependent analysis of sugars and fats* is based on prior research, which has found differences between inbred strains not only in the magnitude of intake (preference), but in the threshold of detection (sensitivity) for these palatable substances (sensitivity) (e.g., Capeless & Whitney, 1995; Stockton & Whitney, 1974). In addition, there is not necessarily a linear relationship between detection and intake. Thus, some strains may be 'super-tasters' and thereby detect sugar and fat at low concentrations, but still ingest less at a particular, higher concentration than other strains. Therefore, it is essential to consider both magnitude and threshold variables when assessing the role of genetic variance. The same rationale applies for using *a dose-response analysis of 2DG and MA* when assessing intake. In addition, since prior exposure based on the concentration order has been proven to be a potential confounding variable in previous studies (Harder et al., 1989; Tordoff & Bachmanov 2002), a range of concentrations and doses calls for testing within and across strains in *ascending and descending orders, to control for order and carry-over effects.* The choice of *the particular nine concentrations chosen for sucrose* solutions (0.0001, 0.001, 0.01, 0.1, 1, 2.5, 5, 10, 20%) was based on a previous analysis of pre-pro-enkephalin gene knockout animals relative to heterozygous and wild-type mice (Ragnauth et al., 2001), where clear differentiations in sucrose intake was found at these concentrations. The *nine concentrations for fat with the use of Intralipid* (0.00001, 0.0001, 0.001, 0.01, 0.1, 0.5, 1, 2, 5%) were chosen to parallel the nine different sucrose concentrations, so that the findings could be compared to one another. Since it is typically difficult to liquefy fat for

presentation at different concentrations due to its lack of solubility over an extended time course (e.g., 24 h) that is reasonable to study murine intake, Intralipid (Baxter Healthcare Corporation, Deerfield, Illinois) was chosen because it is an emulsified fat solution (20%) made almost exclusively from soybean oil (20 g in 100 ml), and is used clinically for delivery of a fat source to human patients. Therefore, the use of Intralipid insures that the fat is equally distributed in solution across a wide range of concentrations, and indeed Intralipid solutions are readily consumed in a manner similar to sucrose and other palatable solutions (e.g., Higgs & Cooper,1998a; 1998b).

The rationale for *the use of a two-bottle preference test* was to effect the most comprehensive assessment of both preference, as well as sensitivity for a particular macronutrient. Intake alone can be assessed with a one-bottle method or acceptance paradigm. However, this is not the most accurate measurement of ingestive behavior, because it can represent overall drinking behavior and not the intake of sugar or fat solutions per se. Therefore, the two-bottle paradigm was utilized, since it provides a comparison of intake of the palatable solution to water. Preference for the tested solution was defined as its percentage of the total intake volume (solution plus water). For baseline measures, we expected the mice to sample from each water bottle equally, resulting in a percent preference of approximately 50% for each bottle. When the percentage of intake increase was statistically significant for the solution as compared to water intake, that measure indicated a preference for the palatable solution. This two-bottle preference method is also a good measure of sensitivity since the percent preference can be compared for each concentration to evaluate at which concentration each strain is detecting and preferring the palatable solution.

In addition to preference measurements for each bottle, the amount of chow intake was also measured in order to *determine the portion of total kilocalories coming from the solution nutrients relative to chow.* This measurement of solution nutrients was expressed as a percent of total intake of kilocalories (solution plus chow). This measure can help to clarify the issue of whether the intake is due to palatability, caloric necessity or both. In addition, *it allows for the evaluation of compensatory behavior*. Thus, we may see reductions in chow intake when the solution concentrations provide high levels of kilocalories.

The underlying hypothesis of Specific Aim 1 is that divergent responses exist between inbred strains for sucrose intake across concentrations. As indicated in the Background sections, prior published studies found individual differences for the intake of sweet tasting substances in inbred mouse strains (e.g., Bachmanov et al., 1997; Blizard et al., 1999; Capeless & Whitney, 1995; Fuller, 1974; Inoue et al., 2004b; Lush, 1989; Pelz et al., 1973; Ramirez & Fuller, 1976; Reed et al., 2004; Stockton & Whitney, 1974; Tordoff et al., 2002) as well as in humans and primates (see review: Reed et al., 1997). Although these previous studies revealed differences between inbred strains in sucrose intake (e.g., Bachmanov et al., 1997; Blizard et al., 1999; Inoue et al., 2004b; Lush, 1989; Ramirez & Fuller, 1976; Stockton & Whitney, 1974), they employed either limited sucrose concentrations, and/or a limited number of strains, while neglecting to include important controls for bottle position and concentration order. Therefore, the rationale for this hypothesis is that the differences between strains will be more accurately defined using a large number of strains and concentrations, and by employing important controls which will more adequately address the genetic component of this behavior.

The underlying hypothesis of Specific Aim 2, that divergent responses exist between strains for fat intake, is based on the same rationale as that for sucrose intake. Although prior studies have found differences in fat intake with the use of inbred mouse strains (Alexander et al., 2006; Bachmanov et al., 2001; 2002b; Leibowitz et al., 2005; Reed et al., 2003; Sclafani , 2007; Smith et al., 1999; 2000; West et al., 1992), there have been no prior strain surveys of a wide range of inbred strains to evaluate the differences using two-bottle preference tests across a variety of concentrations. Therefore, this paradigm employing liquid Intralipid intake as the fat source was designed to parallel our study of sucrose intake. The differences in responses between the wide range of strains for fat intake can then be compared to those of sucrose intake, to further evaluate the genetic components controlling intake of fat, in particular, and palatable substances in general.

The rationale for the third hypothesis, that divergent responses exist between strains for chow intake across a time course following the induction of a glucoprivic state (through the systemic administration of the anti-metabolic glucose analogue, 2DG), is based on previous studies that found increased feeding following this homeostatic challenge (Berthoud & Mogenson, 1977; Booth, 1972; Miselis & Epstein, 1975; Smith & Epstein, 1969; Smith & Root, 1969; Thompson & Campbell, 1977) in rats, monkeys and humans. Additionally, Davidson et al. (1997) proposed a relationship between sucrose intake and glucoprivation based on findings of increased responses in rats to a conditioned stimulus for sucrose following the induction of glucoprivation. Although there have been no prior studies analyzing feeding associated with glucoprivation using inbred mouse strains, this glucoprivation paradigm is used in order to parallel the two

previous preference studies, while eliminating the orosensory factor inherent in the two-bottle preference paradigm. Thus, the glucoprivation paradigm allows for the evaluation of the genetic role of the metabolic component of ingestion alone.

The analysis of the genetic role of food intake in response to lipoprivation, in Specific Aim 4, is based on a similar rationale to that of Specific Aim 3. Davidson et al. (1997) also proposed a relationship between fat intake and lipoprivation based on findings of increased responses in rats to a conditioned stimulus for peanut oil following the induction of lipoprivation. Whereas, the use of 2DG parallels the sucrose two-bottle preference study, the fourth hypothesis, that divergent responses exist between strains for chow intake across a time course following the induction of a lipoprivic state, through the systemic administration of the free fatty acid oxidation inhibitor, MA, parallels the fat (Intralipid) two-bottle preference study.

IV. General Methods

Subjects: All procedures and experiments were approved by the Queens College Institutional Animal Care and Use Committee. Outbred adult male mice, 12 weeks of age, (CD-1) were acquired from Charles River Laboratories, Wilmington, MA. There was an n=10-20 mice per paradigm. Inbred adult male mice,12 weeks of age; A, AKR, BALB/c, CBA, C3H/He (C3H), C57Bl/6 (BL/6), C57Bl/10 (BL/10), DBA/2 (D2), SJL, SWR, 129P3 (129) [all "J" substrains]; were acquired from Jackson Laboratories, Bar Harbor, ME. There was an n=9-10 mice of each strain for each paradigm. These mice were initially acclimated to the Queens College vivarium for one week. They were housed in a group (5 per cage) and maintained on a 12 hour light: 12 hour dark cycle (lights off at 2000 h), at a constant temperature of 22^{o}C with *ad libitum* access to food and water.

Thereafter, each animal was housed individually in plastic cages (30 x 20 x15 cm) for experimental testing. Given the large number of animals involved (approximately 120 animals across the 12 strains in each paradigm) it was not be possible to test all of them contemporaneously. Therefore, the mice were tested for each of the paradigms in approximately three sequences of approximately 40 animals each, with the outbred strain also tested for each of the sequences in the sucrose paradigm. Importantly, systematic analyses of data from the outbred mice across the sucrose sequences failed to reveal any significant differences in any of the response measures. These data indicate that the time sequence in which a strain was tested is not a potential confounding variable. Therefore, we then used only 10 mice of a given inbred or outbred strain in the remaining Intralipid, 2DG and MA paradigms.

Preference Paradigm for Sucrose and Intralipid: Initially, each animal was provided with a pre-weighed ration of Purina Mouse chow and two calibrated and pre-weighed sipper tubes, each filled with water. Each animal, of each strain, was assessed for chow intake and water intake from each individual bottle over a 24-hour period for 4 days. Although fluid spillage was not quantified systematically during the experimental protocol, there were no malfunctioning sipper tubes that could have contributed to larger amounts of spillage. In addition, as a further control, sipper tubes, placed on empty cages for a 24-hour period and assessed for changes in weight, failed to display detectable (<0.1 g) changes in fluid. The position of the two water bottles were systematically switched across animals and across strains according to a left(L)-right(R)-R-L and a R-L-L-R position, respectively, to minimize potential bottle position preference effects (e.g., Bachmanov et al., 2002b).

Following baseline, each mouse of each strain received chow, one bottle of water and one bottle of either sucrose or Intralipid each day. Nine sucrose concentrations (Study 1: 0.0001%, 0.001%, 0.01%, 0.1%, 1%, 2.5%, 5%, 10% and 20%) and nine Intralipid concentrations (Study 2:0.00001%, 0.0001%, 0.001%, 0.01%, 0.1%, 0.5%, 1%, 2%, 5%) were tested in these two-bottle preference tests. Half of the mice of each strain were tested in an ascending concentration order, and the remaining half were tested in a descending concentration order, with the sucrose or Intralipid bottle position systematically controlled as described previously (LR-R-L and a R-L-L-R). Nine sucrose or Intralipid concentrations were prepared via dilution with the necessary volume of water and were used for testing in these two-bottle preference tests. Chow, sucrose or Intralipid, and water intakes were measured daily for each concentration of solution.

Body weights of the animals were measured every 3 days throughout the paradigm and the mean body weight was chosen for each animal of each strain for further analysis.

Gluoprivation and Lipoprivation Paradigms: Each animal was moved to a test cage from group housing at 4-6 h into the light cycle and was provided with a water bottle and a pre-weighed ration of Purina Mouse chow (5.3 kcal/g) placed on a stainless steel grid on the bottom of the test cage. A brown paper towel was placed below this grid to collect and assess spillage. Animals were acclimated to this test cage prior to and during four days of baseline data collection, in which pre-weighed food pellets were placed on the grid floor, and intake assessed after 1, 2 and 4 h. Food intake ($\pm$0.1 g) was assessed by weighing food pellets prior to, and following, each time interval, and adjusting for any spillage.

After determination of stable baseline food intake, the animals were tested with vehicle (0mg/kg) and four doses of 2DG: 200, 400, 600 and 800 mg/kg, or four doses of MA: 5, 35, 70 and 100 mg/kg, administered intraperitoneally at 4-6 hours into the light cycle (Sigma Chemical Company, St. Louis, MO). The 2DG was dissolved in distilled water at concentrations of 20, 40, 60 and 80 mg/ml and the MA at concentrations of 0.5, 3.5, 7 and 10 mg/ml, and injected intraperitoneally in a 10 ml/kg volume. The interval between each injection was 72 hours, minimally, and one week, maximally. Half of the mice of each strain were tested in an ascending dose order, and the remaining half were tested in a descending dose order.

Statistics: For the preference paradigms, one-way analysis of variance was performed to assess whether any pre-existing differences in total baseline water intake and chow intake were observed across strains in each paradigm. To assess sampling of

the two water bottles under baseline conditions, a two-way randomized block analysis of variance was performed with strains as the between-subject variable and water intake from the two bottles, as a repeated measure in the first two studies. In assessing alterations in sucrose or Intralipid relative to water intake, a three-way randomized block analysis of variance was performed with the 12 strains as the between-subject variable, the ten conditions (baseline and 9 concentrations), as a within-subject variable, and the intake from the sucrose or Intralipid and water bottles, as a second within-subject variable. Further, to assess order effects upon solution intake, another set of three-way randomized-block analyses of variance systematically compared, within each strain, those mice that received an ascending order of concentrations with those that received a descending order. Also, two-way randomized-block analyses of variance were systematically performed across strains and across sucrose concentrations to assess changes in the percentage of palatable solution consumed, the total amount of chow intake, and the percentage of kilocalories consumed as sucrose or Intralipid. Tukey comparisons ($P<0.05$) were performed in the presence of significant effects relative to corresponding baseline values within strains.

To assess the consistency of genetic variance across the different palatable solution concentrations in the 12 tested mouse strains, Pearson product-moment correlation coefficients (r) subject to Bonferroni correction for multiple comparisons were calculated for palatable solution intake and percent consumed as sucrose or Intralipid. Correlations of data from baseline water and chow intakes as well as body weight, were compared for both sucrose and Intralipid studies and with studies from other (Bachmanov, et al., 2002) laboratories.

Further, narrow-sense trait heritability were determined by comparing the between-strain variance to the total variance for each concentration of sucrose and Intralipid in terms of intake itself and intake adjusted for body weight. Since these mice are isogenic (i.e., genetically identical) within individual inbred strains, between-strain variance provides a measure of additive genetic ('allelic') variation (VA), whereas within-strain variance ('error variance') represents environmental variability (VE). An estimate of narrow-sense heritability (h^2) for each trait was obtained using the formula: $h^2 = VA/(VA+VE)$. Since animals in strains are initially chosen at random (by the supplier), these values are likely accurate estimates of the true trait heritabilities.

For the glucoprivation and lipoprivation paradigms, alterations in 2DG- or MA-induced feeding relative to baseline intake was assessed using a three-way randomized block analysis of variance, with the 12 strains as the between-subject variable, the five (vehicle and 4 2DG/MA doses) conditions as a within-subject variable, and the three intake (1, 2, 4 h) times as a second within-subject variable. Baseline and vehicles values did not differ significantly. If significant strain differences were observed in baseline and vehicle food intake across the 4 h time course, Tukey comparisons ($P<0.05$) were performed only relative to corresponding baseline or vehicle values within strains. With the 12 strains as the between-subject variable, the four dose conditions of either 2DG or MA, as a within-subject variable, and the intake times, as a second within-subject variable, subsequent separate three-way randomized-block analyses of variance were performed on difference scores, in which each intake value, at each time point following vehicle, in each animal in each strain was subtracted from each corresponding dose value.

All correlations were calculated using Pearson product-moment correlation coefficients [*r*] subject to Bonferroni correction for multiple comparisons. Correlations were performed among the 4 hour difference scores for the four doses of 2DG and MA-induced intake. Subsequent correlations assessed whether inter-strain differences were observed between the glucoprivic and lipoprivic responses, and between preference responses for sucrose and Intralipid.

V. Specific Aim 1: The Role of Genetic Variance in Sucrose Intake in Inbred and Outbred Mouse Strains

Introduction:

Systematic analyses of rodent strain differences can provide a means of identifying the genetic control of salt and other nutrient intake (see review: Reed et al., 1997). Simple sugars are potent stimulators of intake across a wide variety of species when included in solutions in a concentration-dependent manner (see review: Yamamoto, 2003). Strain intake differences have been observed for sucrose (e.g., Bachmanov et al., 1997; Blizard et al., 1999; Inoue et al., 2004; Lush, 1989; Ramirez & Fuller, 1976; Stockton & Whitney, 1974) and saccharin (e.g., Blizard et al., 1999; Capeless & Whitney, 1995; Fuller, 1974; Inoue et al., 2004; Lush, 1989; Nachman, 1959; Pelz et al., 1973; Reed et al., 2004; Tordoff et al., 2002). A strong preference for a 0.1% saccharin solution relative to water was observed in BALB/cJ, C57BL/6J, and I^S/Bi mice, but not in 101Bag/R1 mice (Pelz et al., 1973). In other studies, C57BL6/J mice displayed greater intake of five (0.005-1 M) glucose and sucrose concentrations than did 101Bag/R1 mice (Stockton & Whitney, 1974), greater intake of a 0.1% saccharin solution than DBA/2J mice (Fuller, 1974), and greater intake of a 4% sucrose solution than 129P3/J mice (Bachmanov et al., 1997; Tordoff et al., 2002). Genetic factors accounted for 78% and 83% of the genetic variation associated with consumption of 0.1% saccharin and 3% sucrose, respectively, in outbred and seven inbred strains (Ramirez & Fuller, 1976).

These studies typically evaluated either a small number of strains or a limited number of palatable concentrations. Comparisons between these studies are potentially

confounded by the use of differing strains and/or concentrations. Approaches comparing large numbers of strains have more recently been adopted. These have demonstrated systematic differences in food, water and mineral intake, as well as spout side preference (Bachmanov et al., 2002a; 2002b). Moreover, up to 30 strains of mice were examined for palatable intake in some studies. Nonetheless, only a single concentration of saccharin (1.6 mM) or sucrose (50 mM) was employed (Lush, 1989; Reed et al., 2004).

Subsequently, eleven mouse strains were examined for alterations in sucrose intake, using seven supra-threshold (1-50%) concentrations (Pothion et al., 2004). However, important limitations in the genetic analysis of sweet intake remain due to the lack of important controls. In the present study the order of sucrose concentrations was controlled by exposing half of the mice to an ascending concentration order and the remainder to a descending concentration order, a variable of importance in prior work (Harder et al., 1989). Bottle positions of the sucrose and water bottles were also systematically switched across animals and across strains, another variable identified to be of importance in prior work (Bachmanov et al., 2002b). Moreover, careful measurement of chow intake was simultaneously assessed to determine strain differences in kilocalorie intake as a function of sucrose relative to chow.

The present study examined strain differences in sucrose intake among 11 inbred and one outbred (CD-1) strains across a far greater range of nine different sucrose concentrations (0.0001%-20%) in two-bottle 24-h preference tests. These concentrations were selected based on their clear differentiations in sucrose intake in mice with distinct genetic genotypes (Ragnauth et al., 2001). By testing a sufficient number of inbred

strains, the present study design also allows for the valid estimation of genetic correlations (Hegmann & Possidente, 1981). The demonstration of a genetic correlation implies the involvement of common (although not necessarily identical) physiological substrates. Thus, genetic commonality in the percentage of sucrose intake among sucrose concentrations was tested. Previously, Lush (1989) reported on intake for 1.6 m*M* saccharin solution in 30 inbred mouse strains, including some of those tested in the present study. Subsequently, Reed and co-workers (2004) identified the sequence variants of the previously identified *Tas1r3* taste receptor gene, which codes for the protein associated with this preference. Their relevance to saccharin intake was demonstrated by genotyping these polymorphisms in 30 inbred strains and comparing their allelic frequencies for these variants with their saccharin preference.

The present study assessed the relationship between sucrose intake of various concentrations with the previously reported saccharin intake (Lush, 1989). In addition, sucrose intake in the present study was correlated with polymorphisms of the *Tas1r3* taste receptor gene associated with 1.6 m*M* saccharin intake (Reed et al., 2004). For all correlations, coefficients of covariation were obtained across a range of sucrose concentrations. The results of this Specific Aim, has been published in Physiology and Behavior (Lewis et al., 2005).

Methods:

Subjects: As detailed in the General Methods section, male outbred CD-1 (n=20), and 11 inbred mouse strains: A, AKR, BALB/c, CBA, C3H/He (C3H), C57Bl/6 (BL/6), C57Bl/10 (BL/10), DBA/2 (D2), SJL, SWR, 129P3 (129) (n=9-10 for each inbred strain) purchased from Charles River and Jackson Laboratories at 12 weeks of age were initially

acclimated to the Queens College vivarium for one week in group housing, and were subsequently housed individually in plastic cages on a 12 h light: 12 h dark cycle at a constant temperature of 22^{o}C with food and water available *ad libitum.*

Sucrose Intake Procedure: As detailed in the General Methods section, each mouse was initially provided with a pre-weighed ration (~20 g) of Purina Mouse chow (5.3 kcal/g) and two calibrated and pre-weighed ($\pm$0.1 g = $\pm$0.1 ml) sipper tubes each filled with water (~40 ml each) for baseline measures. To minimize potential bottle position preference effects (Bachmanov et al., 2002b) the position of the two water bottles were systematically switched according to a L-R-R-L and a R-L-L-R position respectively. Each mouse of each strain was assessed for chow intake, adjusted for spillage, and water intakes from each individual bottle every 24 h, over a 4 day period.

Following these baseline measurements, the same procedures were again employed, except one bottle of water was replaced with a different sucrose concentration each day. Nine sucrose concentrations were tested (0.0001%-20%) in these two-bottle preference tests, with half of the mice of each strain presented with an ascending sucrose concentration order and the remaining half with a descending order (with sucrose bottle position systematically controlled (Harder et al., 1989)). Sucrose and water intakes ($\pm$ 0.1 g), as well as chow, were measured daily for each concentration of sucrose.

Statistics: As detailed previously in the General Statistics section, a one-way analysis of variance assessed pre-existing differences across strains, for body weight, total baseline water intakes, and chow intakes. In this, and all subsequent analyses involving chow, data from 11 of the 12 tested strains are evaluated. Chow data from the

129P3/J strain was corrupted by excessive chow spillage and could, therefore, not be used in these analyses.

To assess sampling of the two water bottles under baseline conditions, a two-way randomized block analysis of variance was also performed, with strains as the between-subject variable and water intake from the two bottles, as a repeated measure. In assessing alterations in sucrose relative to water intake, a three-way randomized block analysis of variance was performed, with the 12 strains as the between-subject variable, the ten (baseline and 9 sucrose concentration) conditions as a within-subject variable, and the intake from the sucrose and water bottles as a second, within-subject, variable. Further, to assess order effects upon sucrose intake, another set of three-way randomized-block analyses of variance systematically compared those mice within each strain that received an ascending order of sucrose concentrations with those that received a descending order.

Two-way randomized-block analyses of variance were also systematically performed across strains and across sucrose concentrations to assess changes in the percentage of sucrose consumed, the total amount of chow intake, and the percentage of kilocalories consumed as sucrose. Finally, since there were significant differences in body weight across strains (Table 1), a two-way randomized block analysis of variance was performed with the 12 strains as the between-subject variable, and the ten conditions as a within-subject variable for transformed sucrose intake per 30 grams of body weight. Tukey comparisons ($P<0.05$) were performed in the presence of significant effects relative to corresponding baseline values within strains.

Table 1: Baseline water (g, ±S.E.M.) and chow (g, ±S.E.M.) intake and body weight (g, ±S.E.M.) in 12 mouse strains

Strain	Water (g)	Chow (g)	Body Weight(g)
A/J	5.6 (0.7)	4.4 (0.3)	26.2 (1.4)
AKR/J	5.7 (0.6)	4.6 (0.1)	33.6 (1.4)
BALB/cJ	5.6 (0.1)	5.4 (0.2)*	27.9 (0.8)
C57BL/6J	4.9 (0.3)	4.1 (0.1)	27.9 (0.6)
C57BL/10J	5.5 (0.3)	3.7 (0.1)	26.8 (0.5)
CBA/J	5.3 (0.3)	3.8 (0.1)	32.3 (1.5)
CD-1	7.8 (0.4)*	5.8 (0.3)*	37.7 (0.8)*
C3H/HeJ	5.2 (0.4)	4.2 (0.1)	28.4 (0.5)
DBA/2J	5.1 (0.1)	4.4 (0.2)	27.0 (0.8)
SJL/J	5.7 (0.3)	3.5 (0.2)	25.6 (0.2)
SWR/J	7.3 (0.3)*	4.6 (0.2)	27.2 (0.3)
129P3/J	7.1 (0.4)*	n.a.	30.5 (0.4)

* Significantly greater relative to all other unmarked strains in column
n.a.: not available

All correlations were calculated using Pearson product-moment correlation coefficients (*r*) subject to Bonferroni correction for multiple comparisons. For all analyses, the following sucrose concentrations were considered: 0.01, 0.1, 1.0, 2.5, 5.0, and 10.0%. Lower and higher concentrations were not subject to correlation analyses, because the respective uniformly low and high percentages of sucrose intake at these concentrations could restrict the range of values and underestimate correlations. One exception was our assessment of the correlation between 1% and 2.5% sucrose intake and the total (sucrose and chow) kilocaloric intake of mice offered a 20% sucrose solution, and between 1% and 2.5% sucrose intake and 20% sucrose intake (per 30 g/ body weight).

For the genetic codetermination between sucrose concentrations, the proportion of sucrose intake relative to total fluid intake (percentage of sucrose intake) for each strain at each sucrose concentration was correlated with one another. To assess the relationship between sucrose and saccharin intake, percentage of sucrose intake for each sucrose concentration was correlated with percentage of 1.6 m*M* saccharin intake, as previously reported (Lush, 1989). The covariation between sucrose intake and *Tas1r3* polymorphisms, previously described (Reed et al., 2004), was achieved by assigning a gene dose value of 1 or 2 to each strain displaying an allele associated with high and low saccharin preference, respectively. *Tas1r3* variants assessed were those with the greatest degree of statistical association with saccharin intake. Since all alleles associated with high and low saccharin intake were represented by a gene dose score of 1 or 2, respectively, correlation coefficients were determined for each sucrose concentration once, to minimize type I error, and were generalized to all three polymorphisms. Data

from all inbred strains were included in the correlation analyses, except C57BL/10J as they have not been genotyped for *Tas1r3* variants and their high genetic similarity to C57BL/6J may overestimate genetic correlations.

Results:

Baseline values in water and chow intake: Significant differences were observed among mouse strains in total baseline water ($F(11,117)= 7.40$, $p<0.0001$) and chow ($10,108)= 13.46$, $p<0.0001$) intakes. As summarized in Table 1, baseline water intake was greatest in CD-1, SWR/J, and 129P3/J strains, with significantly less water intake observed in all other strains. Baseline chow intake was again greatest in CD-1 mice with similar intake observed for the BALB/cJ strain (Table 1). Significant differences were observed among mouse strains in body weight ($F(11,117)= 30.21$, $p<0.0001$). Body weight was significantly greater in CD-1 mice relative to the other 11 strains that in turn failed to display significant differences among one another (Table 1). Analysis of two-bottle baseline water intake revealed significant differences across strains ($F(11,209)= 26.04$, $p<0.0001$) and for the interaction between strains and fluid choice ($F(11,209)= 3.29$, $p<0.0001$), but not for intake for the two fluids ($F(1,19)= 0.10$, n.s.). Importantly, all twelve strains displayed similar patterns of sampling of the two water bottles during baseline (BL) testing (Figure 1), indicating that preferences described for intake of different concentrations of sucrose were not due to some underlying intra-strain preference for intake from one water bottle.

Figure 1. Alterations in sucrose (left ordinate, mean, +SEM), water (left ordinate, mean, +SEM) and chow (right ordinate, mean, +SEM) intake across baseline and nine different sucrose concentrations in one outbred (CD-1) and eleven inbred (A/J, AKR/J, BALB/cJ, C57BL/6J, C57BL/10J , CBA/J, C3H/HeJ, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice.

In this and all subsequent figures, the asterisks (*) denote a significant difference in intake relative to corresponding baseline conditions, and the crosses (+) denote a significant difference in sucrose intake relative to corresponding water intake at that concentration (Tukey comparisons, $P<0.05$). 129P3/J mice had large spillage precluding careful measurement of chow intake. The twelve panels of this figure are found on the following three pages. In these and all succeeding figures, the convention ml is used for fluid intake values, instead of g (grams), since it is more understandable as a solution. However, it should be noted that these values were determined by weighing the fluid in grams, and not by observing changes in the meniscus.

Figure 1 A and B

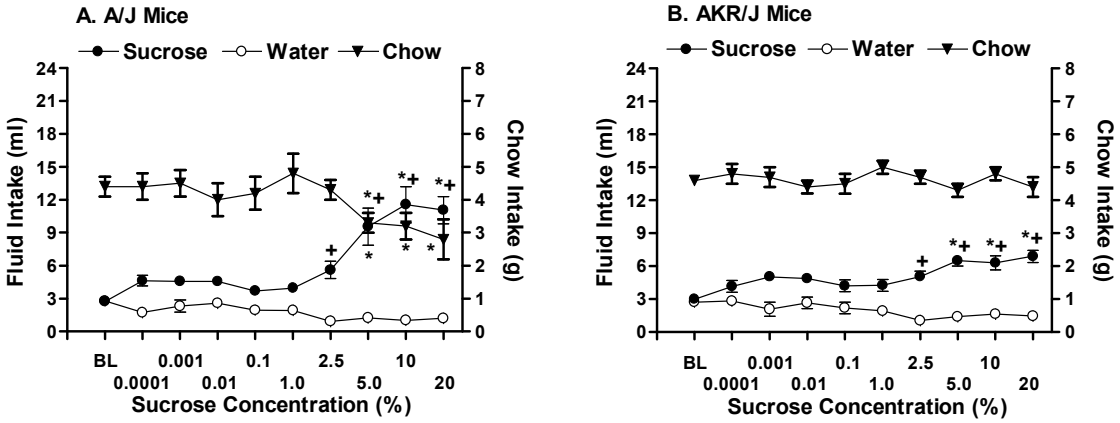


Figure 1 C and D

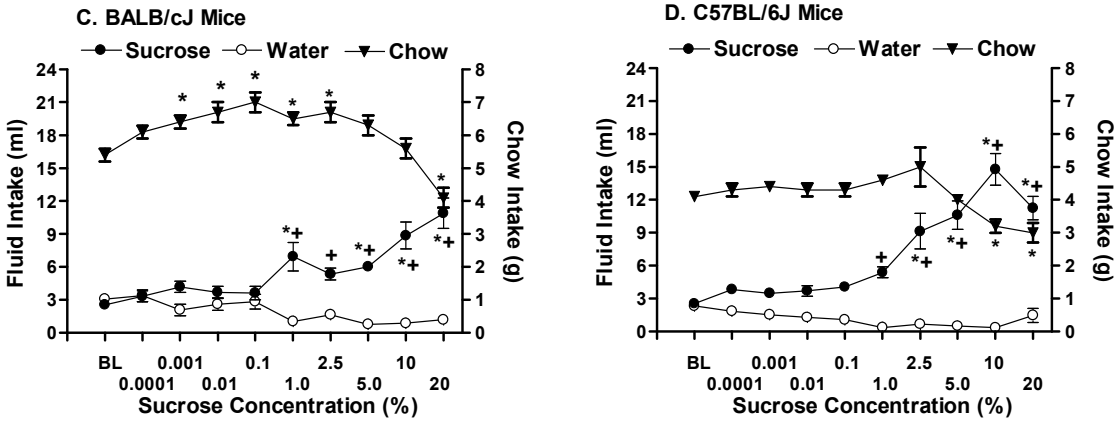


Figure 1 E and F

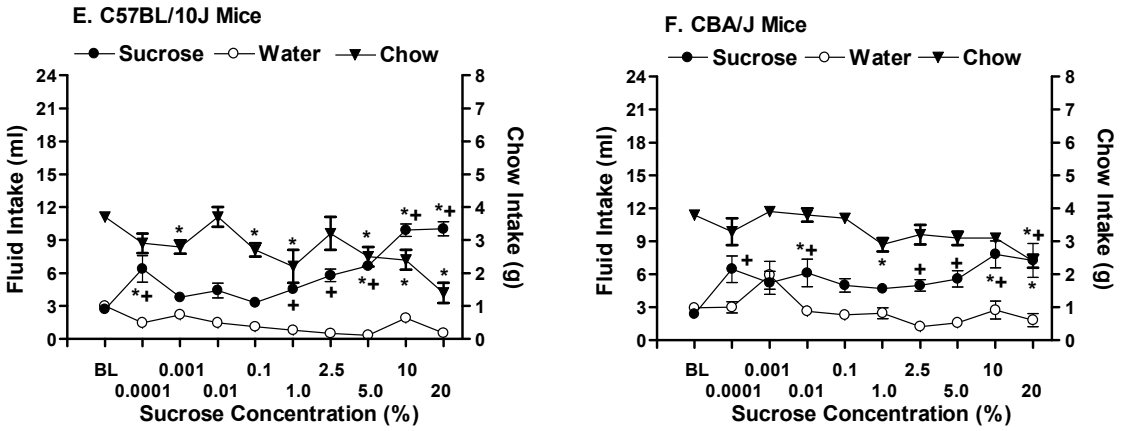


Figure 1 G and H

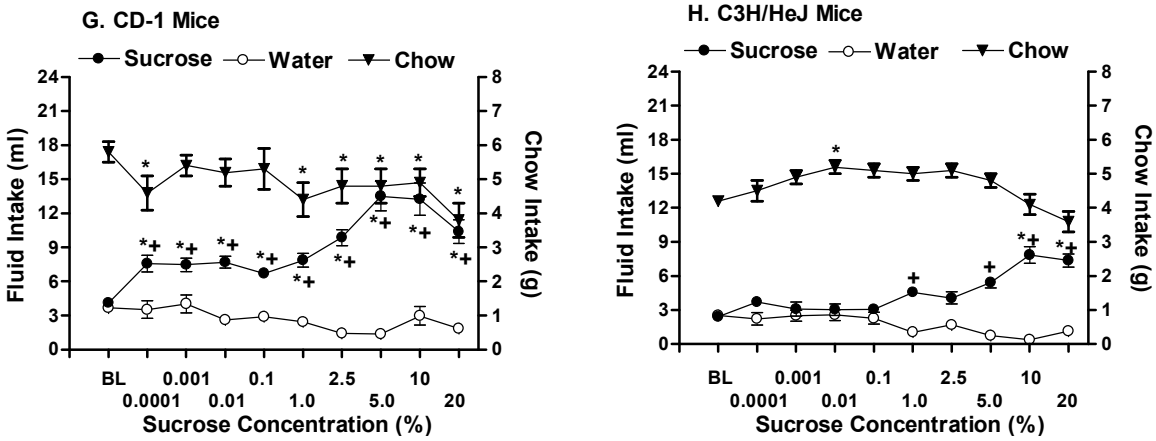
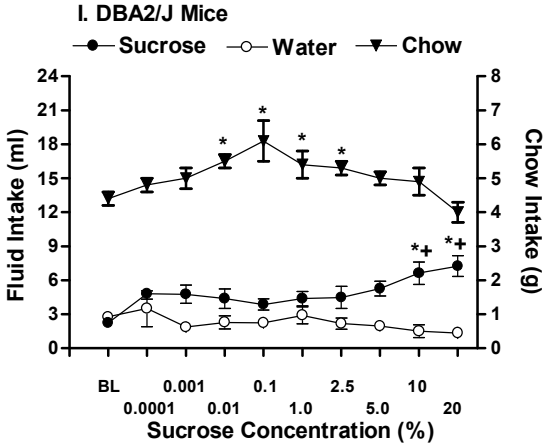


Figure 1 I and J



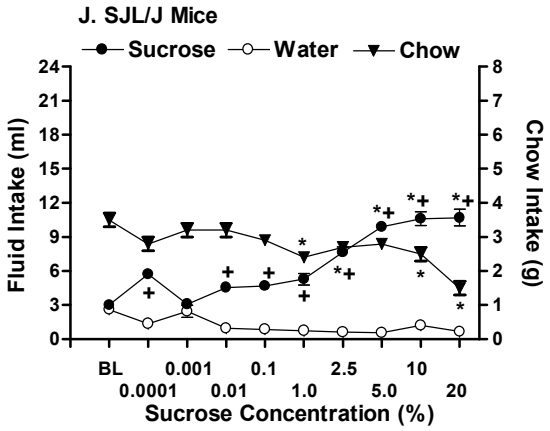
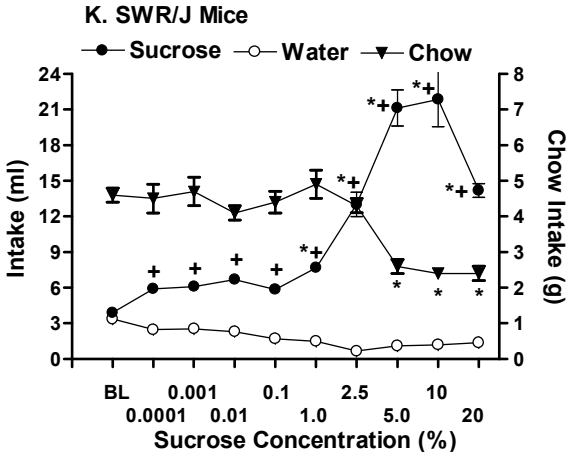
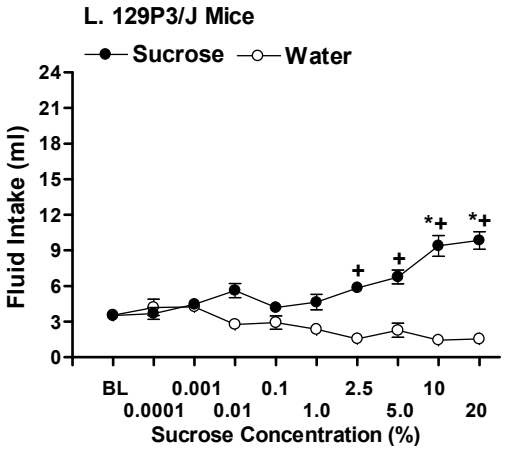


Figure 1 K and L





Sucrose and water intake: In analyzing sucrose and water intake across strains and sucrose concentrations, significant differences in intake were observed among strains ($F(11,209)= 43.46$, $p<0.0001$), across concentrations ($F(9,171)= 239.45$, $p<0.0001$), between the two fluids ($F(1,19)= 1151.48$, $p<0.0001$), and for the interactions between strains and concentrations ($F(99,1881)= 27.45$, $p<0.0001$), strains and fluids ($F(11,209)= 53.34$, $p<0.0001$), concentrations and fluids ($F(9,171)= 378.75$, $p<0.0001$) and among strains, concentrations and fluids ($F(99,1881)= 17.71$, $p<0.0001$). Significant differences in chow intake were observed among strains ($F(10,190)= 52.63$, $p<0.0001$), across concentrations ($F(9,171)= 137.44$, $p<0.0001$) and for the interaction between strains and concentrations ($F(90, 1710)= 12.43$, $p<0.0001$). The effects of sucrose concentration on intake were not generally attributable to the order of sucrose concentration presentation, as mice exposed to ascending and descending orders generally failed to differ in intake. However, CBA/J mice exposed to the ascending order of sucrose concentrations consumed significantly more sucrose at the 0.0001-0.01% concentrations than the same strain exposed to the descending order of sucrose concentrations, indicating the importance of using this order control (Harder et al., 1989).

Among strains, only CD-1 and SWR/J mice significantly increased their sucrose intake relative to either corresponding water intake at every sucrose concentration or to baseline water intake from the "sucrose" bottle (Figures 1G and 1K). For all other strains except the DBA/2J strain, significantly greater sucrose intake relative to water intake was also observed at higher sucrose concentrations (1% or 2.5% – 20%), but significant increases in sucrose intake at lower concentrations was strain-dependent (Figures 1A-1F,

1H, 1J, 1L). DBA/2J mice consumed significantly more sucrose than water at only the highest (10% and 20%) sucrose concentrations (Figure 1I). Thus, inter-strain variability for total sucrose intake was observed across the entire range of sucrose concentrations.

An analysis of variance examining sucrose intake per 30 g of body weight revealed significant differences among strains ($F(11,209)= 61.07$, $p<0.0001$), among sucrose concentrations ($F(9,171)= 430.09$, $p<0.0001$) and for the interaction between strains and concentrations ($F(99,1881)= 32.98$, $p<0.0001$). Table 2 summarizes the strain differences in body weight-corrected sucrose intake with the SWR/J strain displaying significantly greater intake than all other strains at most of the sucrose concentrations. In turn, the C57BL/6J strain displayed significantly greater intake at the four higher concentrations than the DBA/2J, C3H/HeJ, 129P3/J, AKR/J and CBA/J strains, at the three higher concentrations than the BALB/cJ and C57BL/10J strains, and at some concentrations than the CD-1 (10-20%), A/J(2.5%) and SJL/J (10%) strains. Moreover, the SJL/J strain displayed significantly greater intake at the four higher concentrations than the DBA/2J, C3H/HeJ, 129P3/J, AKR/J and CBA/J strains, at the three higher concentrations than the BALB/cJ strain and at some concentrations than the CD-1 (10-20%), C57BL/6J (2.5%) and C57BL/10J (5%) strains. Further, the A/J strain displayed significantly greater intake at the three higher concentrations than the DBA/2J, C3H/HeJ, BALB/cJ, 129P3/J, AKR/J and CBA/J strains and at some concentrations than the CD-1 (10-20%), C57BL/6J (2.5%) and C57BL/10J (5%) strains. The major differences in the pattern of estimated sucrose intake using raw or weight-adjusted intake occurred for the heaviest CD-1 mice (Table 2 vs. Figure 1G).

Table 2: Alterations in Sucrose intake (mean, SEM) each corrected for 30 g of body weight, across baseline and nine different sucrose concentrations in one outbred (CD-1) and eleven inbred (A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C57BL/10J, CBA/J, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice.

Strain	Water*	0.0001%	0.001%	0.01%	0.1%	1%	2.5%	5%	10%	20%
A/J	3.2 (0.4)	5.4 (0.6)	5.3 (0.3)	5.2 (0.4)	4.3 (0.5)	4.6 (0.4)	6.5 (1.0) +	11.1 (2.0) +G	13.5 (2.0) +D	12.8 (1.5) +F
AKR/J	2.8 (0.3)	3.9 (0.6)	4.5 (0.3)	4.4 (0.5)	3.9 (0.6)	3.9 (0.6)	4.7 (0.6)	5.8 (0.4) +	5.8 (0.8)+ +	6.3 (0.7) +
BALB/cJ	2.8 (0.2)	3.7 (0.6)	4.6 (0.6)	4.1 (0.6)	4.0 (0.7)	7.3 (1.2) +L	5.8 (0.6) +	6.6 (0.4) +M	9.7 (1.5) +M	11. 7 (1.3) +I
C57BL/6J	2.7 (0.2)	4.1 (0.5)	3.7 (0.4)	4.0 (0.5)	4.4 (0.4)	5.8 (0.5) +	9.9 (1.7) +E	11.5 (1.4) +E	16.0 (1.6) +C	12.2 (1.3) +F
C57BL/10J	3.0 (0.3)	7.3 (1.2) +	4.2 (0.5)	4.9 (0.7)	3. 7 (0.2)	5.0 (0.4)	6.5 (0.6) +	7.5 (0.4) +	11.1 (0.5) +I	11.2 (0.6) +H
CBA/J	2.3 (0.3)	6.5 (1.5) +	5.1 (1.1) +	5.8 (1.2) +	4.8 (0.7)	4.5 (0.5)	4.7 (0.6)	5.2 (0.6) +	7.7 (1.4) +	7.1 (1.5) +
CD-1	3.3 (0.2)	6.0 (0.6) +	5.9 (0.5) +	6.1 (0.4) +	5.4 (0.4) +N	6.3 (0.5) +	7.8 (0.5) +M	10.1 (1.0) +I	10.5 (1.1) +E	8.2 (0.8) +I
C3H/HeJ	2.6 (0.3)	3.9 (0.4)	3.3 (0.7)	3.2 (0.6)	3.3 (0.4)	4.9 (0.3)	4.3 (0.6)	5.8 (0.6) +	8.3 (0.7) +	8.1 (0.6) +
DBA/2J	2.6 (0.3)	5.3 (0.5) +	5.2 (0.8) +	4.8 (0.9)	4.3 (0.5)	4.8 (0.7)	4.9 (1.0)	5.9 (0.7) +	7.4 (1.0) +	8.2 (1.1) +
SJL/J	3.6 (0.4)	6.7 (0.3) +	3.6 (0.3)	5.3 (0.3)	5.5 (0.3)	6.2 (0.6)	9.0 (0.5) +G	11.6 (0.4) +E	12.4 (0.7) +G	12.5 (0.9) +F
SWR/J	4.3 (0.2)	6.5 (0.5)	6.7 (0.5)	7.4 (0.4) +J	6.4 (0.5) K	8.5 (0.5) +B	14.3 (1.1) +A	23.3 (1.7) +A	24.1 (2.6) +A	15.7 (0.7) +A
129P3/J	3.5 (0.4)	3.6 (0.5)	4.4 (0.4)	5.5 (0.6)	4.1 (0.4)	4.6 (0.6)	5.7 (0.4)	6.7 (0.6) +	9.2 (0.8) +	9.7 (0.7) +

*Baseline water intake from designated "Sucrose" bottle

+Significant increase in Sucrose intake from corresponding baseline value ($p<0.05$)

A: Significantly greater weight-adjusted Sucrose intake than all other eleven strains ($p<0.05$)

B: Significantly greater than A/J, AKR/J, C57BL/6J, C57BL/10J, CBA/J, CD-1, C3H/HeJ, DBA/2J and 129P3/J strains ($p<0.05$)
C: Significantly greater than AKR/J, BALB/cJ, C57BL/10J, CBA/J, CD-1, C3H/HeJ, DBA/2J, SJL/J and 129P3/J strains ($p<0.05$)
D: Significantly greater than AKR/J, BALB/cJ, C57BL/10J, CBA/J, CD-1, C3H/HeJ, DBA/2J and 129P3/J strains ($p<0.05$)
E: Significantly greater than AKR/J, BALB/cJ, C57BL/10J, CBA/J, C3H/HeJ, DBA/2J and 129P3/J strains ($p<0.05$)
F: Significantly greater than AKR/J, CBA/J, CD-1, C3H/HeJ, DBA/2J and 129P3/J strains ($p<0.05$)
G: Significantly greater than AKR/J, BALB/cJ, CBA/J, C3H/HeJ, DBA/2J and 129P3/J strains ($p<0.05$)
H: Significantly greater than AKR/J, CBA/J, CD-1, C3H/HeJ and DBA/2J strains ($p<0.05$)
I: Significantly greater than AKR/J, CBA/J, C3H/HeJ and DBA/2J strains ($p<0.05$)
J: Significantly greater than AKR/J, BALB/cJ, C57BL/6J and C3H/HeJ strains ($p<0.05$)
K: Significantly greater than C57BL/10J and C3H/HeJ strains ($p<0.05$)
L: Significantly greater than A/J and CBA/J strains ($p<0.05$)
M: Significantly greater than the AKR/J strain ($p<0.05$)
N: Significantly greater than the C3H/HeJ strain ($p<0.05$)

The raw data corresponded with the transformed data, as a percentage of sucrose intake and as function of total fluid intake across sucrose concentrations and strains (Table 3). Significant differences in the percentage of fluid intake consumed as sucrose were observed among strains ($F(11,209)= 23.89$, $p<0.0001$), across concentrations ($F(9,171)= 235.88$, $p<0.0001$), and for the interaction between strains and concentrations ($F(99,1881)= 6.43$, $p<0.0001$). Consistent with the data analyzing the amount of sucrose consumed, the percentage of intake consumed as sucrose was significantly higher in CD-1 and SWR/J mice at every concentration, and followed a fairly monotonic function (Figures 2G and 2K). All other strains displayed variability in the significant sucrose percentage effects at the lower (0.0001% - 0.1%) sucrose concentrations (Figures 2A-2F, 2H-2J, 2L). The DBA/2J strain only showed significant sucrose percentage effects at the two highest concentrations (Figure 2I).

Kilocalorie intake as sucrose and chow: Significant differences in the percentage of kilocalorie intake consumed as sucrose were observed among strains ($F(10,190)= 34.74$, $p<0.0001$), across concentrations ($F(8,152)= 1604.73$, $p<0.0001$) and for the interaction between strains and concentrations ($F(80, 1520)= 24.77$, $p<0.0001$). Although all mouse strains consumed a considerable amount of their kilocalories as sucrose, particularly at the higher concentrations, they showed systematic differences in the percentage of kilocalories consumed as sucrose (Table 3, Figure 2). Thus, over 60% of total kilocalorie consumption as sucrose was observed in C57BL/10J and SJL/J strains (Figures 2E, 2J), and over 50% consumption was observed in the A/J and SWR/J strains (Figure 2A, 2K). Moderate (30%-45%) consumption was noted in BALB/cJ, C57BL/6J,

Table 3: Maximal sucrose intake (g), maximal percentage of fluid intake ingested as sucrose, and maximal percentage of kilocalorie intake ingested as sucrose by strain (±S.E.M.) [sucrose concentration for each in brackets].

Strain	Sucrose Intake (g)	% Fluid Intake as Sucrose	% Kilocalorie Intake as Sucrose
A/J	11.6 (1.7) [10%]	91 (1.9) [10%]	50 (0.28) [20%]
AKR/J	6.9 (0.5) [20%]	83 (3.7) [5%]	25 (0.07) [20%]
BALB/cJ	10.9 (1.4) [20%]	90 (2.1) [10%]	35 (0.09) [20%]
C57BL/6J	14.8 (1.5) [10%]	98 (0.4) [10%]	44 (0.10) [20%]
C57BL/10J	10.0 (0.7) [20%]	95 (0.8) [20%]	63 (0.15) [20%]
CBA/J	7.8 (1.2) [10%]	79 (6.6) [2.5%]	36 (0.20) [20%]
CD-1	13.5 (1.3) [5%]	90 (1.6) [5%]	39 (0.17) [20%]
C3H/HeJ	7.9 (0.7) [10%]	95 (1.4) [10%]	31 (0.08) [20%]
DBA/2J	7.3 (0.9) [20%]	88 (3.8) [10%]	28 (0.09) [20%]
SJL/J	10.7 (0.7) [20%]	95 (0.8) [5%]	61 (0.15) [20%]
SWR/J	21.9 (2.3) [10%]	95 (0.6) [5%]	56 (0.08) [20%]
129P3/J	9.8 (0.8) [20%]	87 (2.3) [10%]	n/a

Note: 129P3/J mice had large spillage precluding accurate measurement of chow intake.

Figure 2. Alterations in the percentage of sucrose over total intake(left ordinate, mean, ±SEM) and the percentage of kilocalories consumed as sucrose (right ordinate, mean, ±SEM) across the nine different sucrose concentrations in one outbred (CD-1) and eleven inbred (A/J, AKR/J, BALB/c/J, C57BL/6J, C57BL/6J, CBA/J, C3H/HeJ, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice.

The large spillage by 129P3/J mice precluded measurement of chow intake and therefore, measurement of the percentage of kilocalories consumed as sucrose. The twelve panels of this figure are found on the following three pages.

Figure 2 A and B

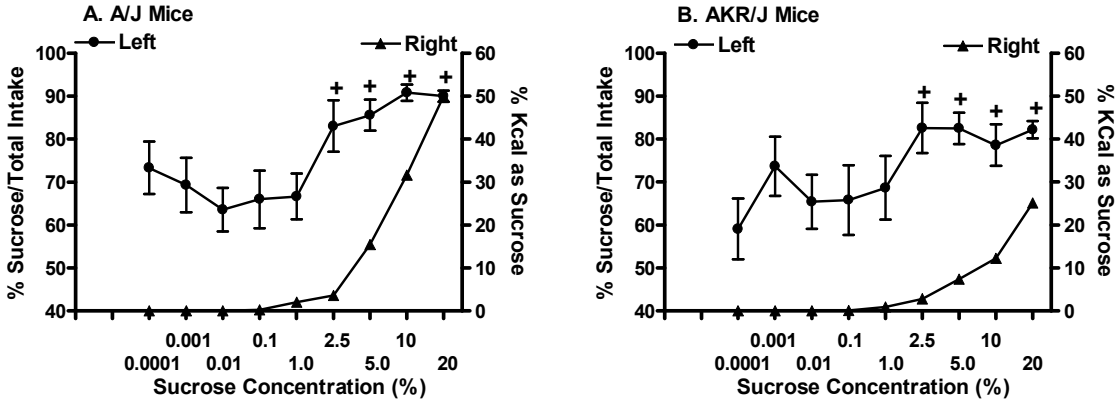


Figure 2 C and D

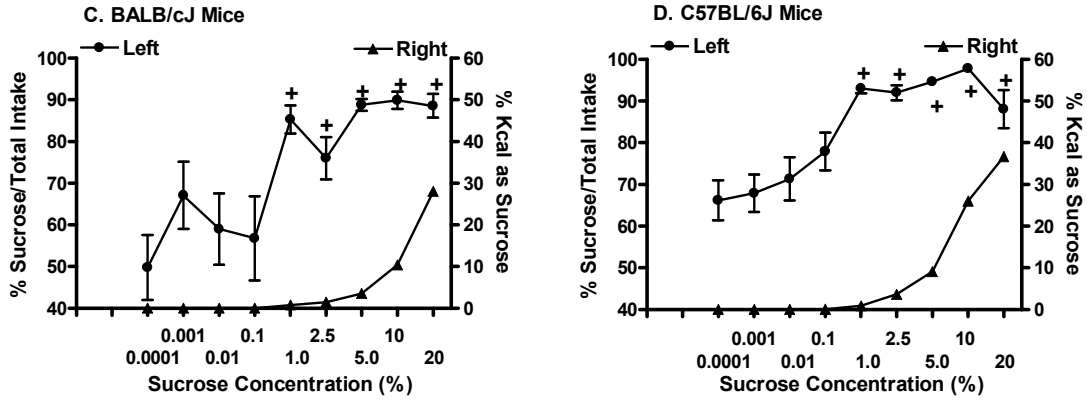


Figure 2 E and F

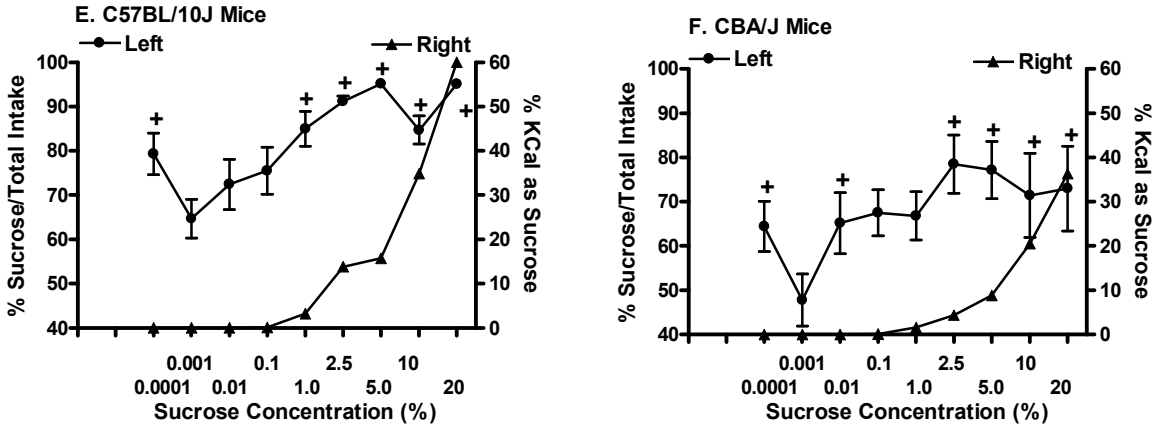


Figure 2 G and H

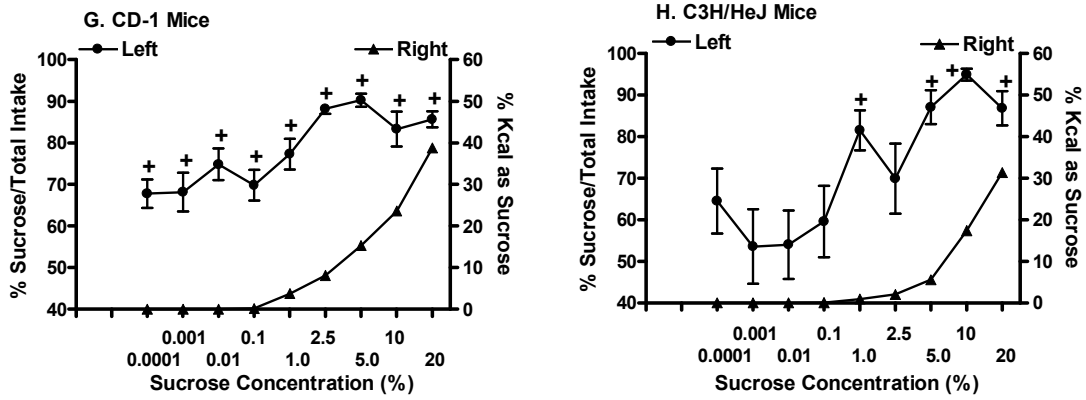


Figure 2 I and J

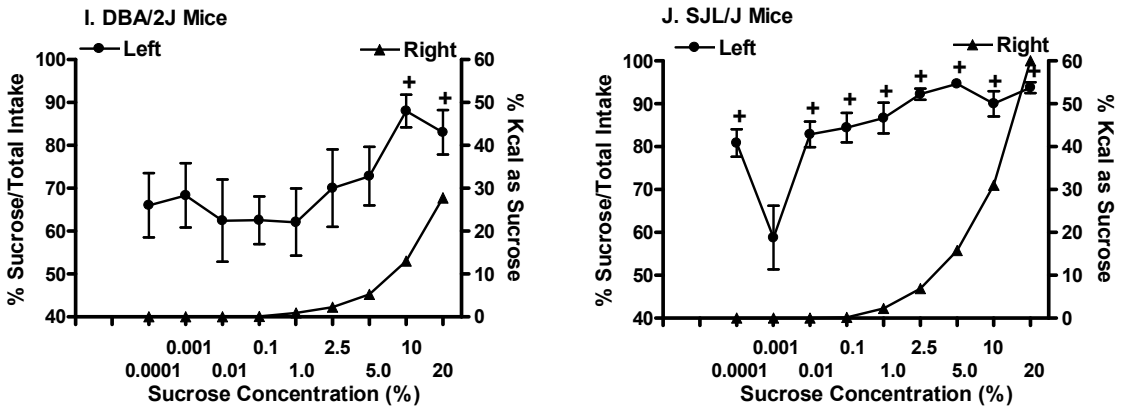
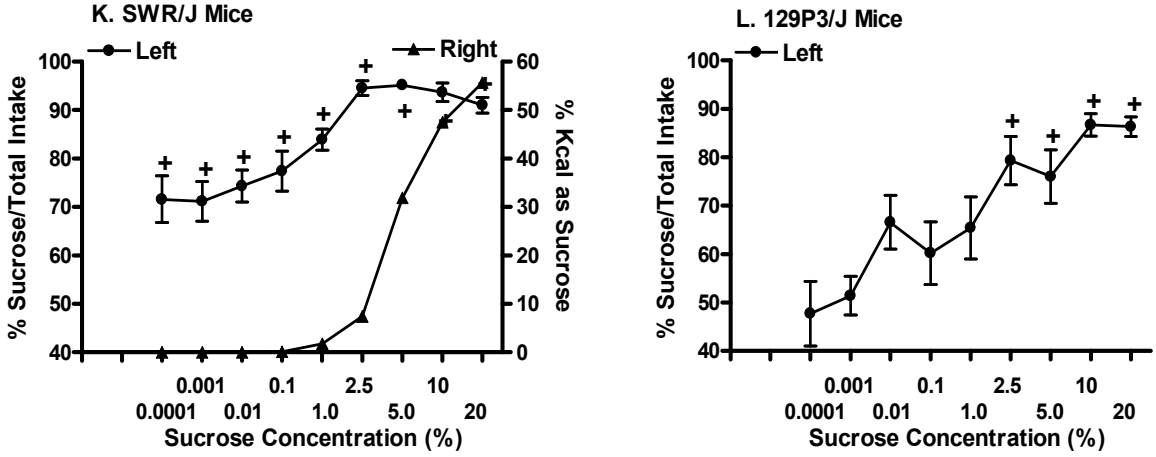


Figure 2 K and L



CBA/J, CD-1 and C3H/HeJ strains (Figures 2C, 2D, 2F-2H), and lower (>30%) consumption was observed in AKR/J and DBA/2J strains (Figures 2B, 2I).

Increased sucrose consumption at the high sucrose concentrations also resulted in a compensatory decrease in chow intake in some strains (A/J, C57BL/6J, C57BL/10J, CBA/J, CD-1, SJL/J, and SWR/J). Except for CD-1 mice in which significant reductions in chow intake was already reduced at a sucrose concentration as low as 1% (Figure 1G), significant reductions in chow intake typically occurred at higher sucrose concentrations (5-20%: Figures 1A, 1D-1F, 1J, 1K). In contrast, chow intake failed to vary at any sucrose concentrations in the AKR/J strain (Figure 1B), and actually significantly increased across ranges of lower sucrose concentrations in BALB/cJ, C3H/HeJ, and DBA/2J mice (Figures 1C, 1H, 1I).

Correlational Data: Table 4 displays the pairwise correlation coefficients between some of the sucrose concentrations. Significant covariation was only occasionally observed, typically between some of the lower concentrations. Sucrose concentrations of 1% and 10% were not correlated with any other sucrose concentration. There was a similar lack of significant correlation between percentage of sucrose intake and percentage of saccharin intake as previously reported by Lush (1989). As evident from Table 5, the percentage intake of a previously-reported (Lush,1989) 1.6 m*M* saccharin solution was correlated only with intake of a 0.1% sucrose solution measured in the present study. Correlation coefficients (Table 5) between strain variation in the percentage of sucrose intake and three *Tas1r3* variants (Reed et al., 2004) appeared to be

Table 4: Pearson product-moment correlation coefficients between sucrose concentrations for percentage sucrose intake in 10 inbred mouse strains

Sucrose Concentration	.01%	0.1%	1.0%	2.5%	5.0%
.01%	---				
0.1%	.91*	---			
1.0%	.36	.51	---		
2.5%	.86*	.86*	.53	---	
5.0%	.49	.62	.91*	.71	---
10.0%	.09	.21	.63	.24	.62

* Significant correlation after Bonferroni corrections ($p < 0.05$).

Table 5: Pearson product-moment correlation coefficients between sucrose concentration and percentage saccharin intake and three polymorphisms in the *Tas1r3* taste receptor gene in 10 inbred mouse strains

	% Saccharin Intake[a]	*Tas1r3* Polymorphisms[b]
Sucrose Concentration		
.01%	.71	.83*
0.1%	.81*	.91*
1.0%	.76	.74
2.5%	.73	.86*
5.0%	.74	.77
10.0%	.51	.50

[a] See Lush (1988) for % saccharin intake data.
[b] See Reed and co-workers (2004) for *Tas1r3* polymnorphisms.
* Significant correlation after Bonferroni corrections ($p < 0.05$).

sucrose concentration-dependent, with instances of significant covariation observed at lower sucrose concentrations. The intake of a 20% sucrose concentration or total kilocaloric intake during its availability was not significantly correlated with either 1% or 2.5% sucrose intake (data not shown).

Discussion:

Strong and systematic strain differences were observed for sucrose intake especially as functions of the total amount of sucrose consumed and the percentage of total kilocalories consumed as sucrose. In this regard, the A/J, C57BL/6J, CD-1 and SWR/J strains consumed the greatest (11.6-22 g) total amounts of sucrose. When adjusted for body weight, the A/J, C57BL/6J, SJL/J and SWR/J strains persisted in consuming the greatest amounts of sucrose per 30 g of body weight (12.5-24.1 g/30 g BW). Further, the A/J, C57BL/10J, SJL/J and SWR/J strains consumed the greatest (50-63%) percentages of kilocalories consumed as sucrose. The BALB/cJ and 129P3/J strains displayed intermediate responsiveness. Alternatively, the AKR/J, CBA/J, C3H/HeJ and DBA/2J strains appeared to consume the least (6.3-8.2 g/30 g BW) amount of sucrose, and displayed lower (25-36%) percentages of kilocalories consumed as sucrose. Interestingly, the A/J, C57BL/6J, C57BL/10J, CD-1, SJL/J and SWR/J strains all displayed the most pronounced compensatory decreases in chow intake as the percentage of kilocalories consumed as sucrose increased. Whereas BALB/cJ and CBA/J mice displayed this effect at the highest sucrose concentration, the AKR/J, C3H/HeJ and DBA/2J strains failed to significantly alter chow intake at any of the sucrose concentrations.

The present study differed from previous studies in terms of the number of strains (12) tested across a wide (9) range of both "sub-threshold" (0.0001-0.1%) and "supra-threshold" (1-20%) sucrose concentrations using two-bottle 24-h preference tests. Moreover, the order of presentation of sucrose concentrations was controlled as suggested from previous work (Harder et al., 1989), and was found to be an important variable for only one (CBA/J) strain. Although sucrose and water bottle positions were also controlled given the previously appreciated relevance of this variable (Bachmanov et al., 2002b), this was done by switching the bottle position every 24 hours. It is important to note a potential limitation of our testing procedure in that it differs from a technique of presenting solutions for two consecutive days ("the 48 h test") and switching the sides of the presentation between the first and second day. Although the one-day test procedure may have added some noise to the data, we do not believe that this invalidates the results.

Finally, systematic measurement of chow, water and sucrose intake allowed the determination of strain differences in kilocalorie intake consumed as sucrose as well as systematic changes in chow intake across sucrose concentrations. Among the A/J, C57BL/6J, CD-1 and SWR/J strains showing the greatest magnitude of sucrose intake, the CD-1 and SWR/J strains also displayed the greatest sensitivities to sucrose, showing significantly greater consumption across all nine sucrose concentrations relative to the corresponding water ration. In contrast, significantly greater sucrose consumption occurred for A/J and C57BL/6J strains at the 2.5% and 5% sucrose concentration respectively. Among the AKR/J, CBA/J, C3H/HeJ and DBA/2J strains showing the smallest magnitude of sucrose intake, the C3H/HeJ and DBA/2J strains also displayed the

least sensitivities to sucrose, showing significantly greater consumption across the two highest (10-20%) sucrose concentrations only.

A number of previous studies have employed the percentage of sweetener consumed as a function of total fluid intake as a measure of preference (e.g., Capeless & Whitney, 1995; Fuller, 1974; Lush, 1989; Pothion et al., 2004). In the present study however, strains that showed both larger (e.g., C57BL/6J, C57BL/10J, SWR/J, SJL/J) and smaller (e.g., C3H/HeJ) magnitudes of sucrose intake invariably showed very high ($\geq$95%) preferences for sucrose. This indicates that sucrose intake is not necessarily a reflection of sucrose preference. Thus, the importance of measuring a variety of intake variables across a wide range of strains and concentrations is apparent.

Interestingly, significant pairwise correlation coefficients between sucrose concentrations were only occasionally observed. It is unlikely that the estimation of covariance was underestimated by restricting the range at the highest (10%) and lowest (0.01%) sucrose concentrations, since significant strain differences were evident at these concentrations. In addition, the range of differences in the percentage of sucrose intake was identical to those obtained for lower and middle concentrations. Furthermore, more moderate 1.0% and 5.0% sucrose concentrations also showed little if any significant correlations. Since we used a sufficient number of inbred strains, allowing for the estimation of genetic correlations (Hegmann & Possidente, 1981), the data suggest that the genetic, and ultimately physiological, regulation of sucrose intake may differ across a range of concentrations.

As noted above, large (26-30) numbers of mouse strains were previously evaluated for sweet intake at only a single saccharin (1.6 mM) or sucrose (50 mM)

concentration (Lush,1989; Reed et al., 2004), limiting the generalizability of effects. Lush (1989) found that the pattern of strong preferences for saccharin and sucrose at these single concentrations were greater in A/J, C57BL/6J, C57BL/10J and SWR/J strains (73-97%) than in AKR/J, CBA/J, C3H/HeJ, DBA/2J and 129P3/J strains (51-61%). We attempted to correlate our data at various sucrose concentrations with that of Lush (1989), who used the 1.6 mM saccharin concentration (Table 5), but found that correlations between these sweetener concentrations were generally not present, except at the relatively low 0.1% sucrose concentration. Since only at very low concentrations is a relationship between these two sweeteners present, sensitivity to higher sucrose concentrations may be under different genetic control than those mediating relatively lower concentrations.

In support of genetic commonality at lower sweetener concentrations, Capeless and Whitney (1995) found order of effects similar to those observed in the present study for strain preference scores in 129P3/J, C57BL/6J, BALB/cJ, C3H/HeJ, and DBA/2J mice across four saccharin concentrations. Furthermore, previous examination (Pothion et al., 2004) of only supra-threshold sucrose concentrations revealed little consistent strain-specific differences in sucrose intake except for 129P3/J mice showing the smallest magnitudes of responses. Therefore, previous studies show strong and similar patterns of results to the present findings.

As the percentage of kilocalories consumed as sucrose increased, we also observed that the compensatory decreases in chow intake differed by strain. Whereas the A/J, C57BL/6J, C57BL/10J, CD-1, SJL/J and SWR/J strains all displayed the most pronounced compensatory decreases in chow intake, the AKR/J, C3H/HeJ and DBA/2J

strains failed to significantly alter chow intake at any of the sucrose concentrations. This very rapid decrease in chow consumption in the presence of sucrose suggests that some strains may be displaying either a greater sensitivity to caloric intake or greater ability to adapt and respond thereto. Divergent responders provide a model for studying and identifying the genetic substrates associated with the ability to regulate kilocalorie intake across a variety of energy sources. Such data might enable us to predict the ability of chronic exposure to concentrated sucrose (as an alternative energy source to chow) on increase in weight gain, obesity and diabetic symptoms in non-compensating strains relative to compensating strains.

Extreme responding strains currently identified for several sucrose intake-related variables may serve as progenitors for QTL analysis and subsequently, the identification of trait relevant genes. Previous QTLs for saccharin and sucrose intake, including *Prp* (Blizard et al., 1999), *Sac* (Fuller, 1974; Lush,1995), and *Tas1r3* (Inoue et al., 2004) have been localized to the distal chromosome 4, using C57BL/6J crosses with DBA/2J or 129P3/J mice. Interestingly, these strains did not display the most divergent responses to sucrose intake from the eleven strains evaluated in this study. Furthermore, correlations between the percentage of sucrose intake and *Tas1r3* taste receptor gene variants were not uniformly significant across sucrose concentrations (Table 5). It is possible that the *Tas1r3* correlation has been underestimated by using the Bonferroni correction for multiple comparisons, reducing the statistical power. Many coefficient values exceeded 0.70, but were not significant. We do not believe this is true for the correlation between different sucrose concentrations since many of those coefficients are quite low and would probably fail to achieve significance even without Bonferroni corrections. Nonetheless,

correlation coefficients between the percentage of sucrose intake of the highest sucrose solution correlated (10%) and the percentage intake of a 1.6 m*M* saccharin, or with *Tas1r3* taste receptor gene variants, were uniformly low. The overall lack of uniform correlations across different sucrose concentrations, support our assertion that distinct genetic mechanisms may underlie the intake of solutions with high and low sweetener concentrations. Thus, a comprehensive understanding of intake of sweeteners like sucrose, will require study across a large range of concentrations.

Sucrose intake is a multi-faceted behavior with separate and dissociable orosensory and post-ingestive mechanisms (see review: Sclafani, 2006b), and thus in all likelihood, is under polygenic control. It is likely that QTL analyses based upon a broader range of sensitivities, such as those characterized in the present study, will allow for identification of additional trait-relevant QTLs, including some with a smaller contribution to the overall genotypic variance. Additionally, using strains identified as good or poor regulators of chow in response to high sucrose ingestion may provide insight into the genetic basis of obesity.

VI. Specific Aim 2: The Role of Genetic Variance in Fat (Intralipid) Intake in Inbred and Outbred Mouse Strains

Introduction:

Systematic analyses of rodent strain differences are important sources regarding the genetic control of all aspects of ingestive behavior (see review: Reed et al., 1997). These studies indicate widespread strain-dependent (i.e., genetic) variance in food, water and mineral intake as well as spout side preference (Bachmanov et al., 2002a; 2002b). Particular orosensory stimuli such as salts (e.g., Bachmanov et al., 2002a; Bachmanov et al., 1998a; 1998b; Beauchamp & Fisher, 1993; Tordoff et al., 2002), bitter tastants (e.g., Bachmanov et al., 1998a; Blizard et al., 1999; Capeless & Whitney, 1992; Harder et al., 1992; Harder & Whitney, 1998; Lush, 1984; Tordoff et al., 2002), saccharin (e.g., 9,10,13,20,26-29,33,39), and sucrose (e.g., Bachmanov et al., 1997; Blizard et al., 1999; Inoue et al., 2004; Lewis et al., 2005; Lush, 1989; Pothion et al., 2004; Ramirez & Fuller, 1976; Stockton & Whitney, 1974) are also subject to intake differences among strains. In addition, these studies help to identify strains with divergent sensitivities for these parameters, offering opportunities for subsequent QTL analyses to localize chromosomal regions, and ultimately genes, critically involved in such differences.

Differences in dietary fat intake are associated with genetic variation (see review: West & York, 1998), and have led to identification of dietary resistance and susceptibility in inbred and outbred strains of rats (e.g., Levin & Sullivan, 1987; Schemmel et al., 1970) and mice (e.g., West et al., 1992; West et al., 1995). The latter studies identified particular strains in which moderate intake of a high-fat diet promoted weight gain and

obesity (e.g., AKR/J mice) and other strains in which large intake of the high-fat diet was not accompanied by weight gain (e.g., SWR/J). Moreover, such weight effects were largely due to variations in the dietary fat content, but this variable weakly correlated with total energy intake. These particular strains displayed similar effects whether the fat source was shortening, lard or granular, and whether the high- and low-fat diets were isocaloric (Smith-Richards et al., 1999). Indeed, whereas AKR/J and C57BL/6J mice self-selected the highest proportion of fat in macronutrient diet selection with epididymal fat correlated with fat consumption, SWR/J and CAST/Ei strains consumed fat that was inversely correlated with epididymal fat (Smith et al., 2000). Moreover, whereas the diet-sensitive AKR/J and DBA/2J strains consumed more fat, displayed more adiposity and displayed elevated levels of leptin and insulin, the C57BL/6J strain showed an equal preference between protein and fat, and displayed normal insulin and leptin levels (Alexander et al., 2006). In contrast, obesity-resistant SWR/J and A/J mice consume more fat than carbohydrate, but fail to gain weight, potentially because of lower insulin levels, increased capacity of skeletal muscle to metabolize fat, enhanced paraventricular galanin and reduced arcuate NPY (Leibowitz et al., 2005). Our laboratory (Lewis et al., 2006a) found genetic variance in the sensitivity and magnitude of feeding responses of mouse strains exposed to the free fatty acid oxidation inhibitor, mercaptoacetate. Inbred DBA/J and outbred CD-1 mice were the most sensitive to mercaptoacetate-induced feeding, whereas mercaptoacetate failed to significantly increase food intake in A/J, C57BL/10J and 129P/3J mice. A series of genetic loci were mapped to explain some of these genetic variations for fat and obesity (e.g., Bachmanov et al., 2001; Fisler et al., 1993; Smith-Richards et al., 2002; Warden et al., 1995; West et al., 1994a; West et al.,

1994b). The above studies used solid fat sources (e.g., shortening, lard, granular), making it relatively difficult to systematically alter the amount of fat in the diet over a wide concentration range, in short-term intake tests, to determine if differences in sensitivity may account for some of the observed genetic variance.

Specific Aim 1 reported on the factors potentially involved in murine genetic variance in sucrose intake among 11 inbred (A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL6/J, C57BL10/J, DBA/2J, SJL/J, SWR/J, 129P3/J) and one outbred (CD-1) strains, thereby allowing for the valid estimation of genetic correlations (Harder & Whitney, 1998). A/J, C57BL/6J, CD-1 and SWR/J strains consumed the greatest (11.6-22 g) amount of sucrose, whereas the A/J, C57BL/10J, SJL/J and SWR/J strains consumed the greatest (50-63%) percentages of kilocalories as sucrose. The AKR/J, CBA/J, C3H/HeJ and DBA/2J strains consumed the least (6.9-7.9 g) amount of sucrose, and displayed lower (25-36%) percentages of kilocalories consumed as sucrose. Whereas A/J, C57BL/6J, C57BL/10J, CD-1, SWR/J and SJL/J strains all displayed the most pronounced compensatory decreases in chow intake as the percentage of kilocalories consumed as sucrose increased, the AKR/J, C3H/HeJ and DBA/2J strains failed to significantly alter chow intake even at high sucrose concentrations. Therefore, in this phase of our study, the use of liquid sucrose solutions over a wide range of different concentrations allowed for analyses of concentration-dependent differences in sensitivities as a function of murine strain.

In contrast to sucrose, liquefied fat sources at different concentrations present a challenging solubility problem. Their inability to stay in solution over a time course (e.g., 24 h) creates a barrier to the study of murine intake. Intralipid (Baxter Healthcare

Corporation, Deerfield, Illinois) is an emulsified fat solution (20%) made almost exclusively from soybean oil (20 g in 100 ml), and is used clinically for delivery of a fat source to human patients. The use of Intralipid provided a fat that is evenly distributed in solution across a wide range of concentrations. Intralipid solutions are readily consumed in a manner similar to sucrose and other palatable solutions (e.g., Higgs & Cooper, 1998a; 1998b).

Assessing Intralipid intake across concentrations (0.00001-5%) can be undertaken by methods parallel to our previous evaluation on intake of different sucrose concentrations (Lewis et al., 2005). Moreover, Intralipid can be compared to water intake in two-bottle, 24-h preference tests for the study of genetic variance in fat intake. A number of the strains that were previously evaluated by our laboratory for sucrose intake (Lewis et al., 2005) displayed three divergent patterns of fat intake in other published studies. These patterns are; high fat intake with weight gain (e.g., AKR/J, C57BL/6J, DBA/2J: Alexander et al., 2006; Smith-Richards et al., 1999; West et al., 1992, 1995); high fat intake without weight gain (e.g., A/J, SWR/J: Leibowitz et al., 2005; Smith et al., 2000); and finally, low fat intake (BALB/cJ, C3H/HeJ SJL/J, 129/J: Alexander et al., 2006; Smith et al., 2000).

The present study examined these same strains for Intralipid intake across a wide range of concentrations in two-bottle 24 h preference tests using all of the dependent measures previously applied to our sucrose studies (Lewis et al., 2005). The results of this Specific Aim were published by our laboratory in Physiology and Behavior in 2007 (Lewis et al., 2007).

Methods:

Subjects: As detailed in the General Methods section, male outbred CD-1 (n=10), and 11 inbred mouse strains: A, AKR, BALB/c, CBA, C3H/He (C3H), C57Bl/6 (BL/6), C57Bl/10 (BL/10), DBA/2 (D2), SJL, SWR, 129P3 (129) (n=9-10 for each inbred strain) purchased from Charles River and Jackson Laboratories at 12 weeks of age, were initially acclimated to the Queens College vivarium for their thirteenth week in group (5 per cage) housing, and were maintained on a 12 h light: 12 h dark cycle (lights off at 2000 h) at a constant temperature of 22^{o}C with *ad libitum* access to food and water. During the ensuing fourteenth week each animal was housed individually in plastic cages to acclimate them to isolated housing. Animals were then tested over an approximate two-week period (weeks 15-17) according to the following paradigm.

Intralipid Intake Procedure: As detailed in the General Methods section, each mouse was initially provided with a pre-weighed (~20 g) ration of Purina Mouse chow (5.3 kcal/g) and two calibrated and pre-weighed ($\pm$0.1 g = $\pm$0.1 ml) sipper tubes each filled with water (~40 ml each) for baseline measures. To minimize potential bottle position preference effects (Bachmanov et al., 2002b) the position of the two water bottles were systematically switched according to a according to a L-R-R-L and a R-L-L-R position, respectively. Each mouse of each strain was assessed for chow intake, adjusted for spillage, and water intakes from each individual bottle every 24 h over 4 days.

Following baseline, the same procedure was employed except one bottle of water was replaced with a different Intralipid concentration each day. Nine Intralipid concentrations were diluted with water from the original 20% concentration (0.00001%-5%) and used for testing in these two-bottle preference tests, with half of the mice of each

strain presented with an ascending Intralipid concentration order, and the remaining half with a descending order (with Intralipid bottle position systematically controlled (Harder et al., 1989)). Intralipid and water intakes ($\pm$ 0.1 g) as well as chow were measured daily for each concentration of Intralipid.

Statistics: As detailed previously in the General Methods section, a one-way analysis of variance assessed pre-existing differences in body weight and in total baseline water intake and chow intake across strains. To assess sampling of the two water bottles under baseline conditions, a two-way randomized block analysis of variance was also performed with strains as the between-subject variable and water intake from the two bottles as a repeated measure. Three-way randomized block analyses of variance were performed for the following variables: Intralipid relative to water intake, with the 12 strains as the between-subject variable, the ten (baseline and nine Intralipid concentrations) conditions as a within-subject variable, and the intake from the Intralipid and water bottles as a second within-subject variable. (The threshold for or sensitivity to Intralipid for each mouse strain was operationally defined as that lowest concentration which Intralipid intake significantly differed from water); and for Intralipid intake in those mice that received an ascending order of Intralipid concentrations with those that received a descending order. Two-way randomized-block analyses of variance were also systematically performed across strains and across Intralipid concentrations to assess changes in the percentage of Intralipid consumed, the total amount of chow intake, and the percentage of kilocalories consumed as Intralipid. Due to the significant differences in body weight across strains (Table 6), a two-way randomized block analysis of variance

Table 6: Baseline water (g, ±S.E.M.) and chow (g, ±S.E.M.) intake and body weight (g, ±S.E.M.) in 12 mouse strains

Strain	Water (g)	Chow (g)	Body Weight (g)
A/J	4.6 (0.2)	3.8 (0.2)	19.6 (0.5)
AKR/J	8.6 (0.2)*	4.1 (0.1)	26.6 (1.0)*
BALB/cJ	7.4 (0.2)	6.7 (0.2)+	23.5 (0.2)
C57BL/6J	4.8 (0.4)	3.6 (0.2)	27.8 (0.8)*
C57BL/10J	6.9 (0.5)	4.8 (0.2)*	22.7 (0.3)
CBA/J	6.7 (0.2)	4.0 (0.2)	22.6 (0.5)
CD-1	9.3 (0.8)*	4.1 (0.4)	37.0 (1.0)+
C3H/HeJ	5.5 (0.2)	4.4 (0.2)	18.5 (1.1)
DBA/2J	6.5 (0.2)	5.3 (0.2)*	23.7 (0.2)
SJL/J	6.6 (0.1)	3.6 (0.2)	21.1 (0.3)
SWR/J	9.2 (0.3)*	3.9 (0.1)	18.3 (0.6)
129P3/J	5.9 (0.4)	5.0 (0.4)*	22.6 (0.4)

*Significantly greater relative to all other unmarked strains in column
+Significantly greater than * strains in column

was performed with the 12 strains as the between-subject variable and the ten conditions as a within-subject variable for transformed Intralipid intake per 30 grams of body weight. This corresponded to previous analyses performed for the sucrose study (Lewis et al., 2005). Tukey comparisons ($P<0.05$) were performed within strains in the presence of significant effects relative to corresponding baseline values.

Pearson product-moment correlation coefficients (*r*) subject to Bonferroni correction for multiple comparisons were calculated for Intralipid intake per se and for the percentage of kilocalories consumed as Intralipid in the 12 tested mouse strains, examining those Intralipid concentrations (0.5, 1, 2 and 5%) producing sizable intake. Further, narrow-sense trait heritability was determined by comparing the between-strain variance to the total variance for each concentration of Intralipid in terms of intake itself and intake adjusted for body weight using the formula: $h^2 = VA/(VA+VE)$.

Results:

Baseline values in water and chow intake: Significant differences were observed among mouse strains in total baseline water ($F(11,106)= 19.66$, $p<0.0001$) and chow ($11,105)= 15.71$, $p<0.0001$) intakes. As summarized in Table 6, baseline water intake was greatest in CD-1, SWR/J and AKR/J strains, with significantly less water intake observed in all other strains. Baseline chow intake was greatest in the BALB/cJ strain relative to all other strains with DBA/2J, 129P3/J and C57BL/10J mice consuming significantly greater amounts of chow relative to the remaining strains as well (Table 6). Significant differences were observed among mouse strains in body weight ($F(11,106)= 58.67$, $p<0.0001$). Body weight was significantly greater in CD-1 mice relative to the 11

inbred strains; C57BL/6J and AKR/J in turn weighed significantly more than the remaining nine inbred strains (Table 6).

Interestingly, the strain-specific effects upon baseline water intake displayed strong positive correlations with previous studies performed in our (r= .647 (23)) and other (r= .666 (Bachmanov et al., 2002b)) laboratories. A similar significant pattern of effects (r=0.77) was observed for strain-specific body weights in the present and our previous (Lewis et al., 2005) study. In contrast, baseline chow intake in the present study failed to display strain-specific correlations with either our previous (Lewis et al., 2005) or other (Bachmanov et al., 2002b) studies.

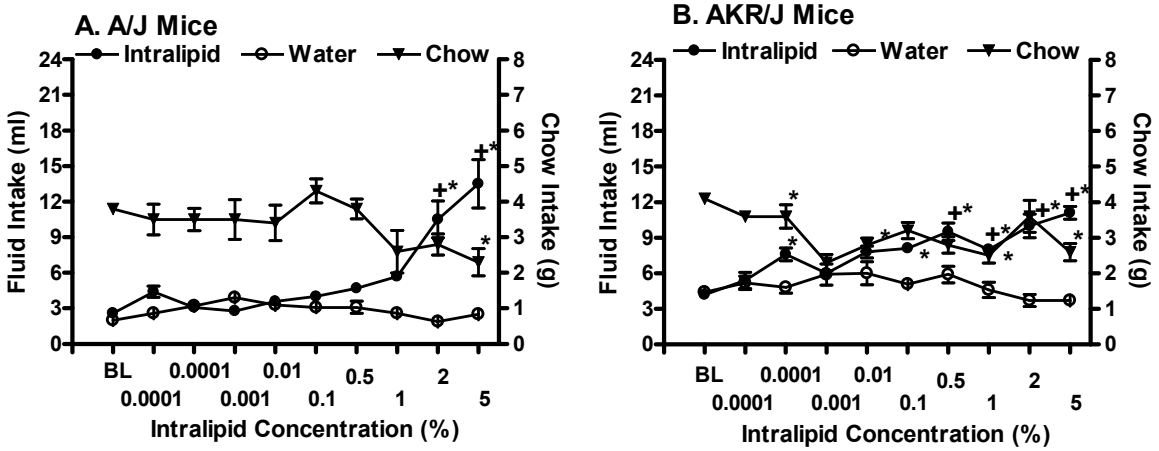
Analysis of two-bottle baseline water intake revealed significant differences across strains ($F(11,99)= 21.28$, $p<0.0001$), for intake for the two fluids ($F(1,9)= 7.24$, $p<0.25$), but not for the interaction between strains and fluid choice ($F(11,99)= 0.45$, n.s.). Importantly, individual comparisons revealed that all twelve strains displayed similar patterns of sampling of their two water bottles during baseline (BL) testing (Figure 3), indicating that preferences described for intake of different concentrations of Intralipid were not due to some underlying intra-strain preference for intake from one water bottle relative to the other.

Intralipid and water intake: In analyzing Intralipid and water intake across strains and Intralipid concentrations, significant differences in intake were observed among strains ($F(11,99)= 46.17$, $p<0.0001$), across concentrations ($F(9,81)= 37.03$, $p<0.0001$), between the two fluids ($F(1,9)= 303.06$, $p<0.0001$), and for the interactions between strains and concentrations ($F(99,891)= 6.84$, $p<0.028$), strains and fluids ($F(11,99)= 16.41$, $p<0.0001$), concentrations and fluids ($F(9,81)= 145.76$, $p<0.0001$) and

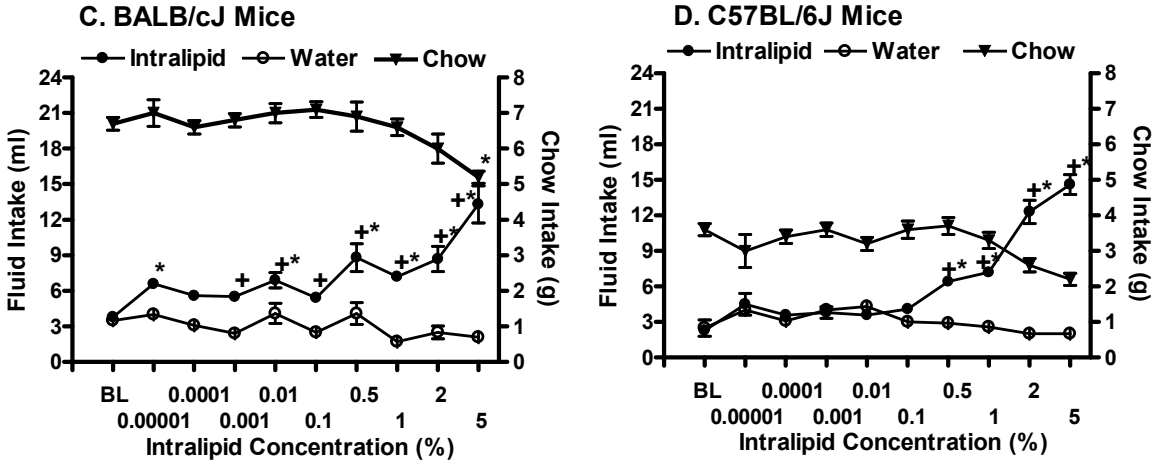
Figure 3. Alterations in Intralipid (left ordinate, mean, ±SEM), water (left ordinate, mean, ±SEM) and chow (right ordinate, mean, ±SEM) intake across baseline and nine different Intralipid concentrations in one outbred (CD-1) and eleven inbred (A/J, AKR/J, BALB/cJ, C57BL/6J, C57BL/10J , CBA/J, C3H/HeJ, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice.

In this and all subsequent figures, the asterisks (*) denote a significant difference in intake relative to corresponding baseline conditions, and the crosses (+) denote a significant difference in Intralipid intake relative to corresponding water intake at that concentration (Tukey comparisons, $P<0.05$). The twelve panels of this figure are found on the following three pages.

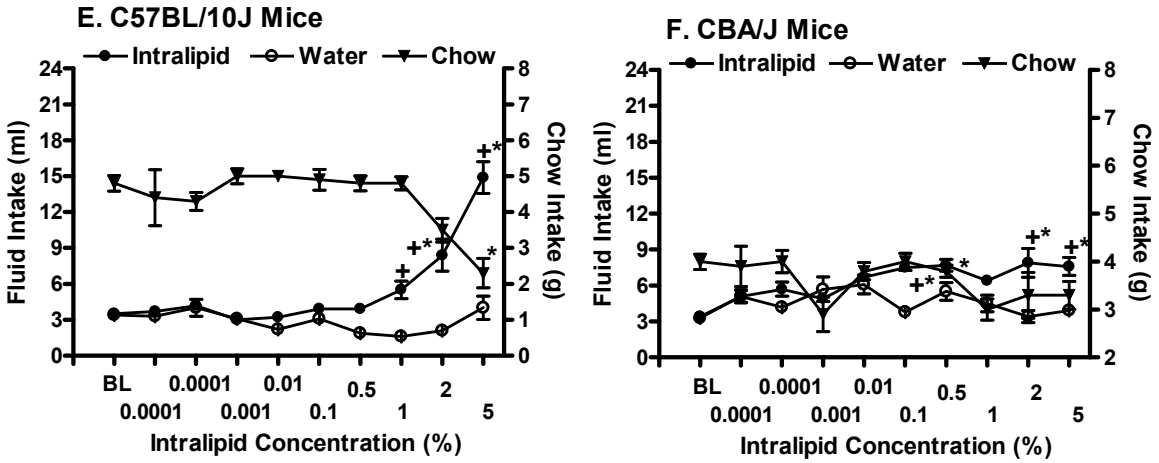
Figures 3A and B



Figures 3C and D



Figures 3E and F



Figures 3G and H

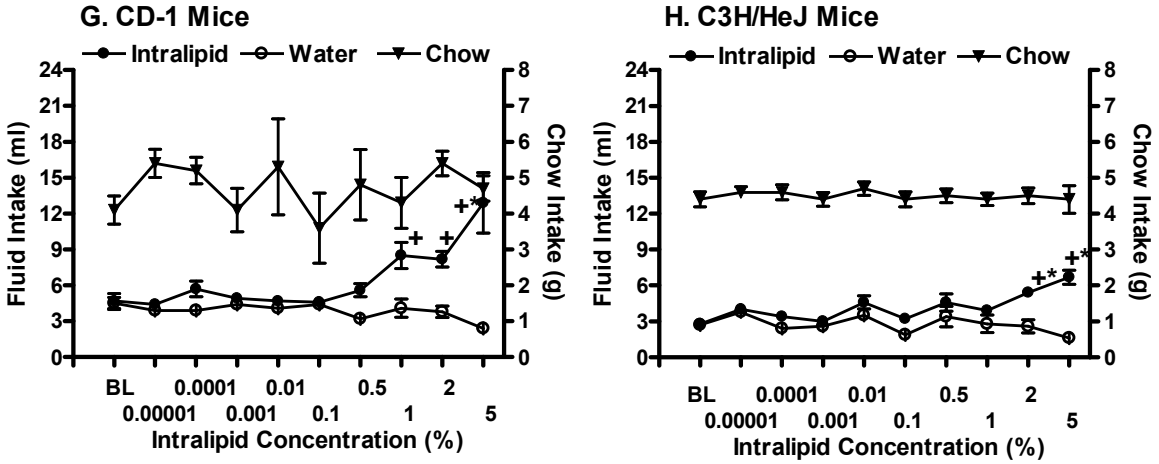
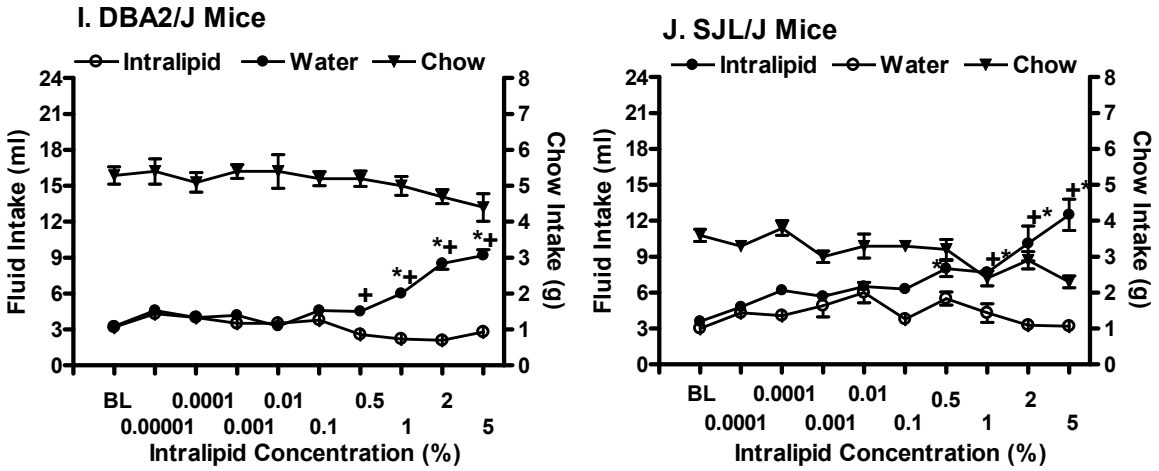
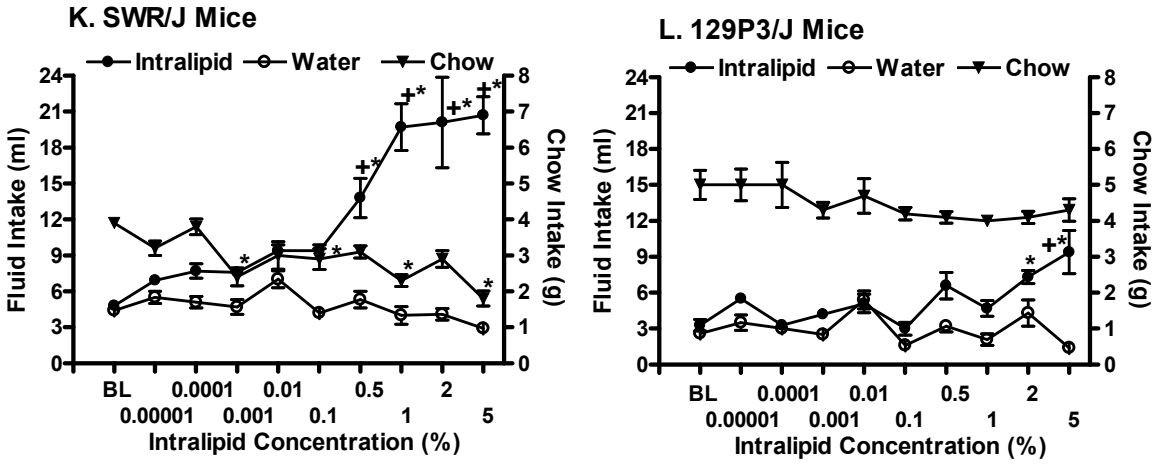


Figure 3I and J



Figures 3K and L



among strains, concentrations and fluids ($F(99,891)= 4.62$, $p<0.0001$). Significant differences in chow intake were observed among strains ($F(11,110)= 43.62$, $p<0.0001$), across concentrations ($F(9,90)= 28.70$, $p<0.0001$) and for the interaction between strains and concentrations ($F(99,990)= 2.80$, $p<0.0001$). First, it is important to note that systematic analyses of intrastrain differences in Intralipid intake at a given concentration were performed as a function of whether the nine Intralipid concentrations were presented in ascending or descending order. This factor failed to produce significant effects in outbred CD-1 mice and the CBA/J, C3H/HeJ, C57BL/6J, DBA/2J and 129P3/J inbred strains. However, significant order effects were observed for the BALB/cJ (main effect: $F(1,4)= 12.18$, $p<0.025$) inbred strain in which mice exposed to the descending order consumed significantly more Intralipid at the 0.1% concentration and less Intralipid at the 0.5, 2 and 5% concentrations. Significant order effects were observed for the A/J (main effect: $F(1,4)= 43.06$, $p<0.003$), AKR/J (main effect: $F(1,4)= 16.62$, $p<0.015$), C57BL/10J (order by concentration interaction: $F(9,36)= 9.18$, $p<0.039$), SJL/J (main effect: $F(1,4)= 17.52$, $p<0.014$) and SWR/J (order by concentration interaction: $F(9,36)= 13.12$, $p<0.022$) inbred strains in which mice exposed to the descending order consumed significantly more Intralipid at the 0.00001% (AKR/J), 0.5% (SJL/J, SWR/J), 1% (AKR/J), 2% (A/J, C57BL/10J, SJL/J, SWR/J) and 5% (A/J) concentrations. This observation emphasizes the importance of controlling for order effects in concentration presentation (Harder et al., 1989; Lewis et al., 2005).

Analysis of the sensitivity to low Intralipid concentrations among strains revealed that BALB/cJ mice significantly increased their Intralipid intake relative to their corresponding water intake at all concentrations from 0.001% to 5% (Figure 3C), thereby

displaying the greatest sensitivity (0.001% threshold). Intralipid intake was significantly increased relative to corresponding water intake between concentrations of 0.5%-5% in AKR/J, C57BL/6J, DBA/2J and SWR/J inbred strains (Figures 3B, 3D, 3I, 3K: 0.5% threshold), of 1%-5% in outbred CD-1 and inbred C57BL/10J and SJL/J strains (Figures 3E, 3G, 3J: 1% threshold), and of 2%-5% in A/J, CBA/J, C3H/HeJ and 129P3/J inbred strains (Figures 3A, 3F, 3H, 3L: 2% threshold), indicating systematic strain-specific sensitivities to lower Intralipid concentrations.

To control for the possibility that the magnitude of Intralipid intake might vary as a function of the significant body weight differences (Table 6), one additional analysis of variance examined Intralipid intake per 30 g of body weight. This revealed significant differences among strains ($F(11,99)= 47.36$, $p<0.0001$), among Intralipid concentrations ($F(9,81)= 83.71$, $p<0.0001$) and for the interaction between strains and concentrations ($F(99,891)= 6.40$, $p<0.0001$). As summarized in Table 7, weight-adjusted Intralipid intake was significantly greater than corresponding water intake following the six highest concentrations in SWR/J mice, the four highest concentrations in SJL/J mice, three high concentrations in AKR/J and BALB/cJ mice, the two highest concentrations in A/J, C57BL/6J, C57BL/10J and DBA/2J mice, and only at one of the highest concentration in CBA/J, CD-1 and 129P3/J mice. When adjusting for weight, the C3H/HeJ strain failed to display significant differences in Intralipid relative to water intake across concentrations.

Profound Strain-specific differences in Intralipid intake were observed in the following six strains. SWR/J mice displayed significantly greater weight-adjusted Intralipid intake from the 0.01 through 5% concentrations relative to the eleven other tested strains, from the 0.0001-0.001% concentrations relative to nine other tested strains,

Table 7: Alterations in Intralipid intake (mean), each corrected for 30 g of body weight, across baseline and nine different sucrose concentrations in one outbred (CD-1) and eleven inbred (A/J, AKR/J, BALB/cJ, C3H/HeJ, C57BL/6J, C57BL/10J, CBA/J, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice.

Strain	Water*	.00001%	.0001%	.001%	.01%	0.1%	0.5%	1.0%	2.0%	5.0%
A/J	3.97	6.55	4.51	3.98	4.76	5.19	5.92	7.03	12.43 +L	15.62 +D
AKR/J	4.80	5.95	8.36 N	6.55	8.51 G	8.54 H	9.76 +J	8.24	10.28 +Q	10.93 +
BALB/cJ	4.87	8.28 Q	6.93	6.74	8.50 G	6.55	10.44 +E	8.46	10.04 +	15.17 +F
C57BL/6J	2.54	4.72	3.65	3.80	3.57	3.96	6.02	6.64	11.25 +L	12.89 +M
C57BL/10J	4.60	4.89	5.46	3.95	4.23	4.96	4.90	6.59	9.22 +	13.99 +I
CBA/J	4.57	6.58	7.21	6.11	7.62	8.55 H	8.61 Q	7.22	8.78 +	8.39
CD-1	3.91	3.64	4.55	3.89	3.74	3.68	4.27	6.49	5.95	9.59 +
C3H/HeJ	4.74	6.30	5.13	4.36	6.19	4.21	5.79	4.82	6.53	8.02
DBA/2J	4.18	5.78	5.08	5.26	4.15	5.69	5.58	7.16	9.68 +	9.54+
SJL/J	5.13	6.71	8.46 N	7.68	8.73 G	7.81 O	9.95 +P	9.53 +R	12.44 +L	14.72 +K
SWR/J	7.92	11.02 C	11.95 B	11.19 B	14.02 +A	13.05 +A	18.81 +A	26.49 +A	26.63 +A	25.89 +A
129P3/J	4.47	7.18	4.11	5.11	6.24	3.65	7.59	5.46	8.47	11.01 +

*Baseline water intake from designated "Intralipid" bottle
+Significant increase in Intralipid intake from corresponding baseline value ($p<0.05$)
A: Significantly greater weight-adjusted Intralipid intake than all other eleven strains ($p<0.05$)
B: Significantly greater weight-adjusted Intralipid intake than A/J, AKR/J, C57BL/6J, C57BL/10J, CBA/J, CD-1, C3H/HeJ, DBA/2J and 129P3/J strains ($p<0.05$)
C: Significantly greater than A/J, AKR/J, C57BL/6J, C57BL/10J, CD-1, DBA/2J and SJL/J strains ($p<0.05$)
D: Significantly greater than AKR/J, CBA/J, CD-1, C3H/HeJ, DBA/2J and 129P3/J strains ($p<0.05$)
E: Significantly greater than A/J, C57BL/10J, CD-1, C3H/HeJ and DBA/2J strains ($p<0.05$)
F: Significantly greater than AKR/J, CBA/J, CD-1, C3H/HeJ and DBA/2J strains ($p<0.05$)
G: Significantly greater than C57BL/6J, C57BL/10J, CD-1 and DBA/2J strains ($p<0.05$)
H: Significantly greater than C57BL/6J, CD-1, C3H/HeJ and 129P3/J strains ($p<0.05$)
I: Significantly greater than CBA/J, CD-1, C3H/HeJ and DBA/2J strains ($p<0.05$)
J: Significantly greater than C57BL/10J, CD-1 and DBA/2J strains ($p<0.05$)

K: Significantly greater than CD-1, C3H/HeJ and DBA/2J strains ($p<0.05$)
L: Significantly greater than CD-1 and C3H/HeJ strains ($p<0.05$)
M: Significantly greater than CBA/J and C3H/HeJ strains ($p<0.05$)
N: Significantly greater than C57BL/6J and 129P3/J strains ($p<0.05$)
O: Significantly greater than CD-1 and 129P3/J strains ($p<0.05$)
P: Significantly greater than CD-1 and DBA/2J strains ($p<0.05$)
Q: Significantly greater than the CD-1 strain ($p<0.05$)
R: Significantly greater than the C3H/HeJ strain ($p<0.05$)

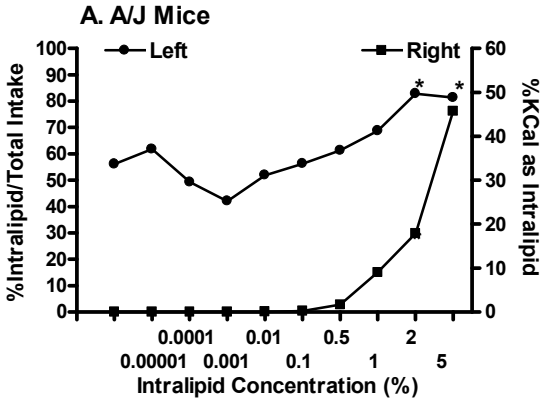
and at the 0.00001% concentration relative to seven other tested strains. A/J mice displayed significantly greater weight-adjusted Intralipid intake at the 5% concentration relative to six other tested strains, and at the 2% concentration relative to two other tested strains. BALB/cJ mice displayed significantly greater weight-adjusted Intralipid intake at the 0.5 and 5% concentrations relative to five other tested strains, at the 0.01% concentration relative to four other tested strains, and at the 0.00001% concentration relative to the outbred CD-1 strain. AKR/J mice displayed significantly greater weight-adjusted Intralipid intake at the 0.01 and 0.05% concentrations relative to four other tested strains, at the 0.5% concentration relative to three other tested strains, at the 0.0001% concentration relative to two other tested strains, and at the 2% concentration relative to the outbred CD-1 strain. SJL/J mice displayed significantly greater weight-adjusted Intralipid intake at the 0.01% concentration relative to four other tested strains, at the 0.5% concentration relative to three other tested strains, at the 5% concentration relative to three other tested strains, at the 0.0001, 0.1, 0.5 and 2% concentrations relative to two other tested strains, and at the 1% concentration relative to the C3H-HeJ strain. The other seven strains displayed more modest differences in Intralipid intake across concentrations relative to each other (Table 7).

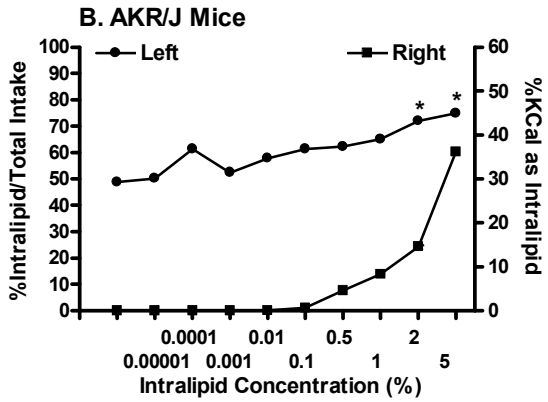
Significant differences in the percentage of fluid intake consumed as Intralipid were observed among strains ($F(11,99)= 5.36$, $p<0.0001$), across concentrations ($F(9,81)= 73.34$, $p<0.0001$), and for the interaction between strains and concentrations ($F(99,891)= 2.20$, $p<0.0001$). There was a great deal of congruence between

Figure 4. Alterations in the percentage of Intralipid over total intake(left ordinate, mean, ±SEM) and the percentage of kilocalories consumed as Intralipid (right ordinate, mean, ±SEM) across the nine different Intralipid concentrations in one outbred (CD-1) and eleven inbred (A/J, AKR/J, BALB/cJ, C57BL/6J, C57BL/6J, CBA/J, C3H/HeJ, DBA/2J, SJL/J, SWR/J, 129P3/J) strains of mice.

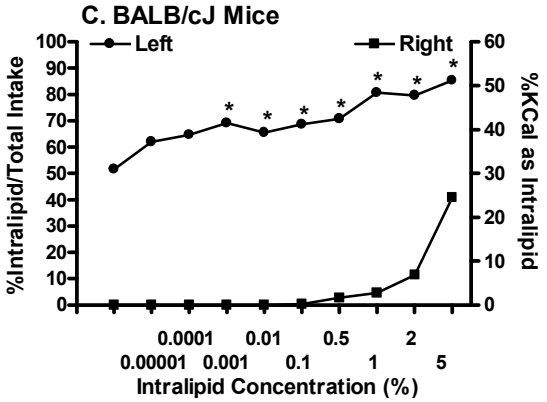
The twelve panels of this figure are found on the following three pages.

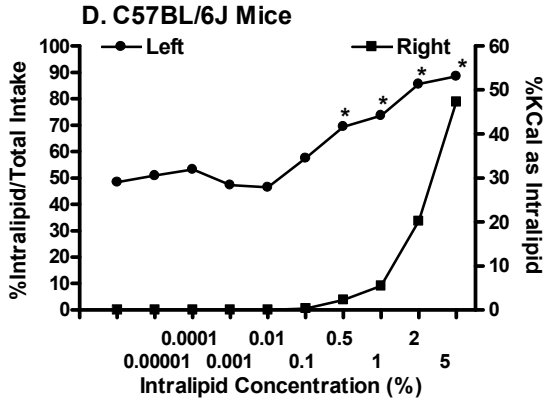
Figures 4A and B



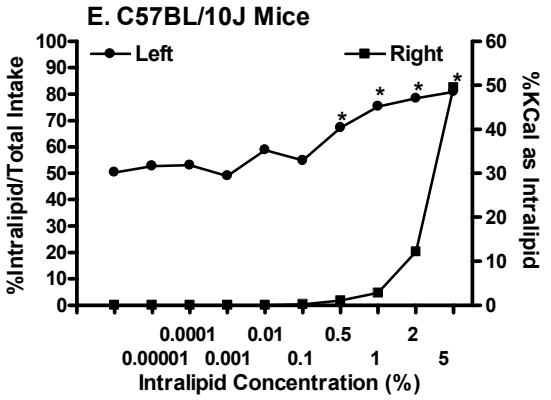


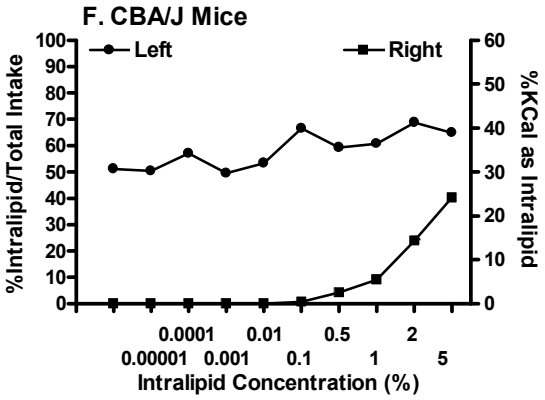
Figures 4C and D



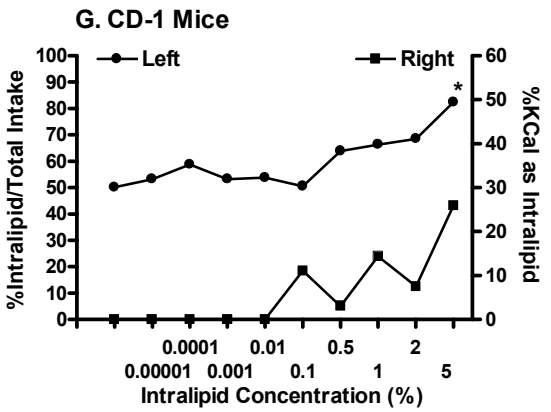


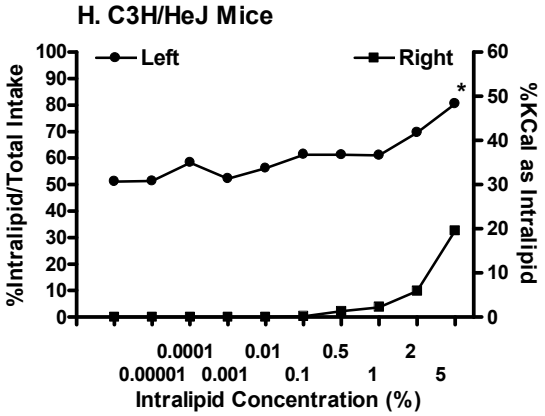
Figures 4E and F



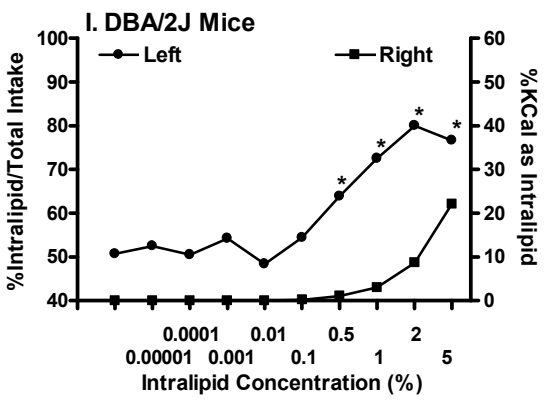


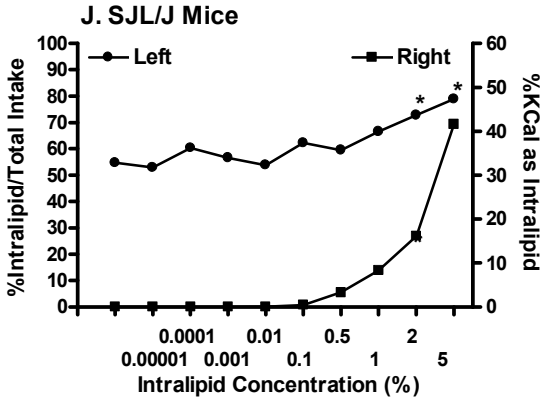
Figures 4G and H



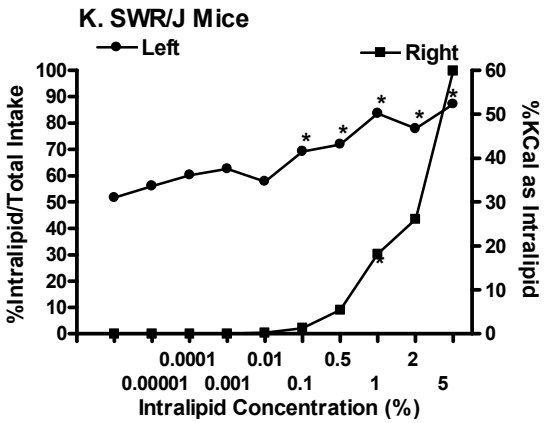


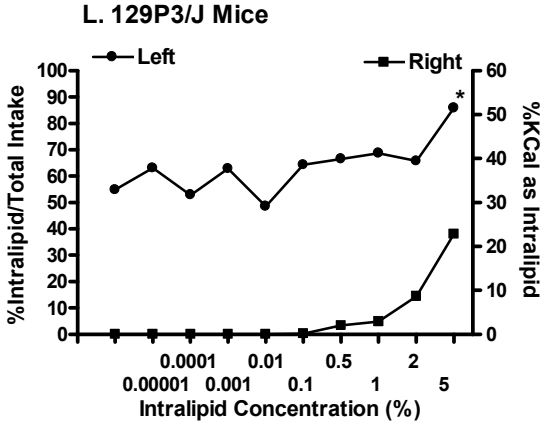
Figures 4I and J





Figures 4K and L





sensitivity to Intralipid as measured by intake per se and the percentage of fluid intake consumed as Intralipid (Figure 4). Thus, the percentage of fluid intake consumed as Intralipid was significantly greater in BALB/cJ mice at the seven highest concentrations (Figure 4C), in SWR/J mice at the five highest concentrations (Figure 4K), in C57BL/6J (Figure 4D), C57BL/10J (Figure 4E) and DBA/2J (Figure 4I) mice at the four highest concentrations, and in A/J (Figure 4A), AKR/J (Figure 4B), and SJL/J (Figure 4J) mice at the two highest concentrations. However, the percentage of Intralipid intake was significantly greater only at the highest concentration in outbred CD-1 (Figure 4G), C3H/HeJ (Figure 4H), and 129P3/J (Figure 4L) mice, but failed to differ at any concentration in CBA/J mice (Figure 4F). This latter group displayed significantly lower percentages of fluid intake consumed as Intralipid at the highest 5% concentration relative to BALB/cJ, C57BL/6J, CD-1, SWR/J and 129P3/J strains.

Kilocalorie intake as Intralipid and chow: Significant differences in the percentage of kilocalorie intake consumed as Intralipid were observed among strains ($F(11,110)= 13.94$, $p<0.0001$), across concentrations ($F(8,80)= 546.96$, $p<0.0001$) and for the interaction between strains and concentrations ($F(88,880)= 7.52$, $p<0.0001$). Although all mouse strains consumed a considerable amount of their kilocalories as Intralipid, particularly at the higher concentrations, they showed systematic differences in the percentage of kilocalories consumed as Intralipid (Figure 4). Thus, SWR/J mice displayed the greatest percentages of kilocalorie intake as Intralipid at ~60% (Figure 4K), and close to 50% consumption was observed in the A/J, C57BL/6J, and C57BL/10J strains (Figure 4A, 4D, 4E). Moderate (35%-45%) consumption was noted in AKR/J and

SJL/J strains (Figures 4B, 4J), and lower (>30%) consumption was observed in BALB/cJ, CBA/J, CD-1, C3H/HeJ, DBA/J and 129P3/J strains (Figures 4C, 4F-I, 4L).

Chow intake changes associated with Intralipid intake: Significant differences in chow intake were observed among strains ($F(11,110)= 43.62$, $p<0.0001$), across concentrations ($F(9,90)= 28.70$, $p<0.0001$) and for the interaction between strains and concentrations ($F(99,990)= 2.80$, $p<0.0001$). Chow intake showed significant decreases at the highest Intralipid concentration in A/J (Figure 3A), AKR/J (Figure 3B), BALB/cJ (Figure 3C), C57BL/10J (Figure 3E) and SWR/J (Figure 3K) strains. The remaining seven strains failed to show significant compensatory decreases in chow intake even at the highest Intralipid concentration. Although significant differences in the total amount of kilocalories were observed among strains ($F(11,99)= 34.95$, $p<0.0001$), across concentrations ($F(9,81)= 5.72$, $p<0.0001$) and for the interaction between concentrations and strains ($F(99,891)= 2.06$, $p<0.0001$), only CD-1 mice displayed significant increases in total kilocalorie intake at the two highest Intralipid concentrations with AKR/J and SWR/J mice displaying transient decreases in total kilocalorie intake at the 0.001% Intralipid concentration.

Correlational and Heritability Data: Table 8 displays the pairwise correlation coefficients between those Intralipid concentrations (0.5-5%) at which the greatest number of strains displayed significant increases in consumption, using the measures of Intralipid intake and the percentage of kilocalories consumed as Intralipid. Stronger and significant correlations were observed among the 0.5, 1, 2 and 5% concentrations

Table 8: Pearson product-moment correlation coefficients between Intralipid concentrations for Intralipid intake (A) and for the percentage of kilocalories consumed as Intralipid in the 12 tested mouse strains (B)

Intralipid Concentration	0.5%	1.0%	2.0%
A. Intralipid Intake (g)			
0.5%	---		
1.0%	.84*	---	
2.0%	.75*	.92*	---
5.0%	.53	.76*	.86*
B. Percent Kilocalories Consumed as Intralipid			
0.5%	---		
1.0%	.75*	---	
2.0%	.62	.51	---
5.0%	.43	.46	.87*

* Significant correlation after Bonferroni corrections ($p < 0.01$).

between Intralipid intakes (0.5 and 1%; 0.5 and 2%; 1 and 2%; 1 and 5%; 2 and 5%) relative to the percentage of kilocalories consumed as Intralipid (0.5 and 1%; 2 and 5%). Table 9 summarizes the narrow-sense heritability estimates for each Intralipid concentration in terms of Intralipid intake itself and Intralipid intake adjusted for body weight. Relatively strong (h^2 = 0.73-0.79) heritability estimates were obtained for weight-adjusted Intralipid intake at those concentrations (0.001-1%) that displayed the largest strain-specific effects in sensitivity to Intralipid. Correspondingly smaller estimates (h^2 = 0.64-0.77) were obtained for Intralipid intake itself at this concentration range. Heritability estimates dropped at both those low concentrations (0.00001-0.0001%: h^2 = 0.32-0.62) that failed to show significant differences in Intralipid intake relative to water in any strain, and at those high concentrations (2-5%: h^2 = 0.41-0.55) at which virtually all strains were consuming Intralipid more than water.

Discussion:

First, it was clear that Intralipid intake relative to water intake was significantly increased in all strains in 24 h, two-bottle preference tests. Second, as expected, dramatic strain differences for Intralipid intake was observed. The most striking increases in Intralipid preferences were observed in BALB/cJ mice across a range of concentrations (0.001-5%). This was observed to progressively lesser degrees in AKR/J, C57BL/6J, DBA/2J and SWR/J inbred strains (0.5-5%), in outbred CD-1 and inbred C57BL/10J and SJL/J strains (1-5%) and to the least degree in A/J, CBA/J, C3H/HeJ and 129P3/J inbred strains (2-5%).

Table 9: Narrow-sense heritability estimates (h^2) for each Intralipid concentration in terms of Intralipid intake itself and Intralipid intake adjusted for body weight.

Concentration Measure	0.00001%	0.0001%	0.001%	0.01%	0.1%	0.5%	1%	2%	5%
Intralipid Intake	0.32	0.59	0.64	0.70	0.77	0.58	0.76	0.41	0.45
Intralipid Intake 30 g BW	0.51	0.62	0.73	0.78	0.79	0.64	0.79	0.47	0.55

Congruent sensitivity data were observed for the percentage of fluid intake consumed as Intralipid. Significant increases were noted in BALB/cJ mice at the seven highest concentrations, in SWR/J mice at the five highest concentrations, in C57BL/6J, C57BL/10J and DBA/2J mice at the four highest concentrations, and in A/J, AKR/J and SJL/J mice at the two highest concentrations. However, the percentage of fluid intake consumed as Intralipid was only significantly greater at the highest concentration in outbred CD-1, C3H/HeJ and 129P3/J mice, and showed no difference at any concentration in CBA/J mice.

In contrast to the observation of limited order effects noted for CBA/J mice in testing ascending and descending concentrations of sucrose (Lewis et al., 2005), a number of strains displayed clear order effects for consumption of Intralipid, an important variable, identified in previous work (Harder et al., 1998). Thus, although Intralipid order effects were not important in outbred CD-1 mice and the CBA/J, C3H/HeJ, C57BL/6J, DBA/2J and 129P3/J inbred strains, Intralipid intake was higher in BALB/cJ mice exposed to the descending order at the 0.1% concentration, but lower at the 0.5, 2 and 5% concentrations. Further, exposure to descending Intralipid concentrations produced greater intake at the 0.00001% (AKR/J), 0.5% (SJL/J, SWR/J), 1% (AKR/J), 2% (A/J, C57BL/10J, SJL/J, SWR/J) and 5% (A/J) concentrations. Such data reinforce the need for this important control in multi-strain analyses of intake across concentrations.

Third, strain differences were noted in the total amount of Intralipid intake consumed, as well as Intralipid intake adjusted for body weight. Thus, SWR/J mice (20.7

g actual; 25.9 g adjusted) consumed by far the most among inbred strains, followed by A/J, BALB/cJ, C57BL/10J and C57BL/6J mice (13.3-14.9 g), followed then in turn by SJL/J, AKR/J and 129P3/J mice (9.4-12.5 g), and finally by DBA/2J, C3H/HeJ and CBA/J mice (6.7-9.2 g). This differential Intralipid consumption across strains remained consistent across the highly effective 0.5, 1, 2 and 5% Intralipid concentrations given the highly significant correlations for intake for the 0.5 and 1% (r=0.84), the 0.5 and 2% (r=0.75), the 1 and 2% (r=0.92), the 1 and 5% (r=0.76) and the 2 and 5% (r=0.86) concentrations.

Moreover, relatively strong (h^2 = 0.73-0.79) heritability estimates were obtained for weight-adjusted Intralipid intake at those concentrations (0.001-1%) that displayed the largest strain-specific effects in sensitivity to Intralipid. This is identical in pattern to the results observed for Intralipid intake itself. Therefore, the close correspondence between actual and the weight-adjusted Intralipid intakes suggests that initial differences in body weight among mouse strains were not an integral factor in the short-term (24 h) Intralipid preference across a range of concentrations in two-bottle choice tests.

It should be noted that a number of previous studies have employed the percentage of the palatable solution consumed per total fluid intake, as a measure of preference (e.g., Capeless & Whitney,1995; Lush, 1989; Pothion et al., 2004). However, in the present study, this did not appear to be a reliable predictor of effects. Indeed, some strains displaying very high (~90%) Intralipid preference show markedly different amounts of Intralipid intake at the identical concentration, e.g., SWR/J (20.7 g), C57BL/6J (14.9 g) and 129P3/J (9.4 g). Moreover, CD-1 (14 g) and C3H/HeJ (7 g) strains displaying great differences in actual Intralipid intake both showed moderately

high (~80%) fluid preferences as Intralipid. This further underscores the need of inbred strain surveys investigating the genetic basis for food intake to be structured across a great range of palatable solution concentrations so as to clearly distinguish between preference and total consumption.

Short-term differences in sensitivity to and consumption of different Intralipid solutions across inbred mouse strains reveal some interesting similarities and differences when compared with studies using solid fat sources, macronutrient selection of different diets, or comparison of low-fat, high-carbohydrate with high-fat, low-carbohydrate diets (e.g., West et al.,1992; 1995). Thus, SWR/J mice consumed by far the greatest overall and weight-adjusted amounts of Intralipid, and among the greatest amounts of kilocalories consumed as Intralipid. This strain also displayed the largest intake of a variety of other high fat diets (Smith et al., 2000; West et al., 1992; 1995), indicating clear similarities for this effect. Yet in prior paradigms which lasted from a week to a month, these animals failed to gain weight despite increased fat intake, potentially because as indicated in the present study, they adjust their intake of other food sources. Other factors are clearly at work given that their fat intake was inversely correlated with epididymal fat (Smith et al., 2000), and since SWR/J mice, like Intralipid-preferring A/J mice, displayed lower insulin levels, increased capacity of skeletal muscle to metabolize fat, enhanced paraventricular galanin and reduced arcuate NPY (Leibowitz et al., 2005). Similarities between Intralipid intake and other forms of fat intake (Alexander et al., 2006; Smith et al., 2000) were also observed in C57BL/6J mice. This strain also avidly consumed Intralipid in the present study, and showed equal preferences between protein and fat while displaying normal insulin and leptin levels (Alexander et al., 2006).

Further, in self-selection studies, C57BL/6J mice consumed some of the highest proportions of fat that correlated with epididymal fat stores (Smith et al., 2000).

Some other strains failed to display similar patterns of fat intake when comparing Intralipid to other fat sources. Thus, AKR/J mice, a strain that consumed appreciably less Intralipid than the strains described above, have been shown to consume moderate intake of high-fat diets that are accompanied by weight gain (Smith-Richards et al., 1999; West et al., 1992; 1995), high proportions of fat in self-selection studies that correlated with epididymal fat (Smith et al., 2000), and, along with the less-sensitive Intralipid DBA/2J strain, consumed more fat, displayed more adiposity, and had elevated levels of leptin and insulin (Alexander et al., 2006). Some of the discrepancies may be due to methodological factors related to the goals of the individual studies. Hence many of the previously-cited studies used solid forms of fat (e.g., shortening, lard or granular fat) at specific concentrations in the diet over longer periods of time to assess differences between small numbers of strains. The present study used an experimental approach allowing for the evaluation of a larger number of strains across a wide range of fat concentrations to assess sensitivity to fat solutions. Since many liquefied fat sources fail to stay in solution when presented at different concentrations over a time course (e.g., 24 h) reasonable to study murine intake, our laboratory chose an emulsified soybean oil solution (Intralipid) capable of equal distribution of fat in solution across a wide range of concentrations. Thus, the texture and other sensory cues presented by Intralipid as compared to the other solid fat sources may be reasons for the differences in strain responsivity to specific aspects of fat intake, and not fat intake per se. However, all of these studies taken together do provide converging information about the potential

genetics of fat intake with the differences in some strains indicating a role for environmental factors interacting with genetic predisposition.

VII. Specific Aim 3: The Role of Genetic Variance in 2DG induced feeding in Inbred and Outbred Mouse Strains

Introduction:

2-deoxy-D-glucose (2DG: Wick et al., 1957) is an anti-metabolic glucose analogue which increases food intake following systemic (e.g., Booth, 1972; Smith & Epstein, 1969; Smith & Root, 1969; Thompson & Campbell, 1977) and cerebro-ventricular (Berthoud & Mogenson,1977; Miselis & Epstein, 1975) administration in rats, monkeys and humans. In rats, this feeding response has been shown to occur in the absence of other signs of glucoprivation (Engeset & Ritter, 1980; Ritter et al., 1978a). The reduced glucose oxidation (Nonavinakere & Ritter, 1983) is also dependent on necessary metabolic changes (Evan & Nicolaidis, 1985) and displays synergistic feeding interactions with central insulin administration (Clegg et al., 2003). Further, glucoprivic feeding in rats is impaired following blockade of central glucoreceptors with alloxan (Murnane & Ritter, 1985a; 1985b; Ritter et al., 1982; Sanders et al., 2004; Woods & McKay, 1978) or stress-induced alterations in noradrenergic function (Ritter et al., 1978b; Rowland, 1992; Scheurink & Ritter, 1993), but see Rowland et al., 1985a). Whereas 2DG-induced feeding is clearly delineated in outbred rats, the presence of its ingestive actions has not been universally observed in other species. Thus, 2DG-induced feeding failed to occur under similar dosing and ingestive conditions in Golden and Siberian hamsters (Angel & Taranger, 1991; Bartness & Clein, 1994; Bartness et al., 1995; Lowy & Yin, 1982; Ritter & Balch, 1978; Rowland, 1983), deermice (Rowland et al., 1985b) and spiny mice (Czech, 1988). 2DG induces feeding occurs only in the

hyperphagic, but not hypophagic phase in ground squirrels (Nizielski et al., 1986), in lean, but not fatty Zucker rats (Tsujii & Bray, 1990) and in rats selectively bred for high, but not low saccharin intake (VanderWheele et al., 2002).

Even within a given species, substantial inter-individual variability in ingestive responses has been demonstrated, and this has been often associated with genetic variance (see review: Reed et al., 1997). Specific Aim 1 reported changes in sucrose intake among 11 inbred strains across a range of sucrose concentrations (0.0001% - 20%) in two-bottle 24-h preference tests (Lewis et al., 2005). Our data show very broad strain-dependent sucrose concentration sensitivities capable of significantly increasing sucrose intake. The magnitude of such intake at different concentrations, and the kilocalories consumed as sucrose, indicates that variation in several sucrose-related measures are also genotype-dependent. In addition to assessing the contribution of genetic background on the variability of ingestive behaviors, inbred mouse strain surveys can also identify strains with highly divergent responses that can serve as progenitors in QTL mapping. This may be employed to localize phenotypic differences to chromosomal regions and, ultimately, the corresponding genes. Such an approach has been successfully applied to differences in the intake of fat, carbohydrate, bitter tastants, saccharin, sucrose, and total Kcal intake (Blizard et al., 1999; Smith-Richards et al., 2002). Identification of QTLs associated with variability in ingestive behaviors like glucoprivation is an important first step in the genetic delineation of obesity.

Although 2DG-induced food intake may reflect glucosensing mechanisms and provide insight into the regulatory control of carbohydrate intake, to our knowledge, no QTL for this response has yet to be identified. In fact, in contrast to other ingestive

processes, it is currently unknown whether this response is subject to response variability at all, and/or whether such potential variability is associated with genetic variability. Although studies have demonstrated a role for genes encoding Na+-coupled glucose transporters (SGLT) and glucose transporter facilitators (GLUT) in glucosensing (see review: Scheepers et al., 2004), it is not known whether allelic frequency of these genes contributes to the quantitative distribution of 2DG feeding responses. Analagously, our laboratory has previously noted the absence of any significant correlations between intake of sucrose at several concentrations and *Tas1r3* taste receptor gene polymorphisms (Lewis et al., 2005).

To begin to provide for the genetic analyses of 2DG-induced feeding, the present study surveyed 11 inbred strains differences for feeding responses across a wide range of previously-determined effective systemic 2DG doses (200, 400, 600, 800 mg/kg) and test times (1-4 h). Since there are no previous 2DG feeding data for inbred strains, standard outbred CD-1 mice were simultaneously tested to provide a point of comparison with other published studies (e.g., Bergen et al., 1996; Czech, 1998; Villanueva et al., 1996) so as to verify our study paradigm. The results of this Specific Aim were published by our laboratory in Physiology and Behavior in 2006 (Lewis et al., 2006b).

Methods:

Subjects: As detailed previously in the General Methods section, outbred CD-1 (n=10), and inbred A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL6/J, C57BL10/J, DBA/2J, SJL/J, SWR/J, 129P3/J male mice (n=9-10 for each inbred strain) purchased from Charles River and Jackson Laboratories at 12 weeks of age were initially acclimated to the Queens College vivarium for one week in group (5 per cage) housing and were

subsequently housed individually in polyethylene cages (30 x 20 x15 cm), on a 12 h light: 12 h dark cycle (lights off at 2000 h) at a constant temperature of 22^{o}C with chow (Purina 5015 Mouse Diet, 5.3 kcal/g) and water available *ad libitum*.

2DG Intake Procedure: As detailed in the General Methods section, each animal was moved to a test cage at 4-6 h into the light cycle and provided with a water bottle and a pre-weighed ration of Purina Mouse chow (5.3 kcal/g). The chow was placed on a stainless steel grid on the bottom of the test cage, with a brown paper towel below the grid to collect chow spillage. Food intake ($\pm$0.1 g) was assessed by weighing food pellets prior to and following each time interval (1, 2 and 4 hours) and adjusting for spillage. After four days of stable baseline data, the mice received an intraperitoneal injection of vehicle (0mg/kg) or one of the four doses of 2DG (200, 400, 600 and 800 mg/kg). Half of the mice of each strain were tested in an ascending 2DG dose order, and the remaining half were tested in a descending order.

Statistics: As detailed previously in the General Methods section, three-way randomized block analyses of variance were assessed for the following variables: alterations in 2DG-induced feeding relative to vehicle intake, with the 12 strains as the between-subject variable, the five (vehicle and 4 2DG doses) conditions as a within-subject variable, and the three intake (1, 2, 4 h) times as a second within-subject variable. Because significant strain differences were observed in vehicle food intake across the 4 hour time course (Table 10), Tukey comparisons ($P<0.05$) were performed in the presence of significant effects only relative to corresponding vehicle values within strains; and for 2DG difference scores. Difference scores were defined as the 2DG intake

Table 10: Food intake difference scores (g, ±S.E.M, minus vehicle values) and percentage change over vehicle intake 4 h following 2DG in 12 mouse strains

2-DG dose/Strain	Vehicle Intake (4 h)	Measures	200 mg/kg	400 mg/kg	600 mg/kg	800 mg/kg
A/J	0.52 (0.04)	Diff. Score % Veh	-0.21# (0.07) 40%↓#	+0.06 (0.06) 12%↑	+0.02 (0.08) 4%↑	+0.52* (0.08) 100%↑*
AKR/J	0.58 (0.08)	Diff. Score % Veh	-0.21 (0.04) 36%↓	-0.08 (0.08) 14%↓	+0.28* (0.11) 48%↑*	+0.35* (0.15) 60%↑*
BALB/cJ	0.74 (0.05)	Diff. Score % Veh	+0.12 (0.10) 16%↑	+0.61* (0.17) 82%↑*	+0.44* (0.08) 59%↑*	+0.71* (0.10) 96%↑*
C57BL/6J	0.39 (0.05)	Diff. Score % Veh	-0.08 (0.06) 21%↓	+0.24* (0.10) 62%↑*	+0.20 (0.09) 51%↑	-0.12 (0.10) 31%↓
C57BL/10J	0.48 (0.09)	Diff. Score % Veh	-0.12 (0.10) 25%↓	-0.08 (0.09) 17%↓	+0.11 (0.10) 27%↑	-0.18 (0.08) 32%↓
CBA/J	0.25 (0.04)	Diff. Score % Veh	-0.04 (0.08) 16%↓	+0.20 (0.03) 80%↑	+0.36* (0.10) 144%↑*	+0.47* (0.07) 188%↑*
CD-1	0.69 (0.06)	Diff. Score % Veh	+0.24* (0.13) 33%↑*	+0.52* (0.15) 77%↑*	+0.42* (0.19) 61%↑*	+0.55* (0.18) 80%↑*
C3H/HeJ	0.46 (0.10)	Diff. Score % Veh	+0.04 (0.13) 9%↑	+0.09 (0.09) 20%↑	+0.19 (0.09) 41%↑	+0.56* (0.11) 122%↑*
DBA/2J	0.48 (0.10)	Diff. Score % Veh	+0.10 (0.12) 21%↑	+0.46* (0.17) 96%↑*	+0.37* (0.15) 77%↑*	+0.39* (0.10) 81%↑*
SJL/J	0.37 (0.04)	Diff. Score % Veh	+0.13 (0.07) 35%↑	+0.22* (0.07) 59%↑*	+0.30* (0.08) 82%↑*	+0.37* (0.08) 100%↑*
SWR/J	0.95 (0.03)	Diff. Score % Veh	-0.40# (0.11) 42%↓#	-0.20 (0.08) 21%↓	+0.01 (0.07) 1%↑	-0.11 (0.08) 12%↓
129P3/J	0.54 (0.04)	Diff. Score % Veh	-0.28# (0.05) 51%↓#	+0.22* (0.08) 41%↑*	+0.28* (0.12) 51%↑*	+0.22* (0.05) 41%↑*

*Significant increase or # decrease in 2DG-induced food intake relative to vehicle values.

value subtracted from the vehicle value at each time point for each dosage, in each animal (with the 12 strains as the between-subject variable, the four 2DG dose conditions as a within-subject variable, and the three intake times as a second within-subject variable).

Pearson product-moment correlation coefficients [*r*], subject to Bonferroni corrections, were performed between mice of 11 inbred and 1 outbred strains, for food intake (4 hour total) during vehicle testing (no 2DG) and after the four 2DG doses.

Results:

Significant differences in food intake were observed among strains ($F(11,209)= 58.31$, $p<0.0001$), among the injection conditions ($F(4,76)= 94.86$, $p<0.0001$), across test times ($F(2,38)= 4476.55$, $p<0.0001$), and for the interactions between strains and conditions ($F(44,836)= 12.85$, $p<0.0001$), strains and times ($F(22,418)= 36.55$, $p<0.0001$), conditions and times ($F(8,152)= 210.81$, $p<0.0001$), and among strains, conditions and times ($F(88,1672)= 13.37$, $p<0.0001$). Significant differences in short-term vehicle intake occurred across strains, with SWR/J mice consuming the most followed by BALB/cJ, CD-1 and AKR/J mice, and then the other strains (Table 10).

Significant differences in the difference scores for 2DG-induced intake were observed among strains ($F(11,209)= 30.67$, $p<0.0001$), among 2DG doses ($F(3,57)= 74.62$, $p<0.0001$), across test times ($F(2,38)= 88.35$, $p<0.0001$), and for the interactions between strains and doses ($F(33,627)= 9.51$, $p<0.004$), strains and times ($F(22,418)= 13.06$, $p<0.0001$), doses and times ($F(6,114)= 254.62$, $p<0.0001$), and among strains, doses and times ($F(66,1254)= 13.52$, $p<0.0001$).

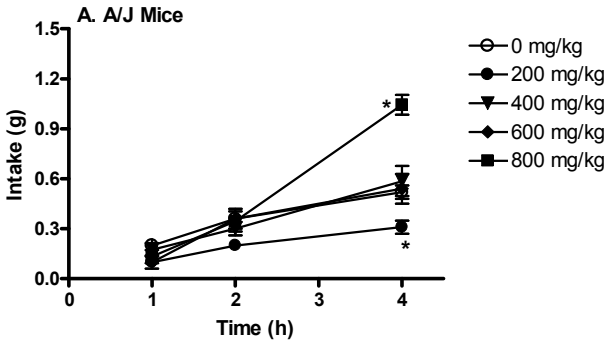
There were clear strain differences in the magnitude and pattern of 2DG-induced intake relative to corresponding vehicle conditions (Figure 5). 2DG produced significant, dose-dependent increases in food intake following each of the four doses in the CD-1 and DBA/2J mouse strains (Figures 5G, 5I), following the three highest doses in the BALB/cJ, SJL/J and 129P3/J mouse strains (Figures 5C, 5J, 5L), and following the two highest doses in the CBA/J and AKR/J mouse strains (Figures 5B, 5F). 2DG produced significant dose-dependent increases in food intake following only the highest dose in A/J and C3H/HeJ mice (Figures 5A, 5H), and following only the 400 mg/kg dose in C57BL/6J mice (Figure 5D).

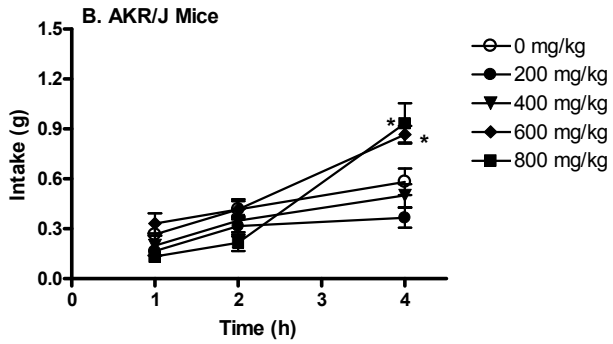
At all doses studied, 2DG failed to alter food intake at any time point in C57BL/10J mice (Figure 5E). In contrast, 2DG significantly reduced food intake at the lowest and highest doses in SWR/J mice (Figure 5K). Table 10 summarizes the food intake difference scores and the percentage changes in intake 4 h following each 2DG dose relative to vehicle treatment in the 12 tested mouse strains.

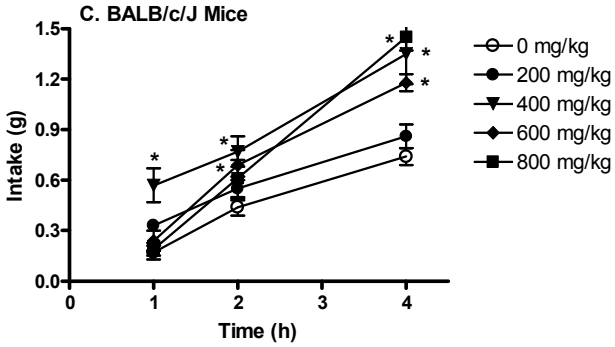
Bonferroni-corrected pairwise correlations were examined for food intake (over 4 h) for vehicle (no 2DG) relative to each of the four 2DG doses, and then among the four 2DG doses (Table 11). Significant correlations were noted for intake across strains between the 200 and 400 mg/kg doses (r= 0.75, $p<0.05$), the 200 and 600 mg/kg doses (r= 0.73, $p<0.05$), and the 400 and 600 mg/kg doses (r=0.82, $p<0.001$). By contrast, significant correlations failed to be observed between food intake after any 2DG dose, compared to vehicle alone or between each of the three lower 2DG doses relative to the highest 800 mg/kg 2DG dose.

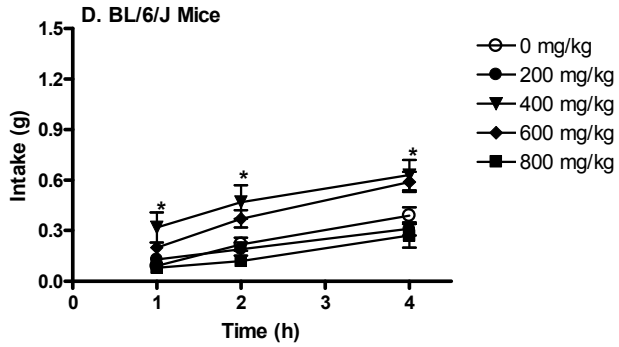
Figure 5. Food intake (g, ±S.E.M.) following four doses of 2-deoxy-D-glucose (2DG) in 12 mouse strains over 4 h.

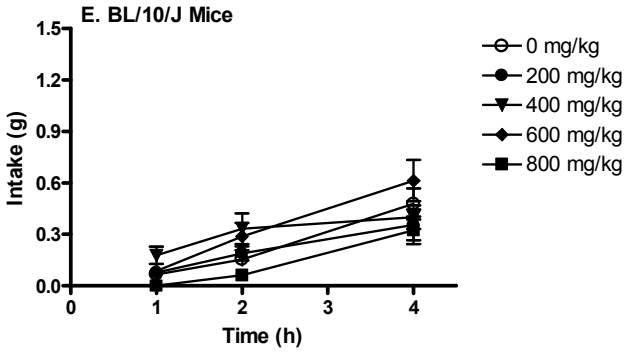
Ordinates are identical to facilitate interstrain comparisons. The asterisks (*) denote significant alterations in food intake relative to the corresponding vehicle value obtained within each strain (Tukey comparisons, $P<0.05$). The twelve panels of this figure are found on the following two pages.

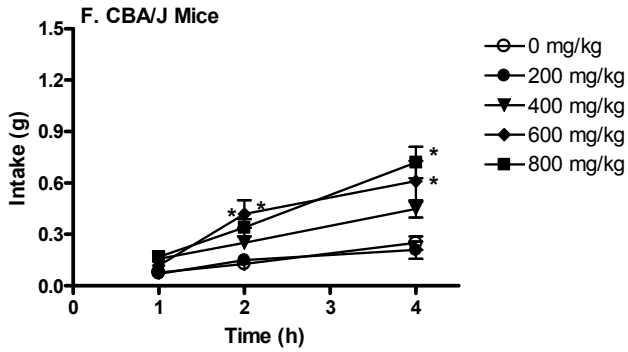

A. A/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg

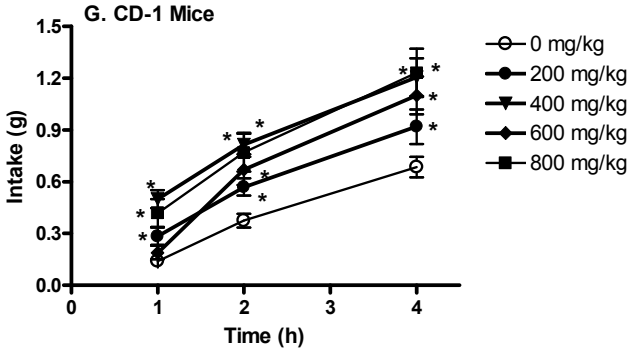

B. AKR/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg

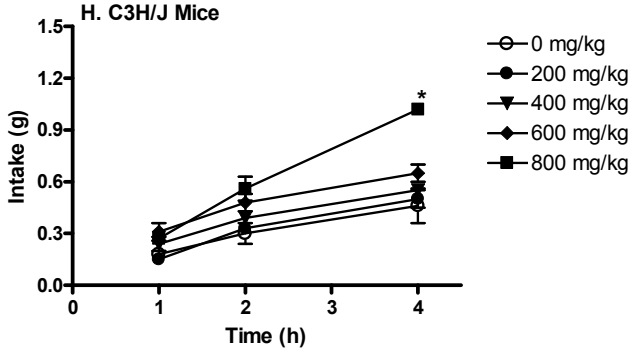

C. BALB/c/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg

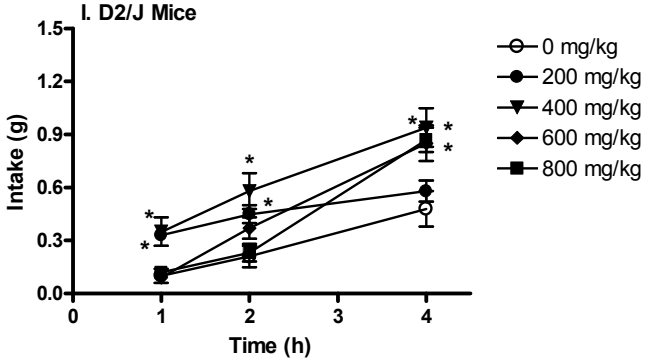

D. BL/6/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg

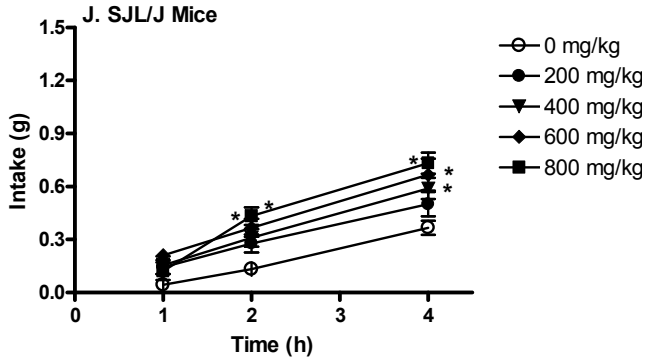

E. BL/10/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg

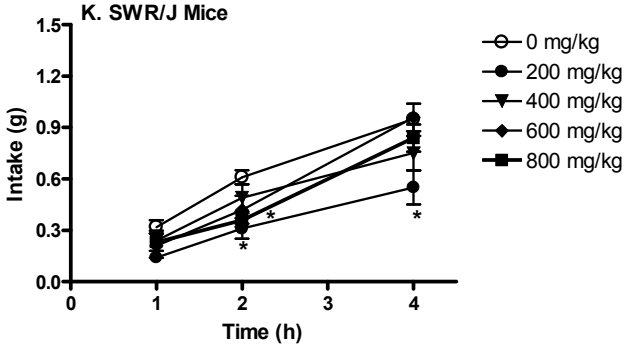

F. CBA/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg


G. CD-1 Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg


H. C3H/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg


I. D2/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg


J. SJL/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg


K. SWR/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg

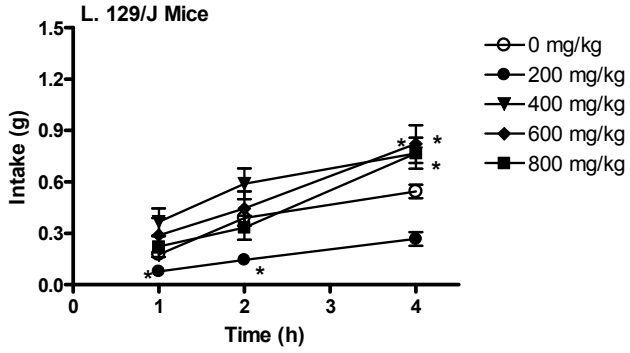

L. 129/J Mice
Intake (g)
Time (h)
0 mg/kg
200 mg/kg
400 mg/kg
600 mg/kg
800 mg/kg

Table 11: Pearson product-moment correlation coefficients for food intake over 4 h in vehicle (0mg/kg) testing and after the four 2DG doses.

	0 mg/kg	200 mg/kg	400 mg/kg	600 mg/kg
0 mg/kg		---		
200 mg/kg	0.28	---		
400 mg/kg	0.08	*0.78	---	
600 mg/kg	0.12	*0.73	**0.82	---
800 mg/kg	0.08	0.57	0.59	0.58

Significant correlation (* $p < 0.05$, ** $p < 0.01$) after Bonferroni correction

Discussion:

Strong and systematic strain differences were observed for 2DG-induced intake as functions of dose and time. Consistent with outbred rats (e.g., Booth, 1972; Ritter et al., 1978; Smith & Epstein, 1969) and mice (e.g., Bergen et al., 1996; Czech 1998; Villaneuva, 1996), our outbred CD-1 mice displayed the most orderly time- and dose-dependent increases feeding associated with 2DG. For inbred mouse strains, orderly dose-dependent increases in feeding associated with 2DG- occurred across all four doses (DBA/2J), across the three highest doses (BALB/cJ, SJL/J and 129P3/J) and across the two highest doses (CBA/J and AKR/J). Some mouse strains displayed very limited instances of 2DG-induced feeding with increases noted following only the highest dose in A/J and C3H/HeJ mice or following the 400 mg/kg dose in C57BL/6J mice. Importantly, although strain-specific effects for feeding following the three lowest 2DG doses produced significant or near significant correlations, intakes following the three lower 2DG doses failed to correlate significantly with intake following the highest 800 mg/kg 2DG dose that typically produced the most pronounced ingestive effects. Just as certain species such as Golden and Siberian hamsters [(Angel & Taranger, 1991; Bartness & Clein, 1994; Bartness et al., 1995; Lowy & Yim, 1982; Ritter & Balch, 1978; Rowland, 1983), deermice (Rowland et al., 1985) and spiny mice (Czeck 1988) fail to display 2DG-induced feeding, the full 2DG dose range failed to alter food intake in C57BL/10J mice. Interestingly, 2DG was associated with significant reductions in food intake in SWR/J mice.

Such strain differences may be related to the observations of the absence of 2DG-induced feeding in mice genetically deficient in dopamine (Hnasko et al., 2004), the

dopamine-3 receptor gene (Benoit et al., 2003), dopamine beta-hydroxylase (Ste Marie & Palmiter, 2003) or neuropeptide Y (Sindelar et al., 2004). Although these findings suggest the importance of these genes in the mediation of glucoprivic feeding, the background strains of the genetically-modified animals might contribute to the absence of this 2DG-mediated effect. In two cases, the knockout and wild type mice were maintained on a mixed C57BL/6J and 129/SvCPJ genetic background (Hnasko et al., 2004; Ste Marie & Palmiter, 2003), while the other studies maintained their knockout mice on either pure 129/SvEv (Sindelar et al., 2004) or pure C57BL/6 (Benoit et al., 2003) backgrounds. Thus, whereas the 129P3/J strain displayed quite robust feeding responses to 2DG across the three highest doses, that were comparable to responses elicited from outbred animals, the C57BL/6J strain displayed a very limited response at only a moderate dose of 2DG. One must consider whether the absence of 2DG-induced feeding in mice with targeted deletions of dopamine (Hnasko et al., 2004), the dopamine-3 receptor gene (Benoit et al., 2003) or dopamine beta-hydroxylase (Ste Marie & Palmiter, 2003) would have been as robust if one used a background strain for 2DG-induced feeding other than the weakly responding C57BL/6J inbred strain. The potential confound of donor strain background has been discussed at length elsewhere (Lariviere et al., 2001).

A common finding of 2G studies is that the presence or absence of increased 2DG feeding depends critically upon the type and consistency of nutrient (Delprete & Scharrer, 1992; Kanarek & Mayer, 1978; Watson et al., 1986)) and timing of the phase of the light-dark cycle (Penicaud et al., 1986; Thompson et al., 1989). Table 10 thus provides the magnitude of 2DG feeding responses with reference to vehicle values.

When all strains are considered, significant correlations failed to occur for food intake between vehicle and any dose of 2DG. Indeed, inbred strains, displaying low (CBA/J: 0.25 g), moderate (C3H/HeJ: 0.46 g) and high (BALB/cJ: 0.74 g) levels of vehicle intake, showed comparable and significant increases in 2DG-induced feeding 4 h following the 800 mg/kg dose; 188%, 122% and 96% respectively (Table 10). Thus, the present data do not indicate a relationship between the magnitude of vehicle food intake and any observed change in food intake after glucoprivation. It is possible that these disparate results may reflect the common use of a single strain for testing. Studies that use a single strain may have caused this relationship to be overestimated and lack the statistical power afforded by assessing the relationship between vehicle intake and 2DG-induced feeding relationships in 11 different genotypes. A more thorough examination of the putative relationship between vehicle food intake and subsequent 2DG-induced feeding should consider several strains at various pre- and post- prandrial intervals.

It is important to note that 2DG administration has also been shown to produce non-specific effects upon food intake, such as lethargy as well as the fact that 2DG-induced feeding can occur in the absence of other signs of glucoprivation. Feeding induced by systemic or cerebroventricular 2DG persists 6 h after injection at which time sympathoadrenal hyperglycemia and reduced glucose oxidation have subsided (e.g., Engeset & Ritter, 1980; Nonavinakere & Ritter, 1983; Ritter et al., 1978a). Moreover, 2DG-induced feeding is reduced by stress (Ritter et al., 1978b), an effect attributable in part to impairment of noradrenergic neuron function (Ritter et al., 1978b; Rowland, 1992; Scheurink &Ritter, 1993, but see Rowland et al., 1985a). Hence, 2DG appears to produce its ingestive effects in rats through selective activation of epinephrine-containing

neurons in dorsal medulla (Ritter et al., 1998), effects attenuated by either immunotoxic destruction (Hudson & Ritter, 2004; Ritter et al., 2001) or prior repeated 2DG treatment (Sanders & Ritter, 2000; 2001). Thus, we can not rule out the possibility that some genetic variation in stress responding and lethargy is associated with the varying strain-specific responses to 2DG injection. Although not a directly-measured variable, we did not observe any strain-specific gross motor or other impairment that might obviously confound food intake. However, such potential confounds are more likely to be pronounced following the higher 2DG doses. In the present study, we reported that there was significant cross-correlation between 2DG doses of 200, 400 and 600 mg/kg. Since the demonstration of genetic correlation between two heritable traits among isogenic (inbred) strains can be used as evidence of the existence of pleiotropic genes with a common influence on both traits (Hegmann & Possidente, 1981), the data suggest that the genetic contribution to strain variance in food intake after these 2DG doses display significant overlap, and provides a genetic validation of 2DG doses across our lower testing range (200-600 mg/kg). Furthermore, the largest 2DG dose of 800 mg/kg was not significantly correlated with any of the lower doses, suggesting a genetic dissociation. It is conceivable that the highest 2DG dose could recruit potentially non-specific systems, possibly including stress and lethargy, that vary among strains and which impact food intake. Clearly, the present data suggest that results from 2DG test doses larger than 600 mg/kg should be interpreted with caution.

VIII. Specific Aim 4: The Role of Genetic Variance in MA induced feeding in Inbred and Outbred Mouse Strains

Introduction:

The free fatty acid oxidation inhibitor, mercaptoacetate (MA: Bauche et al., 1981) significantly increases food intake following systemic administration (Langhans & Scharrer, 1987; Scharrer & Langhans, 1986). This lipoprivic ingestive response has been compared with increases in food intake (Booth, 1972; Smith & Epstein, 1969; Smith & Root, 1969) following systemic administration of the anti-metabolic glucose analogue, 2-deoxy-D-glucose (2DG: Wick et al., 1957) to determine if they share similar circuitry (Friedman & Tordoff, 1986). Functional comparisons between lipoprivic (MA) and glucoprivic (2DG) feeding in rats have revealed similar c-fos responses in the nucleus of the solitary tract, lateral parabrachial nucleus, central nucleus of the amygdala and the dorsal motor nucleus of the vagus (Ritter & Dinh, 1994). Additionally, elevated sympathoadrenal plasma levels of epinephrine and norepinephrine (Scheurink & Ritter, 1993) have been reported with MA and 2DG.

However, there are marked differences in sensitivity between MA-induced and 2DG-induced feeding responses to different physiological manipulations; e.g. following vagotomy (Ritter & Taylor, 1990) and lesions placed in the lateral parabrachial nucleus (Calingasan & Ritter, 1993) or the central nucleus of the amygdala (Ritter & Hutton, 1995).

Within a given species, substantial inter-individual variability in ingestive responses has been demonstrated, and this often associated with genetic variance (e.g.,

see review: Reed et al., 1997). Specific Aim 3 employed a similar strategy in assessing the effect of strain differences in the magnitude of intake across four 2DG doses (200-800 mg/kg) and test times (1-4 h). Orderly strain-specific and dose-dependent increases in 2DG-induced feeding were observed in outbred and inbred mouse strains, with the greatest effects noted for CD-1 and DBA/2J mice. Whereas BALB/cJ, SJL/J, 129P3/J, CBA/J and AKR/J mice displayed moderate levels of increased feeding associated with 2DG, 2DG elicited limited increases in C57BL/6J and C3H/HeJ mice, no changes in C57BL/10J mice and actual reductions in intake in other mouse strains (SWR/J mice). Further, 2DG shows similar responses to feeding in rat strains that are differentially susceptible to dietary obesity, whereas MA is more effective in eliciting feeding in diet-susceptible as compared to diet-resistant rat strains (Singer et al., 1997).

In addition to assessing the contribution of genetic background in the variability of ingestive behaviors, inbred mouse strain surveys can also identify strains with highly divergent responses, to serve as progenitors in QTL mapping (Hegmann & Possidente, 1981). QTL mapping can then be used to map chromosomal regions and potentially genes that contribute to complex traits. This general mapping approach has been applied to differences in the intake of fat, carbohydrate, bitter tastants, saccharin, sucrose, and total Kcal intake (Blizard et al., 1999; Smith-Richards et al., 2002). Thus, identification of QTLs associated with variability in ingestive behaviors, like lipoprivation, is an important first step in the genetic dissection of obesity. To our knowledge, a QTL for this response has yet to be identified. In fact, in contrast to other ingestive processes, it is currently unknown whether this response is subject to response variability at all, and, if present, whether such potential response variability is associated with genetic variability.

To begin to provide for the genetic analyses of MA-induced feeding, the present study surveyed the same 11 inbred (A/J, AKR/J, BALB/cJ, CBA/J, C3H/HeJ, C57BL6/J, C57BL10/J, DBA/2J, SJL/J, SWR/J, 129P3/J) and one outbred (CD-1) strains across a wide range of previously-determined effective systemic MA doses (5, 35, 70, 100 mg/kg) and test times (1-4 h). These strains were used in our other studies (Lewis et al., 2005; 2007) for feeding responses. The order of MA doses was controlled by exposing half of the mice of each strain to an ascending dose order and the remainder to a descending dose order. The results of this Specific Aim were published by our laboratory in Physiology and Behavior in 2006 (Lewis et al., 2006a).

Methods:

Subjects: As detailed previously in the General Methods section, male outbred CD-1 (n=10), and 11 inbred mouse strains: A, AKR, BALB/c, CBA, C3H/He (C3H), C57Bl/6 (BL/6), C57Bl/10 (BL/10), DBA/2 (D2), SJL, SWR, 129P3 (129) (n=9-10 for each inbred strain) purchased from Charles River and Jackson Laboratories at 12 weeks of age, were initially acclimated to the Queens College vivarium for one week in group (5 per cage) housing. They were subsequently housed individually in plastic cages (30 x 20 x15 cm) on a 12 h light: 12 h dark cycle (lights off at 2000 h) at a constant temperature of 22^{o}C with food and water *ad libitum*.

MA Intake Procedure: As detailed in the General Methods section, each animal was moved to a test cage at 4-6 hours into the light cycle and provided with a water bottle and a pre-weighed ration of Purina Mouse chow (5.3 kcal/g). The chow was placed on a stainless steel grid on the bottom of the test cage, with a brown paper towel below it to collect chow spillage. Food intake ($\pm$0.1 g) was assessed by weighing food pellets prior

to and following each time interval (1, 2 and 4 hours) and adjusting for spillage. After four days of stable baseline data, the mice received an intraperitoneal injection of vehicle (0mg/kg) or one or the four doses of MA (5, 35, 70 AND 100 mg/kg). Half of the mice of each strain were tested in an ascending MA dose order, and the remaining half were tested in a descending order.

Statistics: As detailed in the General Methods section, Three-way randomized block analyses of variance were assessed for the following variables; alterations in MA induced feeding relative to vehicle intake, with the 12 strains as the between-subject variable, the five (vehicle and 4 MA doses) conditions as a within-subject variable, and the three intake (1, 2, 4 h) times as a second within-subject variable. Since significant strain differences were observed in vehicle food intake across the 4 hour time course (Vehicle Intake, Table 12), Tukey comparisons ($P<0.05$) were performed within strains for MA difference scores, when there were significant effects relative to corresponding vehicle values. The MA difference score was defined as the vehicle food intake value minus the corresponding MA intake value. This comparison was undertaken for each intake value, at each time point following vehicle, in each animal, for each strain (with the 12 strains as the between-subject variable, the four MA dose conditions as a within-subject variable, and the two later (2-4 hour) intake times as a second within-subject variable). A separate two-way randomized-block analysis of variance was performed for the percent change of food intake after 4 hours following each of the MA doses relative

Table 12: Food intake difference scores (g, ±S.E.M, minus vehicle values) and percentage change over vehicle intake 4 h following MA in 12 mouse strains.

MA dose/ Strain	Vehicle Intake (4 h)	Measures	5 mg/kg	35 mg/kg	70 mg/kg	100 mg/kg
A/J	0.59 (0.09)	Diff. Score % Veh	-0.11 (0.07) 15%↓	0.00 (0.14) 29%	+0.04 (0.04) 19%↑	-0.01 (0.10) 0%
AKR/J	0.80 (0.06)	Diff. Score % Veh	+0.02 (0.11) 1%↓	+0.30* (0.12) 28%↑	+0.38* (0.19) 57%↑	+0.04 (0.17) 16%↑
BALB/cJ	0.73 (0.11)	Diff. Score % Veh	-0.13 (0.16) 22%↓	+0.17 (0.12) 19%↑	+0.02 (0.10) 2%↑	+0.07 (0.11) 6%↑
C57BL/6J	0.40 (0.05)	Diff. Score % Veh	-0.13 (0.09) 24%↓	+0.12 (0.10) 42%↑	+0.00 (0.07) 0%	+0.22* (0.11) 66%↑
C57BL/10J	0.66 (0.09)	Diff. Score % Veh	+0.29 (0.18) 44%↑	+0.22 (0.13) 39%↑	+0.10 (0.07) 32%↑	+0.08 (0.13) 22%↑
CBA/J	0.58 (0.05)	Diff. Score % Veh	+0.05 (0.12) 24%↑	+0.14 (0.09) 31%↑	+0.03 (0.08) 7%↑	-0.07 (0.07) 11%↓
CD-1	1.01 (0.13)	Diff. Score % Veh	+0.08 (0.13) 3%↑	+0.16 (0.22) 30%↑	+0.59* (0.16) 63%↑	+0.65* (0.21) 60%↑
C3H/HeJ	0.42 (0.07)	Diff. Score % Veh	+0.37* (0.17) 80%↑	-0.17 (0.10) 39%↓	-0.14 (0.07) 35%↓	-0.06 (0.10) 2%↑
DBA/2J	0.84 (0.11)	Diff. Score % Veh	+0.17 (0.14) 37%↑	+0.47* (0.06) 63%↑	+0.33*(0.10) 55%↑	+0.61* (0.18) 103%↑
SJL/J	0.78 (0.06)	Diff. Score % Veh	+0.04 (0.06) 8%↑	+0.18 (0.09) 22%↑	+0.11 (0.12) 21%↑	+0.08 (0.10) 10%↑
SWR/J	0.69 (0.08)	Diff. Score % Veh	-0.07 (0.06) 17%↓	+0.06 (0.07) 9%↑	+0.21 (0.09) 39%↑	-0.14 (0.11) 11%↓
129P3/J	0.58 (0.15)	Diff. Score % Veh	+0.11 (0.17) 164%↑	+0.06 (0.17) 173%↑	+0.25 (0.15) 222%↑	+0.16 (0.14) 183%↑

*Significant increase or # decrease in 2DG-induced food intake relative to vehicle values.

to corresponding vehicle values (with the 12 strains as the between-subject variable and the four MA dose conditions as the within-subject variable). Narrow-sense trait heritability was determined by comparing the between-strain variance to the total variance using the formula: h^2 = VA/(VA+VE) (Falconer & Mackay, 1996). Since animals in strains were initially chosen at random, these values are likely accurate estimates of the true trait heritabilities (Hegmann & Possidente, 1981).

Pearson product-moment correlation coefficients [r] subject to Bonferroni corrections were performed among the 4 hour difference scores for the four doses of MA-induced intake. In addition, to assess whether observed differences in vehicle intake were due to enduring strain-specific effects, correlations of strain means at 1, 2 and 4 hours following vehicle treatment in the present study were compared with corresponding vehicle values collected in Specific Aim 3.

Results:

Significant differences in cumulative food intake were observed among strains ($F(11,99)= 16.74$, $p<0.0001$), among the injection conditions ($F(4,36)= 7.30$, $p<0.002$), across test times ($F(2,18)= 803.47$, $p<0.0001$), and for the interactions between strains and times ($F(22,198)= 8.52$, $p<0.0001$) and among strains, conditions and times ($F(88,792)= 2.04$, $p<0.0001$), but not between strains and conditions ($F(44,336)= 3.01$, $p>0.07$) nor conditions and times ($F(8,72)= 2.05$, $p>0.053$). Significant differences in short-term vehicle cumulative intake were observed across strains ($F(11,99)= 5.76$, $p<0.0001$), across test times ($F(2,18)= 490.63$, $p<0.0001$) and for the interaction between strains and times ($F(22,198)= 2.75$, $p<0.0001$). Table 12 summarizes the strain-specific differences in cumulative vehicle intake with CD-1 mice consuming the greatest amount

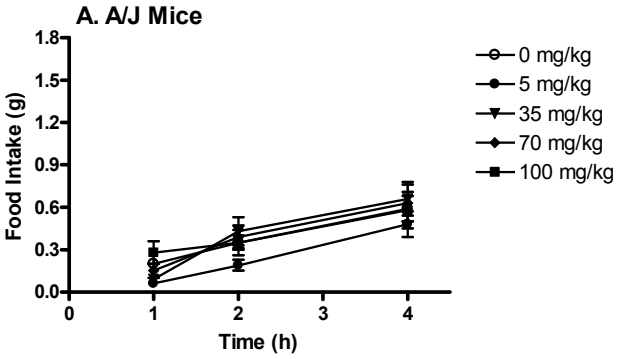
(~1 g/ 4 h) followed by DBA/2J, AKR/J, SJL/J and BALB/cJ mice (~0.7-0.8 g/ 4 h), C57BL/10J and SWR/J mice (~0.6 g/ 4 h), A/J, CBA/J and 129P3/J (~0.5 g/ 4 h) and finally C57BL/6J and C3H/HeJ mice (~0.4 g/ 4 h).

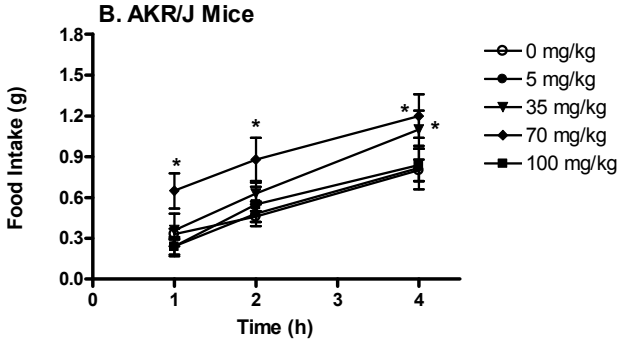
Correlations of strain means for vehicle intakes in the present and our previous glucoprivation study (Lewis et al., 2006b) revealed that significant relationships among strains were not observed for vehicle intakes after 1 ($r=0.32$), 2 ($r=0.21$) or 4 ($r=0.38$) hours in the two studies. This suggests that short-term differences in light cycle intake among strains are probably due to simple inter-animal variability rather than systematic strain-specific effects.

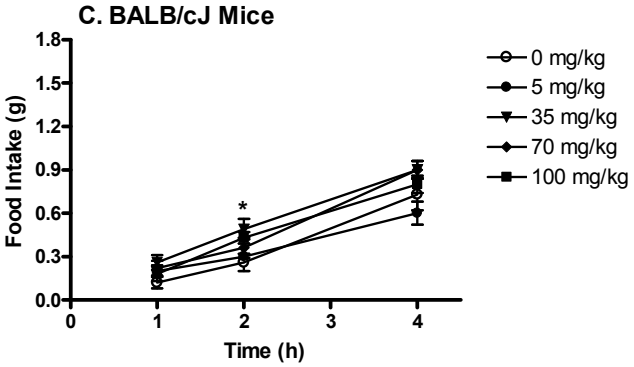
Significant differences in the difference scores for cumulative MA-induced intake were observed among strains ($F(11,99)= 3.03$, $p<0.0016$), among MA doses ($F(3,27)= 3.39$, $p<0.032$), across the two test times ($F(1,9)= 5.31$, $p<0.047$), and for the interactions between strains and times ($F(11,99)= 3.38$, $p<0.0005$) and among strains, doses and times ($F(33,297)= 1.51$, $p<0.041$), but not between strains and doses ($F(33,297)= 2.64$, $p>0.14$) nor doses and times ($F(3,27)= 1.09$, $p>0.41$). There were clear strain differences in the magnitude and pattern of MA-induced intake relative to corresponding vehicle conditions (Figure 6). MA produced significant dose-dependent increases in cumulative food intake following each of the three highest (35 (4 h), 70 (4 h), 100 (2-4 h) mg/kg) doses in DBA/2J mice (Figure 6I), following each of the two highest (70-100 mg/kg, 4h) doses in CD-1 mice (Figure 6G), and following each of the two middle (35 (4 h)-70 (1-4 h) mg/kg) doses in AKR/J mice (Figure 6B). MA produced significant dose-specific increases in food intake following only the lowest (5 mg/kg) dose in C3H/HeJ (1-4 h) mice (Figure 6H), following only the lower middle (35 mg/kg) dose in BALB/cJ (2 h)

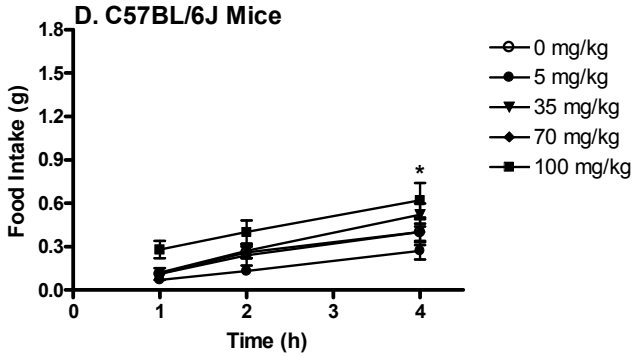
Figure 6. Cumulative food intake (g, ±S.E.M.) following four doses of mercaptoacetate (MA) in 12 mouse strains over 4 h.

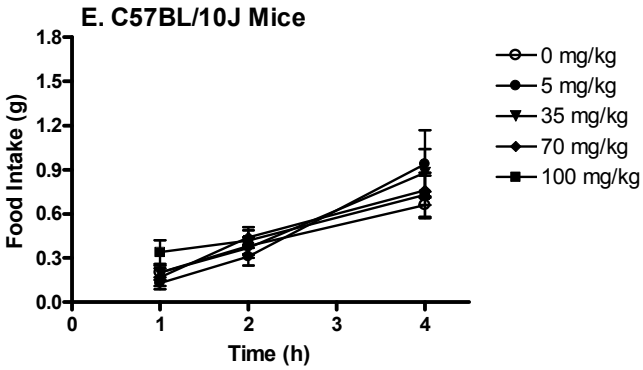
Ordinates are identical to facilitate interstrain comparisons. The crosses (+) denote significant alterations in food intake relative to the corresponding vehicle value obtained within each strain (Tukey comparisons, $P<0.05$). The twelve panels of this figure are found on the following two pages.

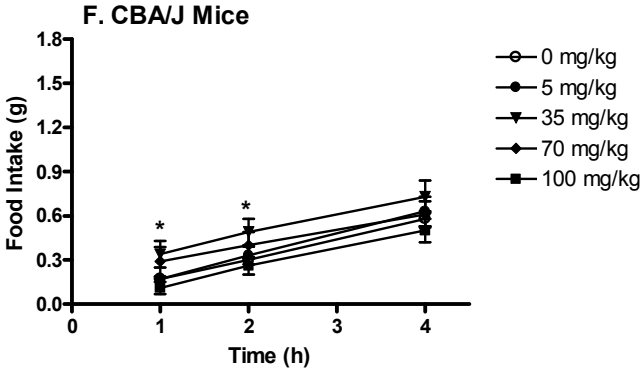

A. A/J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg

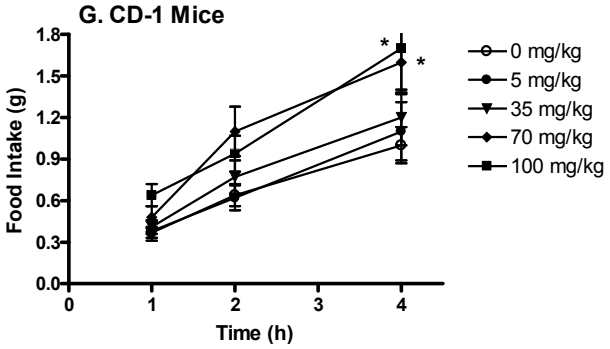

B. AKR/J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg

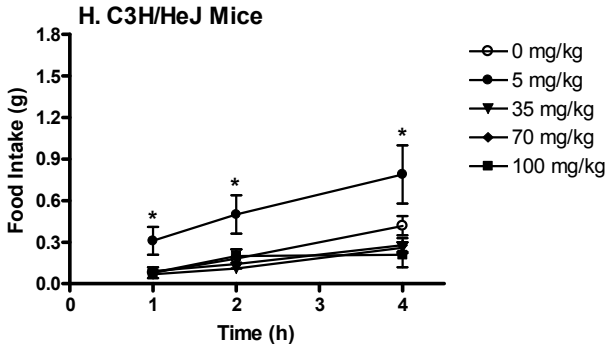

C. BALB/cJ Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg

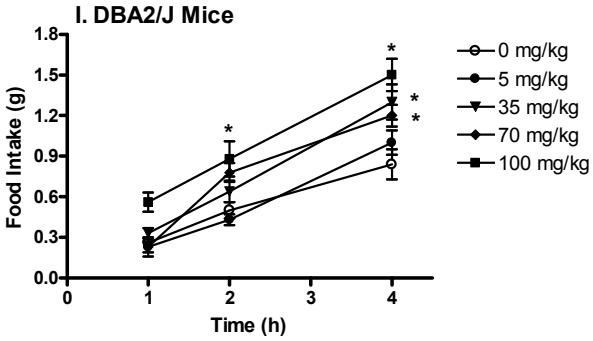

D. C57BL/6J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg

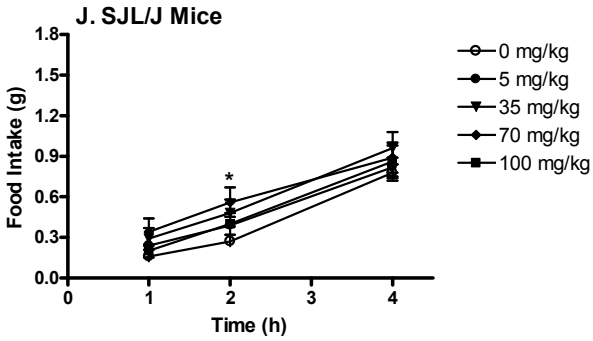

E. C57BL/10J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg

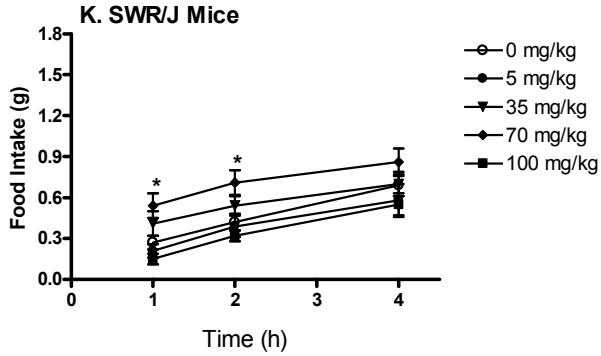

F. CBA/J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg

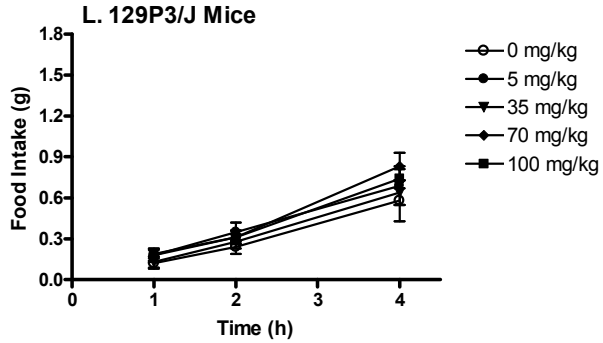

G. CD-1 Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg


H. C3H/HeJ Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg


I. DBA2/J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg


J. SJL/J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg


K. SWR/J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg


L. 129P3/J Mice
Food Intake (g)
Time (h)
0 mg/kg
5 mg/kg
35 mg/kg
70 mg/kg
100 mg/kg

and CBA/J (1-2 h) mice (Figures 1C and 1F), following only the high middle (70 mg/kg) dose in SJL/J (2 h) and SWR/J (1-2 h) mice (Figures 1J and 1K) and following only the highest (100 mg/kg) dose in C57BL/6J (4 h) mice (Figure 6D). In contrast, MA failed to significantly increase food intake in A/J, C57BL/10J and 129P/3J mice (Figures 1A, 1E and 1L).

Table 12 also summarizes the food intake difference scores at 4 hours following each MA dose in the 12 tested mouse strains, allowing for inter-strain comparisons across MA doses. Thus, DBA/2J mice displayed significantly greater magnitudes of MA-induced feeding following the 100 mg/kg dose than all other inbred strains, following the 70 mg/kg dose than A/J, BALB/cJ, CBA/J, C57BL/6J and C3H/HeJ mice, and following the 35 mg/kg dose than A/J, BALB/cJ, CBA/J, C57BL/6J, C3H/HeJ, CD-1, SJL/J, SWR/J and 129P/3J mice. CD-1 mice displayed significantly greater magnitudes of MA-induced feeding following the 100 mg/kg dose than all other inbred (except DBA/2J) strains and following the 70 mg/kg dose than A/J, BALB/cJ, CBA/J, C57BL/6J, C57BL/10J, C3H/HeJ, SJL/J, SWR/J and 129P/3J mice. AKR/J mice displayed significantly greater magnitudes of MA-induced feeding following the 70 mg/kg dose than all other inbred (except C3H/HeJ and DBA/2J) strains and following the 35 mg/kg dose than A/J and C3H/HeJ mice. C57BL/6J mice displayed significantly greater magnitudes of MA-induced feeding following the 100 mg/kg dose than C3H/HeJ, CBA/J and SWR/J mice. Significantly greater magnitudes of MA-induced feeding following the 70 mg/kg dose were observed in SJL/J mice relative to C3H/HeJ and C57BL/6J mice, and in SWR/J mice relative to A/J, C3H/HeJ and C57BL/6J mice. Significantly greater magnitudes of MA-induced feeding following the 35 mg/kg dose were observed in

BALB/cJ and CBA/J mice relative to C3H/HeJ mice. Finally, significantly greater magnitudes of MA-induced feeding following the 5 mg/kg dose were observed in C3H/HeJ mice relative all other strains except 129P/3J mice.

Evaluation of percent change of intake following MA doses relative to vehicle values revealed significant differences among strains ($F(11,99)= 2.62$, $p<0.027$), for the interaction between strains and doses ($F(33,297)= 2.68$, $p<0.0001$), but not among doses ($F(3,27)= 1.63$, $p>0.2$). Table 12 also summarizes the percent change in food intake 4 h following MA doses for all strains. Given that individual 129P/3J mice ate very little (0.1-0.2 g/ 4 h) under vehicle treatment, some of the MA effects were therefore very pronounced, and this strain showed significantly greater increases relative to all other strains. DBA/2J mice displayed significantly greater increases in the percent change of intake at 4 hours following the 100 mg/kg dose relative to C3H/HeJ, BALB/cJ, SWR/J, A/J, SJL/J and CBA/J mice. Finally, C3H/HeJ mice displayed significantly greater increases in the percent change of intake at 4 hours following the 5 mg/kg dose relative to BALB/cJ, C57BL/6J, SWR/J and A/J strains.

Narrow-sense heritability estimates were obtained for 4 hour cumulative food intake following only the highest MA dose (100 mg/kg), where more robust genetic effects are to be expected, and they were compared to 4 hour cumulative intake following vehicle. The obtained h^2 values were moderately high for both MA (0.37) and vehicle (0.43) values. These values are consistent with calculated heritability estimates obtained in our previous study (Lewis et al., 2006b) investigating strain differences in 2DG-induced feeding: 2DG (800 mg/kg at 4 h): $h^2 = 0.51$; vehicle: $h^2 = 0.44$.

In analyzing possible relationships among intakes elicited by the MA doses themselves, Bonferroni-corrected pairwise correlations revealed significant effects only between intakes following the two highest (70 and 100 mg/kg) MA doses across all strains.

Discussion:

First, to our knowledge, this is the first demonstration that outbred CD-1 mice significantly and dose-dependently increase their food intake following the free fatty acid oxidation inhibitor, MA in the same manner as observed for outbred rats (Langhans & Scharrer, 1987; Scharrer & Langhans, 1986). As with different rat strains (Singer et al., 1997), strain-specific effects for MA-induced feeding were observed, that varied as functions of post-injection test time and MA dose. Any dose-dependent effect of MA is important given the fact that the ingestive responses elicited by only the two highest MA doses significantly correlated with each other across strains. Moreover, moderately-high heritability estimates were observed for intake following the largest MA dose ($h^2 = 0.37$) and vehicle ($h^2 = 0.43$). To this end, orderly and significant dose-dependent increases in food intake were observed following the three highest (35-100 mg/kg) MA doses in inbred DBA/2J mice and the two highest (70-100 mg/kg) doses in outbred CD-1 mice. Dose-specific increases in intake were observed following the two middle (35-70 mg/kg) MA doses in AKR/J mice, only the 5 mg/kg MA dose in C3H/HeJ mice, only the 35 mg/kg MA dose in BALB/cJ and CBA/J mice, only the 70 mg/kg dose in SJL/J and SWR/J mice, and only the 100 mg/kg dose in C57BL/6J mice. In contrast, MA failed to significantly increase food intake at any dose in this wide range in A/J, C57BL/10J and

129P/3J mice. The demonstration of genotype-dependent variability in this lipoprivic response may provide the basis for the subsequent identification of trait-relevant genes.

The intake of dietary fat also systematically varies as a function of genetic predisposition among a host of other variables (see review: West & York, 1998), and has led to the identification of dietary resistance and susceptibility phenotypes in inbred and outbred strains of mice (e.g., Smith-Richards et al., 1999; West et al., 1992; 1995). Thus, moderate intake of high-fat diets of shortening, lard or powder promoted weight gain and obesity in AKR/J mice, yet large intake of a high-fat diet was not accompanied by weight gain in SWR/J mice. Notably, AKR/J mice showed significantly increases in food intake following 35 and 70 mg/kg MA doses, and SWR/J mice displayed MA-induced feeding only following the 70 mg/kg dose. Indeed, whereas AKR/J and C57BL/6J mice self-selected the highest proportion of fat in macronutrient diet selection with ependymal fat correlating with fat consumption, SWR/J strains consumed a great deal of fat that was inversely correlated with ependymal fat (Smith et al,. 2000). Although AKR/J and C57BL/6J mice responded similarly in this latter study, the C57BL/6J strain displayed a very muted feeding response following MA in the present study. Moreover, whereas the diet-sensitive AKR/J and DBA/2J strains consumed greater amounts of fat, displayed more adiposity and displayed elevated levels of leptin and insulin, the C57BL/6J strain showed an equal preference between protein and fat, and displayed normal insulin and leptin levels (Alexander et al., 2006). Interestingly, both DBA/2J and AKR/J strains displayed greater feeding sensitivity to MA relative to C57BL/6J mice. In contrast, obesity-resistant SWR/J and A/J mice consume more fat than carbohydrate, but fail to gain weight, potentially because of lower insulin levels, increased capacity of skeletal

muscle to metabolize fat, enhanced paraventricular galanin, and reduced arcuate NPY (Leibowitz et al., 2005). Of importance was the inability of A/J mice to increase food intake at any time point following any of the MA doses. Thus, there appears to be no overall clear picture in which strain differences fully explain lipoprivic responses on the one hand, and predict fat intake on the other hand.

Functional comparisons between lipoprivic feeding induced by MA and glucoprivic feeding induced by 2DG have been examined previously. One similarity between these ingestive responses is that each elicit c-fos responses in the nucleus of the solitary tract, lateral parabrachial nucleus, central nucleus of the amygdala and the dorsal motor nucleus of the vagus (Ritter & Dinh, 1994). Sympathoadrenal plasma levels of epinephrine and norepinephrine are also elevated by 2DG and MA respectively Scheurink & Ritter, 1993). However, there are differences in sensitivity to different physiological manipulations with MA-induced feeding reduced to a far greater degree than 2DG-induced feeding by vagotomy (Ritter & Taylor, 1990), destruction of visceral sensory neurons with capsaicin (Ritter & Taylor, 1989), lesions placed in the lateral parabrachial nucleus (Calingasan & Ritter, 1993) or the central nucleus of the amygdala (Ritter & Hutton, 1995) or administration of the beta-2-adrenoceptor agonist, salbutamol (Nisoli et al., 1996). 2DG, but not MA, increases c-fos responses in the adrenal medulla and sympathetic preganglionic spinal cord neurons (Ritter et al., 1995), and elicits interoceptive sensory signals similar to that of food deprivation (Benoit & Davidson, 1996). Whereas 2DG stimulates intake of all three macronutrients, MA reliably stimulates protein, but not fat intake, and stimulates carbohydrate intake only when carbohydrate palatability is enhanced (Ritter et al., 1999; Singer et al., 1998). A wider

range of intravenous nutrients reduces feeding induced by MA (glucose, lipid, fructose) relative to 2DG (glucose) (Singer & Ritter, 1994), and intraventricular glucose reduces feeding responses following 2DG, but not MA (Singer & Ritter, 1996). Finally, rat strains differentially susceptible to dietary obesity show similar responses to 2DG-induced feeding, but MA is more effective in eliciting feeding in diet-susceptible relative to diet-resistant strains (Singer et al., 1997). In analyzing possible relationships among intakes elicited by the wide dose ranges of MA and 2DG, the present study only found significant correlations between intakes across strains following the highest (100 mg/kg) MA dose and a moderate (400 mg/kg) 2DG dose, and not for any other pair. Thus, the differences among diverse genotypic mouse strains in their ingestive responses to lipoprivation induced by MA and glucoprivation induced by 2DG support the notion that they employ different neural circuitry, and indeed provide evidence that the two responses operate via only partially overlapping genetic mechanisms of action.

IX. General Discussion:

A: Synthesis of Data:

The studies comprising the dissertation utilized inbred mouse strain surveys to parametrically examine genetic variance in sweet (sucrose) intake (Chapter 5; Lewis et al., 2005) and fat (Intralipid) intake (Chapter 6; Lewis et al., 2007), as well as genetic variance in feeding responses elicited by glucoprivic (2DG: Chapter 7; Lewis et al., 2006b) and lipoprivic (MA: Chapter 8; Lewis et al., 2006a) stimuli. To evaluate potential relationships in common or differential genetic variance between sweet and fat intake and between glucoprivic and lipoprivic responses, a correlational analysis was applied to data derived from the different studies. This involved the use of Pearson product-moment correlation coefficients (*r*) subject to Bonferroni correction for multiple comparisons for the following variables.

To evaluate potential relationships between sucrose (Lewis et al., 2005) and Intralipid (Lewis et al., 2007) intake, correlations were performed for baseline water and chow intakes as well as body weights relative to each other; and relative to previously published, independent results (Bachmanov et al., 2002b). Further, correlations were sought for the following dependent variables associated with both palatable solutions: magnitude of intakes, percent of preferences and the percentage of kilocalories consumed as sucrose or Intralipid.

To asses for potential relationships between two forms of glucosensing responses, voluntary sweet intake and feeding following 2DG-induced glucoprivation were

correlated for the percentage of sucrose intake at selected sucrose concentrations (of 0.01%, 2.5%, 10%, and 20%) relative to intake responses following 2DG doses (of 50, 200, 400 and 800 mg/kg). These four sucrose concentrations represent the most preferred concentration (10%), two of the most differentially sensitive concentrations (0.1 and 2.5 %), and the highest concentration tested (20%).

To asses the potential relationships between two forms of liposensing responses, voluntary fat (Intralipid) intake and feeding in response to MA-induced lipoprivation, correlations were performed between Intralipid intake and the percentage of kilocalories consumed as Intralipid, at the highest tested concentration (5%) relative to lipoprivic responses at the two highest and most differentially sensitive 70 and 100 mg/kg MA doses.

To evaluate potential relationships between two forms of privation responses, feeding following 2DG-induced glucoprivation, and feeding following MA-induced lipoprivation, correlations were sought for food intake difference scores at 4 hours following each 2DG or MA dose. The results together, have been reported in a forthcoming book evaluating the role of inbred mice and genetic variance across a range of behaviors (Bodnar et al., in press).

1. Baseline Food, Water and Weight Responses: The two preference studies (Lewis et al., 2005, 2007), have established baseline behaviors for 24 hour intake of chow and water (across two bottles) as well as body weight for the twelve tested strains. A previous analysis (Bachmanov et al., 2002b) of male mice from 28 inbred strains which measured food intake and body weight indicated high narrow-sense heritability estimates,

particularly for body weight (h^2 =0.87). Table 13 summarizes the means of chow, water and body weight for both of our preference studies (Lewis et al., 2005, 2007), and consistent with the high heritability findings described above, strong positive and significant correlations were observed for body weight (r=0.77) and water intake (r=0.65), indicating a close correspondence between our two studies for these variables. Indeed, water intake in common strains tested by Bachmanov (2002b) and by our laboratory (Lewis et al., 2006b) yielded positive and significant correlations as well (r=0.67). Such significant findings indicate that baseline responses elicited by the twelve different strains appear consistent between studies, both in the same laboratory, and across laboratories. This positively addresses the important issue of the reliability of behavioral analyses of strain differences, which can be subject to methodological variation.

2. Sucrose: Our evaluation (Lewis et al., 2005) of genetic variance in sucrose intake revealed strong and marked differences between strains, in terms of sensitivity to sucrose concentrations and the peak magnitude of sucrose intake (Table 14), as well as the amount of kilocalories consumed as sucrose (Table 15). In this regard, sensitivity analysis revealed the greatest sensitivity in CD-1 and SWR/J strains, followed by the SJL/J strain and to progressively lesser degrees in BALB/cJ, C57BL/6J, C57BL/10J, C3H/HeJ strains, in A/J, AKR/J, CBA/J and 129P3/J strains, and the lowest level of sensitivity in DBA/2J mice (Table 14). Similar preference data were observed for the percentage of fluid intake consumed as sucrose (see Figure 2). For measurements of peak magnitude, the A/J, C57BL/6J, CD-1 and SWR/J strains consumed the greatest

Table 13: Baseline water (g, ±S.E.M.) and chow (g, ±S.E.M.) intake and body weight (g, ±S.E.M.) in 12 mouse strains in analyses of sucrose and Intralipid intakes.

Strain	Sucrose Water (g)	Intralipid Water (g)	Sucrose Chow (g)	Intralipid Chow (g)	Sucrose Weight (g)	Intralipid Weight (g)
A/J	5.6 (0.7)	4.6 (0.2)	4.4 (0.3)	3.8 (0.2)	33.6 (1.4)	26.6 (1.0)
AKR/J	5.7 (0.6)	8.6 (0.2)	4.6 (0.1)	4.1 (0.1)	33.6 (1.4)	26.6 (1.0)
BALB/cJ	5.6 (0.1)	7.4 (0.2)	5.4 (0.2)	6.7 (0.2)	27.9 (0.8)	23.5 (0.2)
C57BL/6J	4.9 (0.3)	4.8 (0.4)	4.1 (0.1)	3.6 (0.2)	27.9 (0.6)	27.8 (0.8)
C57BL/10J	5.5 (0.3)	6.9 (0.5)	3.7 (0.1)	4.8 (0.2)	26.8 (0.5)	22.7 (0.3)
CBA/J	5.3 (0.3)	6.7 (0.2)	3.8 (0.1)	4.0 (0.2)	32.3 (1.5)	22.6 (0.5)
CD-1	7.8 (0.4)	9.3 (0.8)	5.8 (0.3)	4.1 (0.4)	37.7 (0.8)	37.0 (1.0)
C3H/HeJ	5.2 (0.4)	5.5 (0.2)	4.2 (0.1)	4.4 (0.2)	28.4 (0.5)	18.5 (1.1)
DBA/2J	5.1 (0.1)	6.5 (0.2)	4.4 (0.2)	5.3 (0.2)	27.0 (0.8)	23.7 (0.2)
SJL/J	5.7 (0.3)	6.6 (0.1)	3.5 (0.2)	3.6 (0.2)	25.6 (0.2)	21.1 (0.3)
SWR/J	7.3 (0.3)	9.2 (0.3)	4.6 (0.2)	3.9 (0.1)	27.2 (0.3)	18.3 (0.6)
129P3/J	7.1 (0.4)	5.9 (0.4)	n.a.	5.0 (0.4)	30.5 (0.4)	22.6 (0.4)
Correlation	r= 0.65	P<0.05	r= 0.42	n.s.	r=0.77	P<0.05

Table 14: Comparison of sensitivity to sucrose and Intralipid and the peak magnitude (g) of intake of both solutions. Sensitivity is defined as the lowest concentration at which a particular sucrose or Intralipid solution produces intake significantly greater than that of water, in a two-bottle preference test.

Strain	Sucrose Sensitivity	Intralipid Sensitivity	Sucrose Magnitude	Intralipid Magnitude
A/J	2.5%	2%	11.6 g	13.5 g
AKR/J	2.5%	0.5%	6.9 g	11.1 g
BALB/cJ	1%	0.001%	10.9 g	13.3 g
C57BL/6J	1%	0.5%	14.8 g	14.6 g
C57BL/10J	1%	1%	10.0 g	14.9 g
CBA/J	2.5%	2%	7.8 g	7.9 g
CD-1	0.0001%	1%	13.5 g	12.9 g
C3H/HeJ	1%	2%	7.9 g	6.7 g
DBA/2J	10%	0.5%	7.3 g	9.2 g
SJL/J	0.01%	1%	10.7 g	12.5 g
SWR/J	0.0001%	0.5%	21.9 g	20.7 g
129P3/J	2.5%	2%	9.8 g	9.4 g
Correlation	r= -0.06	n.s.	r= 0.87	$p<0.01$

Table 15: Comparison of intakes and the percentage of kilocalories consumed as the solution following exposure to Intralipid (I: 5%) or sucrose (S: 20%) across the 12 mouse strains. Significant correlations were observed among strains for both intake ($r= 0.877$, $p<0.01$) and the percentage of kilocalories consumed ($r= 0.763$, $p<0.05$) for these palatable fat and simple carbohydrate stimuli.

Strain		A	AKR	BALB	BL-6	BL10	CBA	CD-1	C3H	DBA	SJL	SWR	129
IL (5%)	Intake	13.5	11.1	13.3l	14.6	14.9	7.6	12.9	6.7	9.2	12.5	20.7	9.4
	%kcal	46%	36%	25%	47%	50%	24%	26%	20%	22%	42%	60%	23%
S (20%)	Intake	11.1	6.9	10.9	11.3	10.0	7.3	10.4	7.7	7.3	10.7	14.2	9.8
	%kcal	50%	25%	35%	44%	63%	36%	39%	31%	28%	61%	56%	n/a

(11.6-22 g) amounts of sucrose (Table 14), whereas the A/J, C57BL/10J, SJL/J and SWR/J strains consumed the greatest (50-63%) percentages of kilocalories as sucrose at the highest (20%) sucrose concentration (Table 15). The BALB/cJ, C57BL/10J SJL/J and 129P3/J strains displayed intermediate magnitudes of intake (9.8-10.9 g). By contrast, the AKR/J, CBA/J, C3H/HeJ and DBA/2J strains consumed the least (6.9-7.9 g) amount of sucrose (Table 14), and displayed lower (25-36%) percentages of kilocalories consumed as sucrose (Table 15).

The consistently higher sucrose responses observed in C57BL6/J mice relative to 129P3/J mice is consistent with prior (Bachmanov et al., 1997; Lush, 1989) and more recent findings (Sclafani, 2006a; 2006c; 2007). A number of previous studies have employed, as a measure of preference, the percentage of sweetener consumed as a function of total fluid intake over a very restricted range of sucrose concentrations (e.g., Capeless and Whitney, 1995; Fuller, 1974; Lush, 1989; Pothion et al., 2004). The findings of our study indicate that this measure of preference is not a reliable measure because strains that showed both larger (e.g., C57BL/6J, C57BL/10J, SWR/J, SJL/J) and smaller (e.g., C3H/HeJ) magnitudes of sucrose intake invariably showed very high ($\geq$95%) preferences for sucrose. This underlines the importance of evaluating a variety of dependent variables across a wide range of strains and concentrations.

A further noteworthy finding of the first study (Lewis et al., 2005) was that increasing sucrose over 24 h produced differential strain-dependent effects on overall kilocaloric intake. Whereas A/J, C57BL/6J, C57BL/10J, CD-1, SWR/J and SJL/J strains all displayed the most pronounced decreases in chow intake as the percentage of kilocalories consumed as sucrose increased, the AKR/J, C3H/HeJ and DBA/2J strains did not

significantly alter chow intake even at high sucrose concentrations. This rapid adjustment in response to the additional calories provided by sucrose suggests that some strains have either a greater sensitivity to caloric changes and/or a quicker ability to both adapt and respond to these changes. Divergent responders may serve as a model for studying and identifying genetic substrates associated with the ability to regulate kilocalorie intake across a variety of energy sources. Such data might predict the ability of chronic exposure to concentrated sucrose (as an alternative energy source to chow) to increase weight gain, obesity and diabetic symptoms in non-compensating strains relative to compensating strains.

Additionally, in our study design, the testing a sufficient number of inbred strains, allows for the valid estimation of genetic correlations (Hegmann and Possidente, 1981). Thus, in determining whether sucrose consumption in the present study correlated with *Tas1R3* polymorphisms in identically-tested mouse strains (Reed et al., 2004), significant correlations were observed between these polymorphisms and moderate (0.01%: r=0.83; 0.1%: r=0.91; 2.5%: r=0.86), but not higher (5-20%) sucrose concentrations (see Table 5 above). This finding, as well as limited correlations at the higher concentrations with a previous survey of sachharin intake (Lush, 1989: see Table 5 above) and transient correlations between the different sucrose concentrations used in our study (Lewis et al., 2005: see Table 4 above) support the assertion that the regulation of sucrose intake is under polygenic control, with distinct genetic mechanisms underlying the intake of solutions with high and low sweetener concentrations. It is plausible therefore that the *Tas1R3* gene is responsible for strain variance in sucrose intakes at the lower concentrations, where taste is a primary cue. At higher sucrose concentrations, there is a

greater involvement of post-oral factors, which are not likely related to polymorphisms of the T1R3 taste receptor, yielding less consistent correlations as described above. This theory is also supported by Sclafani's (2007) most recent findings of the reduction of strain differences upon repeated sucrose testing, when the mice experience the post-oral consequences of sucrose. Thus, it can be argued that a comprehensive understanding of the genetic basis for the intake of sweeteners like sucrose will require study across a range of concentrations.

3. Fat (Intralipid): One of the goals of our parallel study (Lewis et al., 2007) was to assess similarities or differences in genetic variance observed for sucrose intake relative to that for fat. To do so would have required varying the fat concentrations. However, unlike sucrose, solubility difficulties would be encountered when attempting to dilute fat over a variety of concentrations, for a time course (e.g., 24 h) that is reasonable to study murine intake. This led us to employ Intralipid in the next study (Lewis et al., 2007), an emulsified fat solution (20%) made almost exclusively from soybean oil (20 g in 100 ml). This insured that the fat was equally distributed in solution across a wide range of concentrations, enabling the solutions to be readily consumed in a manner similar to sucrose and other palatable solutions (e.g., Higgs and Cooper, 1998a, 1998b). The same strains that were previously evaluated for sucrose intake (Lewis et al., 2005) were analyzed for fat. This selection of strains was supported by findings of three divergent patterns of fat intake for these strains in other studies; high fat intake with weight gain, e.g., AKR/J, C57BL/6J, DBA/2J (Alexander et al., 2006; Smith-Richards et al., 1999; West et al., 1992, 1995); high fat intake without weight gain, e.g., A/J, SWR/J

(Leibowitz et al., 2005; Smith et al., 2000), and low fat intake, e.g., BALB/cJ, C3H/HeJ SJL/J, 129/J (Alexander et al., 2006; Smith et al., 2000).

First, it was clear that all strains displayed significant increases in Intralipid intake relative to water intake in the 24 h, two-bottle preference tests (Table 7; Figure 3). As expected, strong and systematic strain differences were observed for Intralipid preference and intake. Thus, sensitivity analyses (Table 14) revealed significant increases in Intralipid relative to concomitantly-offered water intake to the greatest degree in BALB/cJ mice, to progressively lesser degrees in AKR/J, C57BL/6J, DBA/2J and SWR/J inbred strains, in outbred CD-1 and inbred C57BL/10J and SJL/J strains, and to the least degree in A/J, CBA/J, C3H/HeJ and 129P3/J inbred strains. Similar preference data were observed for the percentage of fluid intake consumed as Intralipid (see Figure 4). Moreover, the peak magnitude of Intralipid intake (Table 14) was greatest for SWR/J mice (20.7 g), followed by A/J, BALB/cJ, C57BL/6J and C57BL/10J mice (13.3-14.9 g), then by AKR/J, CD-1, SJL/J and 129P3/J mice (9.4-12.9 g), and finally by CBA/J, C3H/HeJ and DBA/2J mice (6.7-9.2 g). Correspondingly, strains displayed different responses for the percentage of kilocalories consumed as Intralipid at the highest (5%) Intralipid concentration (Table 15). The SWR/J mice consumed the greatest percentage of kilocalories as Intralipid (60%). Intermediate consumption of kilocalories as Intralipid was observed in A/J, AKR/J, C57BL/6J, C57BL/10J, CD-1 and SJL/J mice (26-50%), while the other five strains displayed the lowest percentages of kilocalories consumed as Intralipid (20-25%).

Further, significant positive correlations for both the magnitude of intake and the percentage of kilocalories consumed as Intralipid were observed among the four highest

(0.5, 1, 2 and 5%) Intralipid concentrations, indicating consistency of the effects. Finally, compensatory decreases in chow intake were noted at the highest Intralipid concentration only in A/J, AKR/J, BALB/cJ, C57BL/10J and SWR/J strains.

Other important points to note include the finding of order effects for several strains with Intralipid (A/J, AKR/J, BALB/cJ, C57BL10J, SJL/J and SWR/J), reinforcing the importance of controlling for order by counterbalancing the ascending verses descending concentrations when assessing intake across different concentrations. Also, as with sucrose, the magnitude of Intralipid intake was not a reliable predictor of the percent preference of Intralipid. This further underscores the need to survey genetic models of food intake across a large range of strains and concentrations, while measuring a variety of dependent variables.

4. Sucrose and Fat Preference Paradigms: Correlational analysis to assess whether genetic variance in sucrose intake was related to genetic variance in Intralipid intake yielded the following interesting results: A highly significant positive correlation (r=0.87) for the peak magnitude of sucrose intake and Intralipid intake was noted among the 12 strains (Table 14). In this regard, SWR/J mice displayed the most pronounced sucrose intake among inbred strains followed by A/J, BALB/cJ, C57BL/6J and SJL/J mice, then by C57BL/10J and129P3/J mice, and finally by AKR/J, CBA/J, C3H/HeJ and DBA/2J mice. Similarly, SWR/J mice consumed by far the most Intralipid among inbred strains, followed by A/J, BALB/cJ, C57BL/6J and C57BL/10J mice, then by AKR/J, SJL/J, and 129P3/J mice, and finally by CBA/J, C3H/HeJ and DBA/2J mice. Moreover, significant positive correlations were also observed when comparing Intralipid (5%) intake with sucrose intake at concentrations of 5% (r= 0.82), 10% (r= 0.85) and 20%

(r=0.88, Table 15). The threshold sensitivity for sucrose intake and for Intralipid intake in these 12 strains did not demonstrate significant relationships.

Additional positive correlations were observed when evaluating the percentage of kilocalories consumed as Intralipid (5%) relative to the percentage of kilocalories consumed as sucrose at concentrations of 5% (r= 0.81), 10% (r= 0.89) and 20% (r= 0.76, Table 15). Thus, the strain-specific pattern of response for the percentage of kilocalories consumed as sucrose was similar to that of the percentage of kilocalories consumed as Intralipid. In this regard, A/J, C57BL/10J, SJL/J and SWR/J strains consumed the highest percentages of kilocalories as sucrose, the BALB/cJ, C57BL/6J, and CD-1 strains were intermediate responders and the AKR/J, CBA/J, C3H/HeJ and DBA/2J strains consumed the least. For percentage of kilocalories consumed as Intralipid, the C57BL/10J and SWR/J strains consumed the highest percentages, the A/J, AKR/J, C57BL/6J, C57BL/10J, CD-1 and SJL/J strains were intermediate responders and the BALB/cJ, CBA/J, C3H/HeJ, DBA/2J and 129P3/J strains consumed the lowest percentages of kilocalories as Intralipid (Table 14).

Finally, compensatory decreases in chow intake were noted as sucrose intake increased for A/J, C57BL/6J, C57BL/10J, SJL/J and SWR/J inbred strains (Lewis et al., 2005). This compares with the compensatory decreases in chow intake only at the highest Intralipid concentration and only in A/J, AKR/J, BALB/cJ, C57BL/10J and SWR/J strains. That C57BL/10J, but not C57BL/6J mice displayed this compensatory decrease in chow intake following Intralipid represents a relatively rare differentiation between these two strains in behavioral assays. This short-term and rapid (within 24 h) reduction in chow intake in the presence of Intralipid, like that to sucrose, suggests that

such strains may be displaying sensitivity to caloric intake and/or a greater ability to both adapt and respond thereto. Divergent responders may provide a model for studying and identifying those genetic substrates associated with this ability to regulate kilocalorie intake across a variety of energy sources. The findings of positive correlations and the similarities of strain specific patterns of responses to sucrose and Intralipid intake support the notion that genetic variance in the consumption of sweets and fats are highly related.

5. Glucoprivic and Lipoprivic Responses: Most prior studies examining genetic variance in ingestive responses employed hedonic and/or orosensory stimuli in distinguishing responsiveness across murine strains. To extend this analysis of genetic variance in ingestive responses to homeostatic mechanisms, our laboratory (Lewis et al., 2006a, 2006b) examined whether different mouse strains varied in their feeding responses, when induced by glucoprivation and lipoprivation. The glucoprivation study surveyed the 11 inbred and one outbred strains for variations in feeding responses following a wide range of systemic doses of the anti metabolic glucose analogue 2DG (200-800 mg/kg), across a 4 hour time course. This paralleled the sucrose study (Lewis et al., 2005).

Genetic variability was observed in the inbred strains with dose-dependent increases in 2DG-induced feeding observed across all four doses (DBA/2J), across the three highest doses (BALB/cJ, SJL/J and 129P3/J) and across the two highest doses (CBA/J and AKR/J). This was similar to outbred CD-1 mice that displayed orderly time- and dose-dependent increases in 2DG-induced feeding. In contrast, some mouse strains displayed very limited feeding increasing following 2DG (A/J and C3H/HeJ: 800 mg/kg; C57BL/6J: 400 mg/kg) while others failed to show any increases (C57BL/10J) and yet

others demonstrated significantly reduced intake (SWR/J). Such effects were not predicted by any baseline differences in intakes. Moreover, although there was significant cross-correlation between 2DG doses of 200, 400 and 600 mg/kg, they in turn failed to correlate with the highest 800 mg/kg 2DG dose. Significant correlations between sucrose intake (Lewis et al., 2005) and 2DG food intake (Lewis et al., 2006b) failed to occur in these 11 inbred strains. Although both experimental paradigms are thought to provide insight into glucosensing processes, these different experimental paradigms present a differential pattern of strain sensitivity, suggesting differential genetic organization. Nonetheless, given the complexity of regulating glucose intake, this lack of correlation does not imply a lack of integration between such systems. Both are likely to contribute to glucosensing in the mouse, although the relative contribution of each may vary with genotype. Further work is needed to provide a model for understanding their integration.

The lipoprivation study (Lewis et al., 2006a) which surveyed the 11 inbred and one outbred strain for variations in feeding responses, following a wide range of systemic MA doses (5-100 mg/kg) across a 4 hour time course, paralleled our study of fat intake (Lewis et al., 2007) by utilizing this free fatty acid oxidation inhibitor to increase food intake following systemic administration (Langhans and Scharrer, 1987; Scharrer and Langhans, 1986). Strain-specific effects for MA-induced feeding were observed following the three highest (35-100 mg/kg) MA doses in inbred DBA/2J mice and the two highest (70-100 mg/kg) doses in outbred CD-1 mice. Dose-specific increases in intake were observed following the two middle (35-70 mg/kg) MA doses in AKR/J mice, following the 5 mg/kg MA dose in C3H/HeJ mice, following the 35 mg/kg MA dose in

BALB/cJ and CBA/J mice, following the 70 mg/kg dose in SJL/J and SWR/J mice, and following the highest, 100 mg/kg, dose in C57BL/6J mice. By contrast, MA failed to significantly increase food intake at any dose in this wide range in A/J, C57BL/10J and 129P/3J mice.

Athough there have been positive functional comparisons in rats between lipoprivic (MA) and glucoprivic (2DG) feeding in terms of similar c-fos responses in the nucleus of the solitary tract, lateral parabrachial nucleus, central nucleus of the amygdala and the dorsal motor nucleus of the vagus (Ritter and Dinh, 1994), and in elevated sympathoadrenal plasma levels of epinephrine and norepinephrine (Scheurink and Ritter, 1993). There have also been findings of marked differences between MA-induced and 2DG-induced feeding responses in terms of sensitivity to different physiological manipulations following vagotomy (Ritter and Taylor, 1990) and lesions placed in the lateral parabrachial nucleus (Calingasan and Ritter, 1993) or the central nucleus of the amygdala (Ritter and Hutton, 1995). Importantly, our correlational analyses of genetic differences in feeding responses elicited by 2DG and MA failed to find significant relationships in terms of either sensitivity or magnitude of effects (Tables 16 and 17). Similarly, significant correlations between genetic variance for Intralipid intake and MA-induced intake were not noted. Thus, the differences between these diverse genotypic mouse strains suggest that they employ different neural circuitry in their ingestive responses to lipoprivation and glucoprivation, and that these are independent homeostatic responses, operating via different genetic mechanisms of action.

Table 16: Pearson product-moment correlation coefficients for the strain means of difference scores for food intake after 4 h across the 11 inbred and one outbred mouse strains subtracted from corresponding vehicle values after the four MA doses, and as compared with previously-derived and published four 2-deoxy-D-glucose (2DG) doses[1] .

MA (mg/kg)	5	35	70	2DG[1] (mg/kg)	200	400	600	800
MA 5	---				+0.21	-0.14	+0.03	-0.01
MA 35	-0.07	---			+0.18	+0.18	+0.45	-0.09
MA 70	-0.30	+0.55	---		+0.10	+0.19	+0.34	+0.02
MA 100	+0.13	+0.55	+0.68*		+0.57	+0.66*	+0.55	+0.14

Significant correlation (* $p < 0.05$) after Bonferroni corrections.
[1]Data derived from (Lewis et al., 2006b).

Table 17: Comparison of sensitivity (minimum dose) to feeding responses to 2DG and MA and the peak magnitude (g) of food intake following the glucoprivic and lipoprivic stimuli. Sensitivity is defined as that dose that significantly increases intake over vehicle values after 4 h. Magnitude is defined as the increased intake after 4 h following 2DG or MA over vehicle values.

Strain	2DG Sensitivity (mg/kg)	MA Sensitivity (mg/kg)	2DG Magnitude	MA Magnitude
A/J	800	>100	0.5 g	0.04 g
AKR/J	600	35	0.4 g	0.4 g
BALB/cJ	400	100	0.7 g	0.7 g
C57BL/6J	800	100	0.2 g	0.1 g
C57BL/10J	>800	>100	0.1 g	0.3 g
CBA/J	600	100	0.5 g	0.1 g
CD-1	200	70	0.5 g	0.7 g
C3H/HeJ	800	5	0.6 g	0.4 g
DBA/2J	200	35	0.5 g	0.6 g
SJL/J	400	100	0.4 g	0.2 g
SWR/J	>800	100	0.01 g	0.2 g
129P3/J	400	>100	0.3 g	0.3 g
Correlation	r= 0.26	n.s.	r= 0.48	n.s.

B: General Implications:

1. Overall Meaning: The demonstration of genetic correlation between the ingestive processes of sucrose and of Intralipid among isogenic strains can be used as evidence for the existence of some common heritable mechanism influencing both traits (see Hegmann & Possidente, 1981). Currently, the sweet taste receptor *Tas1R3* gene is accepted as the mechanism responsible for differences in sucrose preference between inbred mouse strains (Inoue et al., 2004). However, polymorphisms at this genetic locus cannot explain the parallel phenotypic response for Intralipid preference. Furthermore, our use of a wide range of sucrose concentrations calls into question the ubiquitous role of the *Tas1R3* gene for supra-threshold sucrose concentrations (Lewis et al., 20005). This conclusion is based on the lack of uniform correlations for *Tas1R3* polymorphisms and sucrose intake at higher concentrations, lack of significant correlations at the higher sucrose concentrations with other studies investigating sweet taste responsivity (e.g. Lush, 1989) and only transient correlations between the different sucrose concentrations *per se* (see Tables 4 and 5 above). Although fat may be detected by texture, smell and even possibly by taste (Laugerette, 2007; Mattes, 2005), polymorphisms at peripheral receptors cannot fully account for the ingestive responses at higher concentrations since post-oral influences are present at higher sucrose and Intralipid concentrations.

It may be possible to explain the relationship between sweet preference and fat preference in terms of genetic linkages, but there is no evidence at present that the genes responsible for fat preference among inbred mice are proximous to and/or inherited together with the gene responsible for sucrose preference. A theory proposed by Gilbertson (2005) is that fat enhances the taste receptor's response to sweets. Thus,

although Sclafani (2007) noted that the preference for Intralipid may result in part because its free fatty acids enhance the sweet taste of its glycerol content , it does not explain strain differences found for other fat emulsions that do not contain glycerol. Additionally, since strong correlations were found specifically for the voluntary intake of sucrose and Intralipid, and not for the metabolic-driven glucoprivic and lipoprivic intake (Lewis et al., 2006a, 2006b), it is likely that the common hedonic component of sugar and fat preference is rooted in a more centrally mediated mechanism, rather than a peripheral taste and/or texture receptor. Although further investigation is necessary to determine which brain area(s) may be responsible for the intake of sweet substances and fats, areas associated with pleasure, reward and motivation are logical candidates with which to begin.

Since all of the above studies that comprise this dissertation demonstrate divergent responses between strains, these data are important in providing insight into the selection of background strains that might be used for knockout studies. For example, using the C57BL/6J as a background strain for knockout studies involving 2DG elicited feeding responses (e.g. Benoit et al., 2003; Hnasko et al., 2004; Ste Marie & Palmiter, 2003) can confound the results since in our study (Lewis et al., 2006b), this strain proved to be a weak responder to 2DG induced feeding, before any targeted deletions were performed. Therefore, careful consideration of the behavioral responses, as defined by our series of studies in intact inbred strains (Lewis et al., 2005, 2006a; 2006b; 2007), would benefit researchers in selecting an appropriate strain for studies using transgenic mice. The data of the above studies have been submitted to the Mouse Phenome

Database for inclusion in their collection of data of inbred mouse strain characteristics, and for use by other researchers.

In addition, our findings can provide methodological standards for future investigations involving murine ingestive behaviors. Specifically, our data (Lewis et al., 2005, 2007) highlighted the necessity of analyzing both the magnitude of the feeding response and the sensitivity of the response (percent preference at a range of concentrations) because they did not necessarily parallel one another, as well the as the importance of controlling for order effects by counterbalancing ascending versus descending concentrations.

Further, due to the large number of strains and wide range of concentrations employed, our data yielded a diverse range of responses which can inform future QTL analyses for the identification of trait relevant genes. Apropos to this, previous QTL analyses (e.g. Blizard et al., 1999; Fuller, 1974; Lush, 1989; Lush et al., 1995; Inoue et al., 2004) have used strains that did not represent the most divergent responses, according to our surveys of 11 inbred strains with nine concentration conditions. Therefore, a more thorough investigation would involve QTL analysis with more divergent sensitivities such as those characterized in our preference studies in order to allow for the identification of additional trait-relevant QTLs, including those with smaller contributions to the overall genotypic variance. Additionally, QTL analyses based on other divergent responses identified in our series of studies such as the ability to regulate overall kilocaloric intake as well as glucoprivic and lipoprivic induced feeding, can provide further insight into feeding disorders such as obesity and diabetes.

2. Future Directions: This research is the first step in defining phenotypic differences in feeding responses across a wide range of strains and conditions. In addition to influencing future ingestive research, as suggested above, these findings can be used to branch out into other areas such as the functional identification of important brain areas involved in mediating ingestive responses. For example, the analysis of c-fos activation at different brain sites can physiologically validate our findings of strain specific responses to glucoprivic or lipoprivic feeding.

Currently, our laboratory is investigating these strain differences with regard to the effect of pharmacological antagonists including opioid and dopaminergic antagonists, that are known to alter sucrose and fat intake in outbred strains and other species. Thus, our laboratory (Dym et al., Brain Research 2007) found a 37-fold difference in the ability of naltrexone to reduce sucrose intake by 50% in highly-sensitive (e.g., C57BL10J) relative to insensitive (SWR/J) strains.

3. Conclusion: This dissertation has identified strain specific variability in the ingestive processes of sucrose intake, fat intake and food intake in response to the homeostatic challenges of glucoprivation and lipoprivation. Heritability estimates and correlational analysis have further outlined the genetic basis of these behaviors. The positive correlation between the voluntary intake of sucrose and Intralipid is an exciting and intriguing finding that requires further investigation in order to elucidate the source of this relationship. Inbred mouse strain surveys allow for the analysis of genetic contributions to feeding responses in intact, genetically varied strains, and together with studies using knockout and knockdown genetic approaches, can provide feasible

strategies for the understanding of the complex genetic-environmental interactions related to the etiology of both normal and disordered feeding behaviors.

X. References

Alexander, J., Chang, G.Q., Dourmashkin, J.T. and Leibowitz, S.F. (2006). Distinct phenotypes of obesity-prone AKR/J, DBA/2J and C57BL/6J mice compared to control strains. Int. J. Obesity 30, 50-59.

Angel, I., and Taranger, M.A. (1991). Impairment of glucostatic, adrenergic and serotonergic feeding parallels the lack of glucoprivic signals in the golden hamster. Brain Res. Bull. 27, 353-358.

Bachmanov, A.A., Beauchamp, G.K. and Tordoff, M.G. (2002a). Voluntary consumption of NaCl, KCl, $CaCl_2$, and NH_4Cl solutions by 28 mouse strains. Behav. Genet. 32, 445-457.

Bachmanov, A.A., Reed, D.R., Beauchamp, G.K. and Tordoff, M.G. (2002b). Food intake, water intake, and drinking spout side preference of 28 mouse strains. Behav. Genet. 32, 435-443.

Bachmanov, A.A., Reed, D.R., Li, X., Li, S., Beauchamp, G.K. and Tordoff, M.G. (2002c). Voluntary ethanol consumption by mice: genome-wide analysis of quantitative trait loci and their interactions in a C57BL/6ByJ and 129P3/J F_2 intercross. Genome Res. 12, 1257-1268.

Bachmanov, A.A., Reed, D.R., Ninomiya, Y., Inoue, M., Tordoff, M.G., Price, R.A. and Beauchamp, G.K. (1997). Sucrose consumption in mice: major influence of two genetic loci affecting peripheral sensory responses. Mamm. Genome 8, 545-548.

Bachmanov, A.A., Reed, D.R., Tordoff, M.G., Price, R.A. and Beauchamp, G.K. (1996a). Intake of ethanol, sodium chloride, sucrose, citric acid and quinine hydrochloride solutions by mice: a genetic analysis. Behav. Genet. 26, 563-573.

Bachmanov, A.A., Reed, D.R., Tordoff, M.G., Price, R.A. and Beauchamp, G.K. (2001b). Nutrient preference and diet-induced adiposity in C57BL/6JByJ and 129P3/J/J mice. Physiol. Behav. 72, 603-613.

Bachmanov, A.A., Schlager, G., Tordoff, M.G. and Beauchamp, G.K. (1998a). Consumption of electrolytes and quinine by mouse strains with different blood pressures. Physiol. Behav. 64, 323-330.

Bachmanov, A.A., Tordoff, M.G. and Beauchamp, G.K. (1996b). Ethanol consumption and taste preferences in C57BL/6ByJ and 129/J mice. Alcohol Clin. Exp. Res. 20, 201-206.

Bachmanov, A.A., Tordoff, M.G. and Beauchamp, G.K. (1998b). Voluntary sodium chloride consumption by mice: differences among five inbred strains. Behav. Genet. 28, 117-124.

Bachmanov, A.A., Tordoff, M.G. and Beauchamp, G.K. (2000b). Intake of umami-tasting solutions by mice: a genetic analysis. J. Nutr. 130, 935S-941S.

Bachmanov, A.A., Tordoff, M.G. and Beauchamp, G.K. (2001c) Sweetener preference of C57/6ByJ and 129P3/J mice. Chem. Senses 26, 905-913.

Bartness, T.J. and Clein, M.R. (1994). Effects of food deprivation and restriction, and metabolic blockade on food hoarding in Siberian hamsters. Am. J. Physiol. 266, R1111-R1117.

Bartness, T.J., Morley, J.E., and Levine, A.S. (1995). Effects of food deprivation and metabolic fuel utilization on the photoperiodic control of food intake in Siberian hamsters. Physiol. Behav. 57, 61-68.

Bauche, F., Sabourault, D., Giudicelli, Y., Nordmann, J., and Nordmann, R. (1981). 2-mercaptoacetate administration depresses the beta-oxidation pathway through an inhibition of long-chain acyl-CoA dehydrogenase activity. Biochem. J. 196, 803-809.

Beauchamp, G.K. and Fisher, A.S. (1993). Strain differences in consumption of saline solutions by mice. Physiol. Behav. 54, 179-184.

Benoit, S.C., and Davidson, T.L. (1996). Interoceptive sensory signals produced by 24-hr food deprivation, pharmacological glucoprivation, and lipoprivation. Behav. Neurosci. 110, 168-181.

Benoit, S.C., McQuade, J.A., Clegg, D.J., Xu, M., Rushing, P.A., and Seeley, R.J. (2003). Altered feeding responses in mice with targeted disruption of the dopamine-3 receptor gene. Behav. Neurosci. 117, 46-54.

Bergen, H.T., Monkman, N., and Mobbs, C.V. (1996). Injection with gold thioglucose impairs sensitivity to glucose: evidence that glucose-responsive neurons are important for long term regulation of body weight. Brain Res. 734, 232-236.

Berthoud, H.R., and Mogenson, G.J. (1977). Ingestive behavior after intracerebral and intracerebroventricular infusions of glucose and 2-deoxy-D-glucose. Am. J. Physiol. 233, R127-R133.

Blizard, D.A., Kotlus, B. and Frank, M.E. (1999). Quantitative trait loci associated with short-term intake of sucrose, saccharin and quinine solutions in laboratory mice. Chem. Senses 24, 373-385.

Bodnar, R.J., Lewis, S.R. and Kest, B. (in press). Feeding and drinking: Section 3.3., Autonomous and motor behaviors: Section 3, Behavioral Genetics of the Mouse: Volume I. In: Crusio, W.E., Sluyter, F. and Gerlai, R.T. (Eds.) Encyclopedia of Behavior Genetics, Cambridge University Press: Cambridge, United Kingdom.

Booth, D.A. (1972). Modulation of the feeding response to peripheral insulin, 2-deoxy-D-glucose or 3-0-methoxyglucose injection. Physiol. Behav. 8,1069-1076.

Calingasan, N.Y., and Ritter, S. (1993). Lateral parabrachial sub-nucleus lesions abolish feeding induced by mercaptoacetate but not by 2-deoxy-D-glucose. Am. J. Physiol. 265, R1168-R1178.

Capeless, C.G. and Whitney, G. (1995). The genetic basis of preference for sweet substances among inbred strains of mice: preference ratio phenotypes and the alleles of the Sac and dpa loci. Chem. Senses 20, 291-298.

Capeless, C.G., Whitney, G. and Azen, E.A. (1992). Chromosome mapping of Soa, a gene influencing gustatory sensitivity to sucrose octaacetate in mice. Behav. Genet. 22, 655-663.

Chandrashekar, J., Hoon, M., Ryba, N. and Zuker, C. (2006). The receptors and cells for mammalian taste. Nature, 444, 288-294.

Clegg, D.J., Edwards, G.L., and Martin, R.J. (2003). Central insulin potentiates eating elicited by 2-deoxy-D-glucose. Physiol. Behav. 78, 331-336.

Czech, D.A. (1988). Effect of insulin and 2-deoxy-D-glucose on feeding and plasma glucose levels in the spiny mouse. Physiol. Behav. 43, 765-769.

Czech, D.A. (1998). A nitric oxide inhibitor NG-nitro-L-arginine, attenuates glucoprivic feeding and deprivation-induced feeding in the mouse. Pharmacol. Biochem. Behav. 60, 601-607.

Davidson, T. L., Altizer, A. M., Benoit, S.C., Walls, E. K., and Powley, T. L. (1997). Encoding and selective activation of "metabolic memories" in the rat. Behavioral Neuroscience 111, 1014-1030.

Delprete, E. and Scharrer, E. (1992). Effects of 2-deoxy-D-glucose on food intake of rats are affected by diet composition. Physiol. Behav.51, 951-956.

Dym, C.T., Pinhas, A., Ginzberg, M., Kest, B. and Bodnar, R.J. (2007). Genetic variance contributes to naltrexone-induced inhibition of sucrose intake in inbred and outbred mouse strains. Brain Res. 1135, 136-145.

Engeset, R.M. and Ritter, R.C. (1980). Intracerebroventricular 2-DG causes feeding in the absence of other signs of glucoprivation. Brain Res. 202, 229-233.

Even, P. and Nicolaidis, S. (1985). Spontaneous and 2DG induced metabolic changes and feeding: an ischymetric hypothesis. Brain Res. Bull. 15, 429-435.

Eylam, S. and Spector, A.C. (2004). Stimulus processing of glycine is dissociable from that of sucrose and glucose based on behaviorally measured taste signal detection in Sac 'taster' and 'non-taster' mice. Chemical Senses 29, 639-649.

Falconer, D.S. and Mackay, T.F.C. (1996). Introduction to quantitative genetics. Fourth Edition, Longman: Essex, UK.

Finn, D.A., Belknap, J.K., Cronise, K., Yoneyama, N., Murillo, A. and Crabbe, J.C. (2005). A procedure to produce high alcohol intake in mice. Psychopharmacology. 178, 471-480.

Fisler, J.S., Warden, C.H., Pace, M.J. and Lusis, A.J. (1993). BSB: a new mouse model of multigenic obesity. Obesity Res. 1, 271-280.

Friedman, M.I. and Tordoff, M.G. (1986). Fatty acid oxidation and glucose utilization interact to control food intake in rats. Am. J. Physiol. 251, R840-R845.

Fuller, J.L. (1974). Single-locus control of saccharin preference in mice. J. Herid. 65, 33-36.

Geary, N. and Asarian, L. (1999). Cyclic estradidol treatment normalizes body weight and test meal size in ovariectomized rats. Physiol. Behav. 67, 141-147.

Gilbetson, T.A., Liu, L., Kim, I., Burks, C.A. and Hansen, D.R. (2005). Fatty acid responses in taste cells from obesity-prone and –resistant rats. Physiology & Behavior, 86, 681-690.

Gilbertson, T.A., Liu, L., York, D.A. and Bray, G.A. (1998). Dietary Fat Preferences Are Inversely Correlated with Peripheral Gustatory Fatty Acid Sensitivity. Annals of the New York Academy of Sciences 855, 165-168.

Harder, D.B. and Whitney, G. (1998). A common polygenic basis for quinine and PROP avoidance in mice. Chem. Senses 23, 327-332.

Harder, D.B., Azen, E.A. and Whitney, G. (2000). Sucrose octaacetate avoidance in nontaster mice is not enhanced by two type-A Prp transgenes from taster mice. Chem. Senses 25, 39-45.

Harder, D.B., Capeless, C.G., Maggio, J.C., Boughter, J.D., Gannon, K.S., Whitney, G. and Azen, E.A. (1992). Intermediate sucrose octa-acetate sensitivity suggests a third allele at mouse bitter taste loci Soa and Soa-Rua identity. Chem. Senses 17, 391-401.

Harder, D.B., Maggio, J.C. and Whitney, G. (1989). Assessing gustatory detection capabilities using preference procedures. Chem. Senses 14, 547-564.

Hegmann, J.P. and Possidente, B. (1981). Estimating genetic correlations from inbred strains. Behav. Genet. 11,103-114.

Higgs, S. and Cooper, S.J. (1998a). Effects of benzodiazepine receptor ligands on the ingestion of sucrose, Intralipid and maltodextrin: an investigation using a microstructural analysis of licking behavior in a brief contact test. Behav. Neurosci. 112, 447-457.

Higgs, S. and Cooper, S.J. (1998b). Evidence for early opioid modulation of licking responses to sucrose and Intralipid: a microstructural analysis in the rat. Psychopharmacol. 139, 342-355.

Hnasko, T.S., Szczpka, M.S., Alaynick, W.A., During, M.J. and Palmiter, R.D. (2004). A role for dopamine in feeding responses produced by orexigenic agents. Brain Res. 1023, 309-318.

Hudson, B. and Ritter, S. (2004). Hindbrain catecholamine neurons mediate consummatory responses to glucoprivation. Physiol. Behav. 82, 241-250.

Inoue, M., Beauchamp, G.K. and Bachmanov, A.A. (2004a). Gustatory neural responses to umami taste stimuli in C57BL/6ByJ and 129P3/J mice. Chem. Senses 29, 789-795.

Inoue, M., Li, X., McCaughey, S.A., Beauchamp, G.K. and Bachmanov, A.A. (2001). Soa genotype selectively affects mouse gustatory neural responses to sucrose octaacetate. Physiol. Genomics 5, 181-186.

Inoue, M., Reed, D.R., Li, X., Tordoff, M.G., Beauchamp, G.K. and Bachmanov, A.A. (2004b). Allelic variation of the TasIr3 taste receptor gene selectively affects behavioral and neural taste responses to sweeteners in the F_2 hybrids between C57BL/6JByJ and 129P3/J mice. J. Neurosci. 24, 2296-2303.

Kanarek, R.B. and Mayer, J. (1978). 2-deoxy-D-glucose induced feeding: relation to diet palatability. Pharmacol. Biochem. Behav. 8, 615-617.

Langhans, W. and Scharrer, E. (1987). Evidence for a vagally mediated satiety signal derived from hepatic fatty acid oxidation. J. Auton. Nerv. Syst. 18,13-18.

Lariviere W.R. and Chesler E.J., Mogil J.S. (2001). Transgenic studies of pain and analgesia: mutation or background genotype? J Pharmacol Exp Ther. 297, 467-473.

Laugerette1, F., Passilly-Degrace, P., Patris, B., Niot, I., Febbraio, M., Montmayeur, J.P., and Besnard, P. (2005). CD36 involvement in orosensory detection of dietary lipids, spontaneous fat preference, and digestive secretions. Journal of Clinical Investigation 11, 3177-3184**.**

Laugerette F, Gaillard D, Passilly-Degrace P, Niot I, and Besnard P. (2007). Do we taste fat? Biochimie 89, 265-269.

Leibowitz, S.F., Alexander, J., Dourmashkin, J.T., Hill, J.O., Gayles, E.C. and Chang, G.Q. (2005). Phenotypic profile of SWR/J and A/J mice compared to control strains: possible mechanisms underlying resistance to obesity on a high-fat diet. Brain Res. 1047, 137-147.

Levin, B.E. and Sullivan, A.C. (1987).Glucose-induced norepinephrine levels and obesity resistance. Am. J. Physiol. 253, R475-R481.

Lewis, S.R., Ahmed, S., Dym, C., Khaimova, E., Kest, B., and Bodnar, R.J. (2005). Inbred mouse strain survey of sucrose intake. Physiol. Behav. 85, 546-556.

Lewis, S.R., Dym, C., Ginzberg, M., Kest, B. and Bodnar, R.J. (2006a). Genetic variance contributes to ingestive processes: a survey of mercaptoacetate-induced feeding in eleven inbred and one outbred mouse strains. Physiol. Behav., 88, 516-522.

Lewis, S.R., Khaimova, E., Israel, Y., Singh, A., Kandov, Y., Kest, B. and Bodnar, R.J. (2006b). Genetic variance contributes to ingestive processes: a survey of 2-deoxy-D-glucose-induced feeding in eleven inbred mouse strains. Physiol. Behav. 87, 595-601.

Lewis, S.R.., Dym, C., Chai, C., Singh, A., Kest, B., and Bodnar, R.J. (2007). Genetic variance contributes to ingestive processes: a survey of eleven inbred mouse strains for fat (Intralipid) intake. Physiol. Behav., 90, 82-94.

Lowy, M.T. and Yim, G.K. (1982). Drinking, but not feeding, is opiate-sensitive in hamsters. Life Sci. 30,1639-1644.

Mattes, R.D. (2005). Fat Taste and Lipid Metabolism in Humans. Physiology & Behavior, 86, 691-697.

Lush, I.E. (1981). The genetics of tasting in mice. I. Sucrose octaacetate. Genet. Res. 38, 93-95.

Lush, I.E. (1982). The genetics of tasting in mice. II. Strychnine. Chem. Senses 7, 93-98.

Lush, I.E. (1984). The genetics of tasting in mice. III. Quinine. Genet. Res. 44, 151-160.

Lush, I.E. (1989). The genetics of tasting in mice. VI. Saccharin, acesulfame, dulcin and sucrose. Genet. Res. 53, 95-99.

Lush, I.E., Hornigold, N., King, P. and Stoye, J.P. (1995). The genetics of tasting in mice. VII. Glycine revisited, and the chromosomal location of Sac and Soa. Genet. Res. Camb. 66, 167-174.

Miller, C.C., Martin, R.J., Whitney, M.L. and Edwards, G.L. (2002). Intracerebroventricular injection of fructose stimulates feeding in rats. Nutr. Neurosci. 5, 359-362.

Miselis, R.R. and Epstein, A.N. (1975). Feeding induced by intracerebroventricular 2-deoxy-D-glucose in the rat. Am. J. Physiol. 229, R1438-R1447.

Mogil, J.S. and Grisel, J.E. (1998). Transgenic studies of pain. Pain 77, 107-128.

Murname, J.M. and Ritter, S. (1985a). Alloxan-induced glucoprivic feeding deficits are blocked by D-glucose and amygdalin. Pharmacol. Biochem. Behav. 22, 407-413.

Murname, J.M. and Ritter, S. (1985b). Intraventricular alloxan impairs feeding to both central and systemic glucoprivation. Physiol. Behav. 34, 609-613.

Nachman, M. (1959). The inheritance of saccharin preference. J. Comp. Physiol. Psychol. 52, 451-457.

Nelson, G., Hoon, M.A., Chandrashekar, J., Zhang, Y., Ryba, N.J.P., and Zuker, C.S. (2001). Mammalian sweet taste receptors. Cell, 106, 381-390.

Nie, Y., Vigues, S., Hobbs, J.R., Conn, G.L. and Munger, S. D. (2005). Distinct contributions of T1R2 and T1R3 taste receptor subunits to the detection of sweet stimuli. Current Biology, 15, 1948-1952.

Nisoli, E., Garosi, V., Blundell, J.E., and Carruba, M.O. (1996). Salbutamol antagonizes insulin- and sodium mercaptoacetate-induced but not 2-deoxy-D-glucose-induced hyperphagia. Pharmacol. Biochem. Behav. 54, 409-413.

Nizielski, S.E., Morley, J.E., Bartness, T.J., Seal, U.S. and Levine, A.S. (1986). Effects of manipulations of glucoregulation on feeding in the ground squirrel. Physiol. Behav. 36, 53-58.

Nonavinakere, V.K. and Ritter, R.C. (1983). Feeding elicited by 2-deoxy-D-glucose occurs in the absence of reduced glucose oxidation. Appetite 4, 177-185. Nutr. Rev. 61, S5-S9.

Pelz, W.E., Whitney, G. and Smith, J.C. (1973). Genetic influences on saccharin preference in mice. Physiol. Behav. 10, 263-265.

Penicaud, L., Thompson, D.A., and LeMagnen, J. (1986). Effects of 2-deoxy-D-glucose on food and water intake and body temperature in rats. Physiol. Behav. 36, 431-435.

Pothion, S., Bizot, J. C., Trovero, F. and Belzung, C. (2004). Strain differences in sucrose preference and in the consequences of unpredictable chronic mild stress. Behav. Brain Res. 155, 135-146.

Ragnauth, A., Schuller, A., Morgan, M., Chan, J.,Ogawa, S., Pintar, J.; Bodnar, R.J. and Pfaff, D.W. (2001). Female preproenkephalin-knockout mice display altered emotional responses. Proc. Natl. Acad. Sci (USA) 98, 1958-1963.

Ramirez, I. and Fuller, J.L. (1976). Genetic influence on water and sweetened water consumption in mice. Physiol. Behav. 16, 163-168, 1976.

Reed, D.R., Bachmanov, A.A., Beauchamp, G.K., Tordoff, M.G. and Price, R.A. (1997). Heritable variation in food preferences and their contribution to obesity. Behav. Genet. 27, 373-387.

Reed, D.R., Li, S., Li, X., Huang, L., Tordoff, M.G., Starling-Roney, R., Taniguchi, K., West, D.B., Ohmen, J.D., Beauchamp, G.K. and Bachmanov, A.A. (2004). Polymorphisms in the taste receptor gene (Tas1r3) region are associated with saccharin preference in 30 mouse strains. J. Neurosci. 24, 938-946.

Reed, D.R., Li, X., McDaniel, A.H., Lu, K., Li, S., Tordoff, M.G., Price, R.A. and Bachmanov, A.A. (2003). Loci on chromosomes 2, 4, 9 and 16 for body weight, body length, and adiposity identified in a genome scan of an F_2 intercross between the 129P3/J and C57BL/6ByJ mouse strains. Mamm. Genome 14, 302-313.

Rhodes, J.S., Best, K., Belknap, J.K., Finn, D.A. and Crabbe, J.C. (2005). Evaluation of a simple model of ethanol drinking to intoxication in C57BL/6J mice. Physiol. Behav. 84, 53-63.

Ritter, R.C. and Balch, O.K. (1978). Feeding in response to insulin but not to 2-deoxy-D-glucose in the hamster. Am. J. Physiol. 234, E20-E24.

Ritter, R.C., Roelke, M. and Neville, M. (1978a). Glucoprivic feeding behavior in absence of other signs of glucoprivation. Am. J. Physiol. 234, E617-E621.

Ritter, S., Bugarith, K., and Dinh, T.T. (2001). Immunotoxic destruction of distinct catecholamine subgroups produces selective impairment of glucoregulatory responses and neuronal activation. J. Comp. Neurol. 432, 197-216.

Ritter, S. and Dinh, T.T. (1994). 2-mercaptoacetate and 2-deoxy-D-glucose induce fos-like immunoreactivity in rat brain. Brain Res. 641, 111-120.

Ritter, S. and Hutton, R. (1995). Mercaptoacetate-induced feeding is impaired by central nucleus of the amygdala lesions. Physiol. Behav. 58, 1215-1220.

Ritter, S., Llewellyn-Smith, I. and Dinh, T.T. (1998). Subgroups of hindbrain catecholamine neurons are selectively activated by 2-deoxy-D-glucose induced metabolic challenge. Brain Res. 805, 41-54.

Ritter, S., Murname, J.M., and Ladenheim, E.E. (1982). Glucoprivic feeding is impaired by lateral or fourth ventricular alloxan injection. Am. J. Physiol. 243, R312-R317.

Ritter, S., Pelzer, N.L. and Ritter, R.C. (1978b). Absence of glucoprivic feeding after stress suggest impairmant of noradrenergic neuron function. Brain Res. 149, 399-411.

Ritter, S., Ritter, J.B. and Cromer, L. (1999). 2-deoxy-D-glucose and mercaptoacetate induce different patterns of macronutrient ingestion. Physiol. Behav. 66, 709-715.

Ritter, S., Scheurink, A. and Singer, L.K. (1995). 2-deoxy-D-glucose but not 2-mercaptoacetate increases Fos-like immunoreactivity in adrenal medulla and sympathetic preganglionic neurons. Obes. Res. 3, 729S-734S.

Ritter, S. and Taylor, J.S. (1989). Capsaicin abolishes lipoprivic but not glucoprivic feeding in rats. Am. J. Physiol. 256, R1232-R1239.

Ritter, S. and Taylor, J.S. (1990). Vagal sensory neurons are required for lipoprivic but not glucoprivic feeding in rats. Am. J. Physiol. 258, R1395-R1401.

Rowland, N.E. (1983). Physiological and behavioral responses to glucoprivation in the golden hamster. Physiol. Behav. 30, 743-747, 1983.

Rowland, N.E. (1992). Effects of glucose and fat antimetabolites on norepinephrine turnover in rat hypothalamus and brainstem. Brain Res. 595, 291-294.

Rowland, N.E., Bellush, L.L. and Carlton, J. (1985a). Metabolic and neurochemical correlates of glucoprivic feeding. Brain Res. Bull. 14, 617-624.

Rowland, N.E., Watkins, L. and Carlton, J. (1985b). Failure of 2-deoxy-D-glucose to stimulate feeding in deermice. Physiol. Behav. 34, 155-157.

Salter, D., and Watts, A.G. (2002). Differential suppression of hyperglycemic, feeding and neuroendocrine responses in anorexia. Am. J. Physiol. 284, R174-R182.

Sanders, N.M., Dunn-Meynell, A.A. and Levin, B.E. (2004). Third ventricular alloxan reversibly impairs glucose counterregulatory responses. Diabetes 53, 1230-1236.

Sanders, N.M., and Ritter, S. (2000). Repeated 2-deoxy-D-glucose-induced glucoprivation attenuates expression and glucoregulatory responses during subsequent glucoprivation. Diabetes 49, 1865-1874.

Sanders, N.M. and Ritter, S. (2001). Acute 2DG-induced glucoprivation or dexamethasone abolishes induced glucoregulatory responses to subsequent glucoprivation. Diabetes 50, 2831-2836.

Scharrer, E. and Langhans, W. (1986). Control of food intake by fatty acid oxidation. Am. J. Physiol. 250, R1003-R1006.

Scheepers, A., Joost, H.G. and Schurmann A. (2004). The glucose transporter families SGLT and GLUT: molecular basis of normal and aberrant function. J. Parenteral and Enteral Nutr. 28, 364-371.

Schemmel, R., Mickelson, O. and Gill, J.L. (1970). Dietary obesity in rats: body weight and body fat accretion in seven strains of rat. J. Nutr. 100, 1041-1048.

Scheurink, A. and Ritter, S. (1993). Sympathoadrenal responses to glucoprivation and lipoprivation in rats. Physiol. Behav. 53, 995-1000.

Sclafani, A. (2006a). Enhanced sucrose and Polycose preference in sweet "sensitive" (C57BL/6J) and "subsensitive" (129P3/J) mice after experience with these saccharides. Physiol. Behav. 87, 745-756.

Sclafani, A. (2006b). Oral, post-oral and genetic interactions in sweet appetite. Physiol Behav. 89, 525-30.

Sclafani, A. (2006c). Sucrose motivation in sweet "sensitive" (C57BL/6J) and "subsensitive" (129P3/J) mice measured by progressive ratio licking. Physiol. Behav. 87, 734-744.

Sclafani, A. (2007). Fat and sugar flavor preference and acceptance in C57BL/6J and mice: experience attenuates strain differences. Physiol. Behav. 90, 602-611.

Sclafani, A. and Glendinning, J.I. (2005). Sugar and fat conditioned flavor preferences in C57BL/6J and 129 mice: oral and postoral interactions. Am. J. Physiol. 289, R712-R720.

Sindelar, D.K., Ste Marie, L., Miura, G.I., Palmiter, R.D., McMinn, J.E., Morton, R. and Schwartz, M.W. (2004). Neuropeptide Y is required for hyperphagic feeding in response to neuroglucopenia. Endocrinol. 145, 3363-3368.

Singer, L.K. and Ritter, S. (1994). Differential effects of infused nutrients on 2-deoxy-D-glucose- and 2-mercaptoacetate-induced feeding. Physiol. Behav. 56, 193-196.

Singer, L.K. and Ritter, S. (1996). Intraventricular glucose blocks feeding induced by 2-deoxy-D-glucose but not mercaptoacetate. Physiol. Behav. 59, 921-923.

Singer, L.K., York, D.A. and Bray, G.A. (1997). Feeding responses to mercaptoacetate in Osborne-Mendel and S5B/PL rats. Obes. Res. 5, 587-594.

Singer, L.K., York, D.A. and Bray, G.A. (1998). Macronutrient selection following 2-deoxy-D-glucose and mercaptoacetate administration in rats. Physiol. Behav. 65, 115-121.

Smith, B.K., Andrews, P.K. and West, D.B. (2000). Macronutrient diet selection in thirteen mouse strains. Am. J. Physiol. 278, R797-R805.

Smith, B.K., Andrews, P.K., York, D.A. and West, D.B. (1999). Divergence in proportional fat intake in AKR/J and SWR/J mice endures across diet paradigms. Am. J. Physiol. 277, R776-R785.

Smith, B.K., Volaufova, J. and West, D.B. (2001). Increased flavor preference and lick activity for sucrose and corn oil in SWR/J vs. AKR/J mice. Am. J. Physiol. 281, R596-R606.

Smith, G.P. and Epstein, A.N. (1969). Increased feeding response to decreased glucose utilization in the rat and monkey. Am. J. Physiol. 217, R1083-R1087.

Smith, G.P. and Root, A.W. (1969). Effect of feeding on hormonal responses to 2-deoxy-D-glucose in conscious monkeys. Endocrinol. 85, 963-966.

Smith-Richards, B.K., Andrews, P.K., York, D.A. and West, D.B. (1999). Divergence in proportional fat intake in AKR/J and SWR/J mice endures across diet paradigms. Am. J. Physiol. 277, R776-R785.

Smith-Richards, B.K., Belton, B.N., Poole, A.C., Mancuso, J.J., Churchill, G.A., Li, R., Volaufova, J., Zuberi, A. and York, B. (2002). QTL analysis of self-selected macronutrient diet intake: fat, carbohydrate and total kilocalories. Physiol. Genomics 11, 205-217.

Ste Marie, L. and Palmiter, R.D. (2003). Norepinephrine and epinephrine-deficient mice are hyperinsulinimic and have lower blood glucose. Endocrinol. 144, 4427-4432.

Stockton, M.D. and Whitney, G. (1974). Effects of genotype, sugar and concentration on sugar preference of laboratory mice (Mus musculus). J. Comp. Physiol. Psychol. 86, 62-68.

Stricker, E.M. (1990). Handbook of behavioral neurobiology: neurobiology of food and fluid intake. Volume 10, Plenum: New York, NY.

Thompson, C.I., Fleming, R.L., Franken, K.A., Hornback, D.A. and Boha, S.P. (1989). Dual 24-hour feeding response to 2DG in rats: daytime increase and nighttime decrease. Physiol. Behav. 45, 155-161.

Thompson, D.A. and Campbell, R.G. (1977). Hunger in humans induced by 2-deoxy-D-glucose: glucoprivic control of taste preference and food intake. Science 198, 1065-1067.

Tordoff, M.G. and Bachmanov, A.A. (2002). Influence of test duration on the sensitivity of the two-bottle choice test. Chem. Senses 27, 759-768.

Tordoff, M.G. and Bachmanov, A.A. (2003b). Influence of the number of alcohol and water bottles on murine alcohol intake. Alcohol Clin. Exp. Res. 27, 600-606.

Tordoff, M.G., Pilchak, D.M., Williams, J.A., McDaniel, A.H. and Bachmanov, A.A. (2002). The maintenance diets of C57BL/6JJ and 129X1/SvJ mice influence their taste solution preferences: implications for large-scale phenotyping projects. J. Nutr. 132, 2288-2297.

Torfdoff, M.G. and Bachmanov, A.A. (2003a). Mouse taste preference tests: why only two bottles? Chem. Senses 28, 315-324.

Tsujii, S. and Bray, G.A. (1990). Effects of glucose, 2-deoxyglucose, phlorizin and insulin on food intake of lean and fatty rats. Am. J. Physiol. 258, E476-E481.

VanderWheele, D.A., Dess, N.K. and Castonguay, T.W. (2002). Ingestional responses to metabolic challenges in rats selectively bred for high and low saccharin intake. Physiol. Behav. 75, 97-104.

Villanueva, I., Racotta, I.S. and Racotta, R. (1996). Glucoprivation attenuates the hypophagia induced by epinephrine in mice. Physiol. Behav. 60, 1383-1386.

Warden, C.H., Fisler, J.S., Shoemaker, S.M., Wen, P.Z., Svenson, K.L., Pace, M.J. and Lusis, A.J. (1995). Identification of four chromosomal loci determining obesity in a multifactorial mouse model. J. Clin. Invest. 95, 1545-1552.

Warren, R.P. and Lewis, R.C. (1970). Taste polymorphism in mice involving a bitter sugar derivative. Nature 227, 77-78.

Watson, P.J. and Biderman, M.D. (1982). Failure of rats deprived of water to increase food intake during glucoprivation induced by 2-deoxy-D-glucose. Pharmacol. Biochem. Behav. 17, 955-959.

Watson, P.J., Rhodes, D.H., Rhea, K., McKinney, J., Hawkins, C. and Bartles, R.R. (1996). Intake of a liquid diet after 2-deoxy-D-glucose injections in rats. Pharmacol. Biochem. Behav. 25, 1153-1158.

West, D.B. and York, B. (1998). Dietary fat, genetic predisposition, and obesity: lessons from animal models. Am. J. Clin. Nutr. 67, 505S-512S.

West, D.B., Boozer, C.N., Moody, D.L. and Atkinson, R.L. (1992). Obesity induced by a high-fat diet in nine strains of inbred mice. Am. J. Physiol. 262, R1025-R1032.

West, D.B., Goudey-Lefevre, J., York, B. and Truett, G.E. (1994a). Dietary obesity linked to genetic loci on chromosomes 9 and 15 in a polygenic mouse model. J. Clin. Invest. 94, 1410-1416.

West, D.B., Waguespack, J. and McCollister, S. (1995). Dietary obesity in the mouse: interaction of strain with diet composition. Am. J. Physiol. 268, R658-R665.

West, D.B., Waguespack, J., York, B., Goudey-Lefevre, J. and Price, G.A. (1994b). Genetics of dietary obesity in AKR/J x SWR/J mice: segregation of the trait and identification of a linked locus on chromosome 4. Mamm. Genome 5, 546-552.

Wick, A.N., Drury, D.R., Nakada, H.I. and Wolfe, J.B. (1957). Localization of the primary metabolic block produced by 2-deoxy-D-glucose. J. Biol. Chem. 224, 963-969.

Woods, S.C. and McKay, L.D. (1978). Intraventricular alloxan eliminates feeding elicited by 2-deoxy-D-glucose. Science 202, 1209-1211.

Yamamoto, T. (2003). Brain mechanisms of sweetness and palatability of sugars. Nutr. Rev. 61, S5-9.

Zhao, G., Zhang, Y., Hoon, M., Chandrashekar, J., Erlenbach, I., Ryba, N. and Zuker, C. (2003). The receptors for mammalian sweet and umami Taste. Cell, 115, 255-266.