One size does not fit all: Assessing the impact of genetic background and diet on obesity and hepatic gene expression in the Collaborative Cross

By

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For my mother,

who always did the best with what she had

For my grandmother and her late husband,

who made our lives here possible

For Uncle Alvin,

whose kindness I will always treasure

and

In memory of Soulmate A,

my dearest and truest friend

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Abstract

Obesity is a complex disease characterized by excessive fat accumulation that leads to decrease in health and increased risk of developing numerous health complications such as metabolic syndrome, type 2 diabetes mellitus, cardiovascular diseases, and other pathological conditions. Fundamentally, obesity is a consequence of long-term energy imbalance where energy intake surpasses energy expenditure, but the mechanisms behind energy imbalance are influenced by numerous biological and environmental factors, such as genetics and diet. Both the complex etiology and heterogeneous nature of obesity present challenges to effective long-term prevention and treatment of obesity at the population level. For example, population-based diet recommendations have had limited success in mitigating obesity because of the variation in other factors that differ at the individual level to impact physiological response to diet and obesity development, such as differences in genetic background. Since genetics and diet are crucial determinants in the regulation of energy balance, it is necessary to broaden our understanding of how genetic background and diet interact relative to the development of obesity for improving recommendations for weight loss.

Animal models are indispensable for discerning the effect of genetic factors from environmental factors on the manifestation of the phenotype of interest. The murine model has been especially crucial for the discovery of mechanisms that influence energy balance and obesity development, such as appetite signaling. Of all available mouse models, the Collaborative Cross (CC) mouse panel is a particularly excellent model system for comparing the effects of genetic background to environmental effects. Derived from elaborate intercrosses of 5 classically inbred mouse strains and 3 wild-derived mouse strains, the CC is a large recombinant inbred mouse population with the degree of genetic and phenotypic diversity reflective of the human population. The CC simultaneously provides both tremendous genetic diversity and the ability to use genetic “replicates” which can mimic twin studies.

In this work, replicates from 22 CC strains were placed on either a high protein diet or high fat high sucrose diet challenge for eight weeks. Body composition and circulating analyte levels were assessed both at baseline before the diet challenge and post-diet to compare the impact of genetic background (strain) and diet on adiposity and clinical traits associated with metabolism. The second

chapter of this work focused on determining how much genetic background and diet contribute to the development of obesity, whether diet alters susceptibility to developing obesity, and whether differences in diet macronutrient composition result in more beneficial phenotypic outcomes. Both at baseline and post-diet, the CC exhibited a wide range of phenotypic variation for adiposity and circulating analytes by strain; after the diet challenge, phenotypic differences were much larger between strains than diet, suggesting that genetics play a much bigger role in the development of obesity than diet. Similar to the observation in humans, the individual CC strains responded differently to diet where certain strains gained weight on one diet or the other, while others stayed consistently lean or consistently fat regardless of diet, indicating that genetics largely determines whether an individual will become obese, but the effect of diet can be larger or smaller depending on specific genetics. When examining the effect of diet by itself, certain traits differed significantly by diet such as body weight and cholesterol levels, while others did not differ by diet such as adiposity and triglyceride levels, demonstrating that whether and how macronutrient composition influences phenotypic change depends on the trait. Surprisingly, when correlations were performed between adiposity and traditional markers of metabolic syndrome (such as circulating triglycerides, glucose, cholesterol, and insulin), only the correlation between insulin and adiposity stayed significant both before and after the diet challenge.

The third chapter of this work explored the relationship between genetics, diet, and hepatic gene expression relative to obesity since the liver regulates biological processes that impact adiposity accumulation, such as lipogenesis and metabolism of macronutrients. To relate the phenotypic results and findings from chapter two to hepatic gene expression, correlations were performed using phenotype data and microarray data, revealing 2,552 genes whose expression levels were significantly correlated with adiposity. In general, the effect of strain was much stronger than diet on hepatic gene expression as demonstrated by differential gene expression analysis which found over 9,000 genes differentially expressed by strain compared to 1,344 genes differentially expressed by diet. Interestingly, diet differentially expressed genes (DEGs) were enriched for many biological pathways associated with substrate metabolism, whereas strain DEGs were enriched for pathways less sensitive to environmental perturbations. Because common obesity is caused by multiple genes, weighted gene co-expression network analysis (WGCNA) was performed to identify clusters of related genes grouped into “modules”.

Multiple gene modules were found that differed in average expression by both diet and strain, where three of the gene modules were correlated with adiposity and enriched for biological pathways related to obesity development. By combining all the analyses above and searching in the genome-wide association studies (GWAS) catalog, the list of obesity candidate genes found via (GWAS) in humans can be narrowed down to increase the success of future functional validations studies.

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Chapter 1:
The Consequences and Causes of Obesity

Viewed as a detrimental global epidemic in the 21st century (NCD Risk Factor Collaboration [NCD-RisC], 2016; Williams et al., 2015), obesity is a serious disease defined by a disproportionately increased ratio of body weight to height and an excessive accumulation of body fat resulting in negative health outcomes (González-Muniesa et al., 2017; World Health Organization [WHO], 2021). Various methods have been developed to determine whether an individual is obese and to quantify the extent of obesity, such as assessments using anthropometry and techniques to measure body composition, including densitometry and imaging-based methods (Duren et al., 2008; Fosbøl & Zerahn, 2015; Heymsfield et al., 2015). To formally classify the status of obesity for adults, body mass index (BMI) is the most often used tool to estimate adiposity (Javed et al., 2015) based on the weight of the individual measured in kilograms (kg) divided by the square height in meters (m^2). Using BMI as the metric, the World Health Organization (WHO) classifies undernutrition as <18.5 kg/m^2, normal weight as 18.5–24.9 kg/m^2, overweight as 25–29.9 kg/m^2, obesity as ≥30 kg/m^2, and extreme obesity as ≥40 kg/m^2 (Després, 2012). The broad estimation of adiposity provided by measuring BMI can be further classified with the additional measurement of waist circumference and calculating waist-to-hip circumferences and waist-to-height ratios to differentiate between subcutaneous obesity and visceral obesity (Cerhan et al., 2014), which differ in physiological adipose deposition, function, and associated mortality (Ibrahim, 2010).

The benefits of indirect anthropometric methods of assessing obesity include their simplicity, low cost, non-invasive nature, universal standardization, and ease of practical execution, all of which make BMI the most commonly used tool for estimating obesity (Javed et al., 2015). Although indirect anthropometric estimations of obesity are incredibly useful, direct assessments of body composition that can quantify an individual's ratio of fat tissue to lean mass such as dual energy X-ray absorptiometry (DXA scans), computed tomography (CT scans), or magnetic resonance imaging (MRI) should be used in conjunction with anthropometric measurements when possible for the most accurate diagnosis of obesity, especially in a clinical setting to monitor the loss of weight attributed to fat compared to water when an individual is on a weight loss program to treat obesity (Duren et al., 2008).

Prevalence and Detrimental Effects of Obesity

The most recent global estimates of obesity in 2016 indicate that 39% of adults aged 18 years and over (39% of men and 40% of women) were overweight, while about 13% of the world's adult population (11% of men and 15% of women) were obese; in terms of a population count, more than 1.9 billion adults were overweight, of which over 650 million adults were obese (World Health Organization [WHO], 2021). Alarmingly, the worldwide prevalence of obesity nearly tripled between 1975 and 2016 (González-Muniesa et al., 2017; World Health Organization [WHO], 2021) with estimates predicting that 57.8% of the world's adult population (3.3 billion people) will be obese by 2030 (Finkelstein et al., 2012; Kelly et al., 2008). Increases in the prevalence of obesity have been observed in both developed and developing countries (Ng et al., 2014) with the rate of increase higher in developed countries than in developing countries (Apovian, 2016).

Given the high prevalence of obesity, public health efforts have been made over the past four decades to slow the increase of obesity at the population level. For example, the United States Department of Agriculture (USDA) and Health and Human Services (HHS) issued the first official version of dietary guidelines in 1980 describing principles for helping people make healthy food choices and providing guidance on limiting certain dietary components such as sugar, fat, cholesterol, and sodium, which were suspected of being risk factors for various chronic diseases; this document was subsequently revised about every five years by the Dietary Guidelines Advisory Committee based on reviewing updated findings in nutrition and health, and is now known as the Dietary Guidelines for Americans (DGA) (USDA, 2021). Similarly, the WHO has published several reports since 2004 detailing actions needed to support healthy diets and regular physical activity at the population level to control and reduce both adult and childhood obesity (World Health Organization [WHO], 2021). In the past decade, several countries around the world have implemented taxes on sugar-sweetened beverages and front-of-package nutrition labels to promote healthier food choices and discourage excessive consumption of calories, sugar, saturated fat, and sodium (Malik et al., 2013, 2020), which resulted in reductions in sales of sugar-sweetened beverages in the US and Mexico (Colchero et al., 2017; Silver et al., 2017) and reformulations of beverages to reduce sugar content in the United Kingdom (Bandy et al., 2020).

Despite these efforts, the prevalence rate for being overweight or obese increased 27.5% for adults and 47.1% for children between 1980 and 2013 for a total of 2.1 billion overweight or obese individuals worldwide (Ng et al., 2014) with BMI rising at the same rate or faster in rural areas as urban areas, especially in low- and middle-income countries (NCD Risk Factor Collaboration [NCD-RisC], 2019). These increases in the prevalence of obesity have resulted in a global health care cost equivalent of approximately $2 trillion US dollars, or 2.8% of the world's gross domestic product (Dobbs et al., 2014). In the United States alone, about $40 billion in increased medical spending is attributed to obesity (Finkelstein et al., 2009), as people with obesity have increased annual health care costs of 36% and medication costs of 77% compared to people of average weight (Sturm, 2002). Furthermore, health care costs related to obesity-associated diseases are forecasted to reach $48-66 billion per year by 2030 in the US (Y. C. Wang et al., 2011). Clearly, the high prevalence of obesity incurs substantial costs to the world at large and individuals alike.

In addition to bearing the increased financial burden of obesity, individuals with obesity often have adversely impacted physical and mental health, and overall decreased quality of life. Obesity has been well established as a risk factor for the development of a plethora of adverse pathological conditions such as certain types of cancer, cardiovascular diseases, type 2 diabetes mellitus, and metabolic syndrome (Alberti et al., 2009; González-Muniesa et al., 2017; Williams et al., 2015). Although a subset of individuals with obesity may be metabolically healthy (Dobson et al., 2015; Peppa et al., 2013; Schulze, 2019), they may still suffer from the negative influence of obesity on mental health, as obesity has been associated with an increased risk of depression, body image dissatisfaction, suicide ideation, and low self-esteem (Avila et al., 2015; Carpenter et al., 2000; C. Dong et al., 2006; R. Puhl & Suh, 2015; Sarwer & Polonsky, 2016). Furthermore, individuals with obesity may experience various forms of social stigmatism, including employment discrimination (e.g. being paid less than their non-obese counterparts for an equivalent job, being less likely to be hired or receive promotions), receiving reduced quality of health care due to explicit and implicit bias of medical professionals, and subjugation to shame or ridicule by peers – frequently spouses, friends, and family members (Fulton & Srinivasan, 2021; R. Puhl & Brownell, 2001; R. M. Puhl & Heuer, 2010; Schwartz et al., 2003; Seacat et al., 2016).

Prevention and Treatment of Obesity

Effective prevention and treatment of obesity requires interventions on multiple levels of the social continuum, from individual efforts to systemic changes in the community and public policy. With the currently available knowledge on the etiology of obesity and the limitations dictated by practicality and health care systems, health professionals encourage the implementation of low-risk treatments for weight management or weight loss at the individual level, including behavioral modifications, dietary improvements, and maintaining moderate levels of physical activity. As the “first line of defense”, recommendations for weight management or weight loss should account for multiple facets of energy balance. Lifestyle programs for effective weight loss or weight management that focus on behavior modification encourage individuals to monitor their eating behavior and educate individuals about the different factors that shape their eating behavior to improve their understanding of dietary patterns and make appropriate changes, such as eating type, location, speed, and other triggers of unhealthy eating (Burke et al., 2011; Robinson et al., 2014).

As the “calorie in” component of energy balance, employing dietary interventions to achieve negative energy balance through calorie restriction is advised for weight loss with either a restriction of 500 kcal per day or -30% energy restriction as the typical recommendation; alternative general dietary recommendations are diets with 1,200 kcal per day for women and 1,500 kcal per day for men (González-Muniesa et al., 2017). Any healthy diet can induce weight loss since differences in macronutrient composition do not favor any one diet over others at the population level (Gardner et al., 2018; Johnston et al., 2014), though the effects of differences in diet macronutrient composition at the individual level requires further elucidation; for example, a meta-analysis of many popular diets demonstrated a negligible difference in weight loss after 12 months between low-carbohydrate diets (-7.25 kg) and low-fat diets (-7.27 kg) (Johnston et al., 2014). Interestingly, improving the overall dietary quality of calories consumed without caloric restriction can also facilitate weight loss (e.g. consuming the Mediterranean diet) (Mozaffarian, 2016a, 2016b). Specifically, differences in macronutrient composition may facilitate maintenance of long-term weight loss (Abete et al., 2010); for example, diets higher in carbohydrates may result in weight gain, while low fat diets higher in protein and foods with low glycemic index may prevent regaining weight (Bray & Siri-Tarino, 2016; Larsen et al., 2010).

In terms of changes in weight from a population perspective, there is high inter-individual variability in response to diet, where some individuals lose weight while others actually gain weight on the same diet (Berry et al., 2020; Dansinger et al., 2005), which may be affected by genetics (Bray & Siri-Tarino, 2016). Because the more important determinant of weight loss is adherence to the chosen calorie-restricting weight loss diet more than differences in macronutrient composition alone (Dansinger et al., 2005), effective dietary recommendations for weight control should take into consideration the different individual factors that impact eating behaviors and long-term health, such as food preferences, clinical history, culture, lifestyle, and socioeconomic status. In the future, clinicians may be able to use a "precision nutrition" approach to improve dietary adherence in patients by integrating individualized genetic, nutrigenomic, phenotypical, and environmental information when making dietary recommendations for obesity management (Ferguson et al., 2016; Goni et al., 2015).

When enacted along with dietary intervention, regular physical activity has been shown to facilitate weight loss (T. Wu et al., 2009) and improve health independent of weight loss by lowering risk of developing diabetes mellitus and cardiovascular disease (WHO, 2010). The current recommendation for sedentary individuals is to gradually increase aerobic physical activity to reach a goal of >150 minutes per week, although 60-90 minutes of exercise per day may be required for long term weight maintenance (Donnelly et al., 2009; Jakicic, 2008; WHO, 2010). Because the response to any treatment may differ depending on the individual, clinicians may tailor their recommendations related to behavior modifications, dietary improvements, or physical activity according to whether the goal is to lose or maintain weight (Bray & Siri-Tarino, 2016).

For individuals with moderate- to high-risk obesity (BMI of >30 kg/m^2 or >27 kg/m^2 with comorbidities present) who have a history of struggling and failing to lose and maintain weight loss, clinicians may prescribe drugs to assist in weight loss in tandem with diet interventions and exercise programs (Apovian et al., 2015; Bray et al., 2016; Solas et al., 2016). Most medications used for treating individuals with obesity facilitate weight loss by helping patients adhere to their diets (usually via appetite suppression), including phentermine/topiramate, naltrexone/bupropion, and liraglutide, which are available and approved for use in the US only for adults who are not pregnant or nursing (Bray et al.,

2016). Similar to diet recommendations, there is no single ideal medication so far and any of these medications may help patients successfully lose weight. Because of the variation in individual response to specific compounds in each of these drugs and associated side effects, the efficacy of these medications may vary depending on the specific individual. The current recommendation is to stop the drug treatment and switch to an alternative approach if the patient fails to lose 4-5% of their body weight after three months; individual patients with obesity may be on medication that influences weight gain for treating other conditions such as diabetes or depression, which further necessitates using a personalized approach for prescribing weight loss medication (Apovian et al., 2015).

Advances in surgical interventions have led to the development of a wide range of lower risk laparoscopic procedures each with different degrees of weight loss, benefits, and risks (Courcoulas, 2013; Inge et al., 2016), which has increased the use of bariatric surgery to treat severe obesity recently, with nearly half a million procedures performed in 2013 (Bray et al., 2016). As criteria for determining patient eligibility to receive bariatric surgery as a treatment for severe obesity, “severe obesity” is defined as having either a BMI of >40 kg/m^2, a BMI of >35 kg/m^2 with comorbidities such as hypertension or dyslipidemia, or a BMI between 30 and 35 kg/m^2 with pre-diabetic symptoms or recent-onset diabetes (Schauer et al., 2014). Two examples of current bariatric surgical techniques are the abdominal gastric band and the sleeve gastrectomy (Naik et al., 2016). The abdominal gastric band results in less weight loss than some other procedures but is technically easier to implement since weight loss depends on the size of the gastric pouch determined by the circumference of the band placed below the gastro-esophageal junction. In the sleeve gastrectomy, the size of the patient’s stomach is reduced by placing a staple line “down” the curvature of the stomach, followed by excision of the closed segment; though technically more demanding than the abdominal gastric band, this method typically results in greater weight loss and is one of the most widely performed procedures. Bariatric surgery can generally produce important improvements in health (e.g. glucose control in patients with type 2 diabetes mellitus) and reduction in risk for myocardial infarction, cancer, and other comorbidities (Sjöström, 2013). Although bariatric surgery can potentially provide many benefits, there are also risks and potential adverse side effects that may be challenging to treat like dumping syndrome, gastro-esophageal reflux, and hypoglycemia. Furthermore, proper aftercare is crucial to the long-term success of the initial procedure,

which requires adhering to huge dietary changes to prevent weight regain and may include lifelong replacement therapy for vitamin or mineral deficiencies after malabsorptive operations (Chang et al., 2014).

Systemic changes in the community and public policies are necessary to support individuals in overcoming the barriers of obesity prevention and treatment established by socioeconomic status and other environmental influences. At the population level, public policies can be adjusted to improve food environments for encouraging individuals to ameliorate longstanding eating behavior, while improving physical environments would aid individuals in incorporating more physical activity into daily habits. Some examples of current efforts in various countries such as the United States, Mexico, United Kingdom, and Chile to prevent and treat obesity include the implementation of taxes on sugar-sweetened beverages, the inclusion of calorie labels on menus, and the addition of front-of-package nutrition labels to promote healthier food choices and discourage excessive consumption of calories, sugar, saturated fat, and sodium (Malik et al., 2013, 2020). Still, the prevention and treatment of obesity would benefit from more attention and financial support (Malik et al., 2013) to maintain and expand current efforts such as education programs that integrate public and personalized nutrition to support individuals in long-term adherence to behavior modifications that are part of healthy lifestyles (Enright et al., 2016; Kelley et al., 2016).

Etiology of Obesity

To effectively prevent and treat obesity, it is necessary to understand its etiology. The first law of thermodynamics provides the simplest explanation of how obesity occurs: excessive fat accumulation results from the disruption of energy balance, where an individual's caloric intake exceeds their energy expenditure (J. O. Hill et al., 2012; Oussaada et al., 2019; Romieu et al., 2017). According to this view, obesity results from overconsumption of food relative to energy expenditure, as even small daily positive energy balance contributes to weight gain (F. B. Hu, 2008). Thus, the seemingly obvious solution for obesity prevention and treatment would be to simply "eat less, move more." In reality, the implementation of such a simple solution is limited by a myriad of complex factors that dictate the mechanisms behind energy balance (or lack thereof), including socioeconomic status, culture, environment, personal behavior, physiology, and genetics, as well as the interactions between these factors (Batis et al., 2011;

C. G. Bell et al., 2005; Bhupathiraju & Hu, 2016; Leng et al., 2021; Sanchez-Vaznaugh et al., 2019; Sellayah et al., 2014; Shahnazaryan et al., 2019; Singh et al., 2017).

Part of the “eat less, move more” solution includes the encouragement to consume a “healthy” diet, since the type and quality of calories defined by macronutrient type (fat, carbohydrates, and protein) and the sources of these macronutrients has been shown to affect energy balance and long-term body weight (Ebbeling et al., 2012; Ludwig, 2016; Mozaffarian et al., 2011). Although it is unclear whether the impacts of dietary distribution and thermogenic effects of specific macronutrients affect overeating or have direct metabolic effects to influence body weight (J. A. Martinez et al., 2014), studies have shown associations between higher weight gain and increased consumption of “unhealthy” foods such as sugar-sweetened beverages, potato chips, French fries, trans-fats, processed meats, ultra-processed foods, and added sugars (González-Morales et al., 2020; Juul et al., 2018; Mozaffarian et al., 2011), which are calorically dense and alter insulin/satiety signaling if consumed in excess. Conversely, a high-quality dietary pattern with increased consumption of fruits, nuts, vegetables, and whole grains has been shown to be inversely associated with weight gain (Razquin et al., 2009; Schwingshackl et al., 2015), while several longitudinal studies echo the importance and benefits of consuming meals comprised of these “healthy” foods and complex carbohydrates with a low glycemic index for the prevention obesity (Mozaffarian, 2016b; J. D. Smith et al., 2015; Tobias et al., 2015). In fact, the current DGA recommends implementing these findings in practice, essentially to consume just enough calories according to one’s caloric need in the form of nutrient-dense food that have little or no added sugars, saturated fat, and sodium while providing vitamins, minerals, and other health-promoting components (USDA and HHS, 2020).

Despite the simplicity behind the ideas of “eat less, move more” and “eat a healthy diet”, numerous elements associated with socioeconomic status, culture, environment, physiology, and genetics that shape personal behaviors of food choice, overeating, and reduced physical activity prevent the practice of these tenets. From an individual’s perspective, genetic and biological factors that influence food choice such as taste perception (Callaway, 2012; L. Eriksson et al., 2019; Knaapila et al., 2012; Risso et al., 2018; Roudnitzky et al., 2015) are often further driven by: preferences in personal tastes –

food choices are often made depending on taste perception (Duffy et al., 2010; Feeney et al., 2011; Graham et al., 2021; J. L. Smith et al., 2020); convenience – both ease of food acquisition in the local environment and ease of food preparation in the context of accommodating work schedules are important determinants of healthy eating patterns (Bell et al., 2021; Lima et al., 2021; Martinho et al., 2021; Nicholls et al., 2017); and cost – higher quality nutrient-dense food generally costs more than the "unhealthy" food (Aggarwal et al., 2011; Darmon & Drewnowski, 2015; Drewnowski, 2018; Monsivais et al., 2012). Essentially, the choice to consume unhealthy foods over healthy foods is often a result of increased accessibility, affordability, and availability to unhealthy foods over healthy foods (Matsuzaki et al., 2020; Mylona et al., 2020; Sanchez-Vaznaugh et al., 2019). Furthermore, changes in lifestyle from the past to the present have fostered habits that are often associated with overeating, such as: watching television while eating which was shown to extend time eating (Alblas et al., 2021; Kegler et al., 2021; Mougharbel et al., 2020); stress which can lead to emotional eating as a coping mechanism (Caner & Evgin, 2021; D. C. Hill et al., 2018; Richardson et al., 2015), and increased frequency of eating away from home (Bes-Rastrollo et al., 2010; Bhutani et al., 2018; H. J. Kim et al., 2019; Nago et al., 2014).

Environmental factors especially can discourage physical activity, including perceived and objective lack of safety in the built environment (e.g. neighborhood of residence) or decreased proximity to safe spaces for exercise (Elshahat et al., 2020; Jáuregui et al., 2016; Rees-Punia et al., 2018) and excessive screen time, e.g. playing video games or using the internet (Islam et al., 2020; Matthews et al., 2021). Conversely, environment can also increase physical activity; for example, availability of healthy transportation options such as public transit or bike sharing programs can facilitate the incorporation of physical activity during daily transportation time (Centers for Disease Control and Prevention (CDC), 2018; L.-T. Chen & Hsu, 2020; Koehler et al., 2018; Saelens et al., 2014). Ultimately, one of the biggest challenges in preventing and treating obesity is the long-term adherence to overall healthy lifestyle changes and habits that result in an energy deficit for weight loss, or at least energy balance for the prevention of weight gain.

Heterogeneous Phenotypes of Obesity

Exploring the biology behind the etiology of obesity illustrates the complexity of its development, both in terms of the heterogeneity in phenotypes as well as variety of underlying causes. Numerous studies have

clearly demonstrated the increased risk of individuals with obesity developing related common comorbidities that contribute to premature death compared to normal-weight individuals as the extent of obesity increases, including endocrine disorders, type 2 diabetes mellitus, respiratory problems such as sleep apnea, cardiovascular diseases, cancer, and features of metabolic syndrome (Cornier et al., 2011; Nordestgaard et al., 2012; Park et al., 2013; Renehan et al., 2010), but the degree of disease risk can differ widely among individuals with obesity. The International Diabetes Federation, the American Heart Association, and the US National Heart, Lung, and Blood Institute define the diagnosis of metabolic syndrome as the simultaneous occurrence of three of five clinical criteria in a patient (Alberti et al., 2009): waist circumference of ≥94 cm in men and ≥80 cm in women in Western countries as an estimate of visceral obesity (thresholds vary by country); hypertriglyceridemia (≥150 mg/dl or on triglyceride-lowering medication); low levels of high-density lipoprotein (HDL) cholesterol (<40 mg/dl for men, <50 mg/dl for women); elevated blood pressure (systolic blood pressure of ≥130 mmHG, diastolic blood pressure of ≥85 mmHg, or on an antihypertensive drug treatment); increased glucose levels (fasting glucose levels of ≥100 mg/dl or on glucose-lowering medication). In the context of these clinical criteria, obesity phenotypes differ by severity of cardiometabolic health impairment based on how many of these risk factors occur concurrently in an individual with obesity, where individuals with few or none of these conditions have metabolically healthy obesity (MHO) (Stefan et al., 2013; Teixeira et al., 2015) and individuals afflicted with many of these conditions have metabolically unhealthy obesity (MUHO). Although individuals with MHO have increased fat mass compared to normal-weight individuals, they may not necessarily be at an increased risk of developing cardiovascular complications (Schulze, 2019), since the magnitude of myocardial dysfunction appears to be more correlated with poor metabolic health than just BMI or fat mass alone (Dobson et al., 2015). Yet the metabolically healthy obese phenotype may not be entirely benign since it tends to be a transient state (Stefan et al., 2013), where higher baseline BMI or waist circumference and extended duration of obesity are related to the conversion from MHO to MUHO (Achilike et al., 2015; Appleton et al., 2013; Mongraw-Chaffin et al., 2016; Moussa et al., 2019); thus, it may only be a matter of time until cardiometabolic complications arise.

Because cardiovascular disease risk increases with decline in metabolic health, a subgroup of individuals may be metabolically unhealthy and at high risk of developing cardiometabolic complications

despite belonging to the normal weight BMI category (18.5-24.9 kg/m^2) i.e. metabolically unhealthy normal-weight (MUHNW) (Schulze, 2019). For instance, metabolically “unhealthy” phenotypes were characterized by higher levels of insulin resistance biomarkers, hepatic steatosis and inflammation, and higher indices of central (abdominal) adiposity, but not total adiposity in both obese and nonobese postmenopausal women (Peppa et al., 2013). This highlights the importance of using additional methods to quantify obesity in conjunction with BMI to accurately assess cardiovascular disease risk in a clinical setting. To overcome the limitations of only using BMI to appraise disease risk, clinicians also utilize other tools including obesity staging systems that take into account estimations of metabolic health (e.g. blood pressure, dyslipidemia, and glucose homeostasis) and physiological fat distribution, such as waist-to-hip ratio derived from measuring waist circumference to quantify body shape, the Framingham risk score, the Cardiometabolic Sating System, and the Edmonton Obesity Staging System (B. Dong et al., 2015; F. Guo et al., 2014; Savva et al., 2013).

In addition to the degree of excessive adiposity or fat accumulation, the regional distribution of fat deposition also confers differences in the degree of the manifestation of associated health risks and their severity (González-Muniesa et al., 2017; Hiuge-Shimizu et al., 2012). Across populations, there is high heterogeneity in terms of the ways that individuals with obesity accumulate body fat, with subcutaneous obesity on one end of the spectrum and visceral obesity on the other. Characterized by excess fat deposition under the skin around the hip and thigh areas, subcutaneous obesity typically results in a pear-like body shape and is also known as gynoid obesity, which tends to be more common in pre-menopausal women (Abildgaard et al., 2021; Pan & Chen, 2021). On the other hand, visceral obesity is characterized by the deposition of mainly mesenteric adipose tissue concentrated in the abdominal region, typically resulting in an apple-like body shape. Also known as android obesity, visceral obesity tends to be more common in men and more harmful to cardiovascular health than subcutaneous obesity (Hernández-Conde et al., 2019; Hiuge-Shimizu et al., 2012; Pan & Chen, 2021; Ruiz-Castell et al., 2021).

Remarkably, differences in the region of fat accumulation are associated with differences in the pathological effects of adipose tissue, where the presence of additional inner fat deposits (e.g. visceral adipose tissue stored in the abdomen) and stores of adipose tissue in and around normally lean tissues

such as the heart, liver, and kidneys (also known as ectopic fat deposition) (Després & Lemieux, 2006; Rosen & Spiegelman, 2014; Shulman, 2014) influences an individual's cardiometabolic risk profile, insulin sensitivity, and dyslipidemia due to dysregulated oxygen tension from pathological adipocyte growth and the way adipose tissue manages the excess of energy (González-Muniesa et al., 2016). In fact, these differences in adipose tissue function are so profound that adipose tissue can be broadly classified based on their location in the body, where the two main types are subcutaneous adipose tissue located in the hypodermis just under the skin and visceral adipose tissue located in the abdominal cavity packed between organs (Marieb & Hoehn, 2008); visceral adipose tissue can be further categorized as intra-abdominal (mesenteric), perirenal, or pericardial adipose tissue. Imaging-based methods for measuring body composition such as CT or MRI scans are used to estimate the amount of abdominal fat stored in different physiological compartments and to distinguish abdominal fat from ectopic fat (Després & Lemieux, 2006; Rosito et al., 2008; Thanassoulis et al., 2010; Thomas et al., 2012), but these methods are usually limited to laboratory settings and not typically used to diagnose obesity (Heymsfield et al., 2015; Seabolt et al., 2015).

Given the clear correlations between visceral adipose tissue accumulation and development of metabolic abnormalities that characterize metabolic syndrome as well as obesity-related cardiovascular risk, intra-abdominal adipose tissue biology has been a topic of particular interest to researchers and clinicians (Hiuge-Shimizu et al., 2012; Kotani et al., 1994). Indeed, visceral obesity and excess fat in the liver, heart, and kidneys often occur simultaneously (the degree to which this occurs with inter-individual variation), but this relationship is less clear between subcutaneous obesity and fat accumulation in organs (Fabbrini et al., 2009; Ross et al., 2008). Moreover, studies have shown that visceral fat can create a physiological state of low-grade inflammation which imparts systemic metabolic and cardiovascular impairment via the release of increased amounts of pro-inflammatory adipokines and suppression of anti-inflammatory adipocytokine secretion, leading to increased risk of diabetes, metabolic syndrome, and/or cardiovascular disease (de Heredia et al., 2012; Ouchi et al., 2011).

In contrast, the nature of subcutaneous adipose tissue function and pathology varies greatly relative to obesity-associated disease risk depending on how the adipose tissue handles excess energy

stores. For example, if excess energy stores induce subcutaneous adipose tissue expansion by increasing the number of adipose cells in the tissue (cell hyperplasia), then this proper expansion of adipose tissue can protect lean tissues and organs against harmful ectopic fat deposition by functioning as a “metabolic sink” (Després & Lemieux, 2006; Karpe & Pinnick, 2015); in this state, subcutaneous adipose tissue may function normally with the expected release of anti-inflammatory adipokines and may even be protective against unwanted health outcomes such as diabetes and cardiovascular disease (Neeland et al., 2015). However, if subcutaneous adipose tissue instead expand via the enlargement of individual adipose cell size (cell hypertrophy), adipocytes become saturated with triglyceride molecules, which may lead to adipocytes rupturing when they reach their size limit and can no longer expand; this is often accompanied by the increased release of pro-inflammatory adipokines, decreased release of anti-inflammatory adipokines, and/or macrophage invasion (de Heredia et al., 2012; Hammarstedt et al., 2018; Ouchi et al., 2011). Once adipocytes are fully saturated, ectopic fat deposition increases and creates a physiological environment conducive to atherosclerosis, diabetes, and inflammation, since excess triglyceride molecules will subsequently be stored at inappropriate sites such as the liver, heart, and kidneys if the subcutaneous adipose tissue lack the physical capacity to store the triglyceride molecules (Rosen & Spiegelman, 2014); ectopic intrahepatic fat in particular may be an even better marker of metabolic dysfunction associated with obesity than visceral adipose tissue (Fabbrini et al., 2009).

As master regulators of energy balance and nutritional homeostasis, adipocytes can also be categorized into “types” based on their function: brown adipocytes which play a role in energy expenditure by maintaining body temperature through thermogenesis (Sacks & Symonds, 2013); white adipocytes which store energy in the form of triglycerides packed into large lipid droplets and secrete various adipokines that affect inflammation, appetite mediation, and fat deposition (Matsuzawa, 2006); and “beige” adipocytes which have morphology and gene expression patterns similar to brown adipocytes but are recruited from white adipose depots (Rosen & Spiegelman, 2014). Although brown and white adipocytes are considered the two major types of adipose tissue compared to beige adipocytes, beige adipocytes also help regulate energy balance since they can either store energy or turn on heat production when the receive the appropriate signals or thermogenic stimuli (Wu et al., 2012).

Physiological Basis of Energy Balance

Many physiological systems contribute to the regulation of the complex biological mechanisms that control energy balance, beginning with the central nervous system – specifically, the hypothalamus which senses hormonal and neuronal signals communicating when and whether energy intake should occur (B. Xu et al., 2003). In particular, the arcuate nucleus in the hypothalamus contains two sets of neurons crucial to the regulation of energy balance: one set of neurons produces agouti-related protein (AGRP) and neuropeptide Y (NPY), and sends orexigenic signals to downstream effector neurons promoting food intake and reducing energy expenditure, while the other set of neurons produces pro-opiomelanocortin (POMC) and cocaine- and amphetamine-related transcript (CART), and relays anorexigenic signals to downstream effector neurons reducing food intake and promoting energy expenditure (Barsh & Schwartz, 2002). Important downstream effector neurons that enact the actions dictated by the arcuate nucleus include melanin-concentrating hormone neurons, thyrotrophin-releasing hormone neurons, and γ-aminobutyric acid (GABA)-releasing interneurons (Flier et al., 2000; Spiegelman & Flier, 2001). The dopamine, serotonin, and endocannabinoid signaling systems also provide input to modify appetite signals.

A myriad of signaling molecules play important roles in the feeding behavior regulated by the central nervous system through different mechanisms, including the appetite inhibitors POMC, alpha-melanocyte stimulating hormone (α-MSH), insulin, leptin, and adiponectin as well as the appetite stimulators NPY, AGRP, and ghrelin (Singh et al., 2017). In the brain, POMC is processed to form α-MSH, which interacts with melanocortin receptors (MCRs) widely expressed in the hypothalamus such as melanocortin-3-receptor (MC3R) and melanocortin-4-receptor (MC4R) to regulate metabolic functions and inhibit appetite (Begriche et al., 2011; D'Agostino & Diano, 2010; Garfield et al., 2009; Pritchard et al., 2002). Secreted by pancreatic β-cells, insulin interacts with specific receptors in the arcuate nucleus of the hypothalamus to reduce food intake and indicates adiposity levels over a moderate- to long-term period (Air et al., 2002); in the liver, insulin decreases the release of glucose while stimulating the uptake

of glucose and deposition of glycogen in the liver (Chavez et al., 1995). Leptin and adiponectin are both secreted by white adipose tissues but inhibit appetite using different mechanisms. Known as the “satiety hormone”, leptin secreted from white adipocytes conveys information regarding the amount of energy stored in adipose tissue directly to the hypothalamus – inhibiting orexigenic NPY/AGRP neurons and stimulating anorexigenic POMC/CART neurons - and its levels in plasma are highly correlated to adipocyte numbers (Friedman & Halaas, 1998). In addition to suppressing appetite, leptin also stimulates energy expenditure and activates AMP-activate protein kinase to influence other metabolic processes such as fatty-acid oxidation (Friedman & Halaas, 1998; Minokoshi et al., 2002). In contrast, adiponectin also influences fatty-acid oxidation and glucose homeostasis among other metabolic pathways, but adiponectin inhibits food intake by decreasing insulin resistance (González-Muniesa et al., 2017; Heilbronn et al., 2003) instead of direct communication through the central nervous system.

The main appetite stimulators in satiety and hunger signaling via the central nervous system also employ different mechanisms to increase food intake. Both NPY and AGRP are produced in the arcuate nucleus, but NPY sends direct signals to the paraventricular nucleus in the hypothalamus to stimulate appetite (Olza et al., 2013) while AGRP mediates food intake through antagonist interactions with MC3R and MC4R (Jackson et al., 2006; Lu et al., 1994; Yang et al., 1997). Unlike NPY and AGRP, ghrelin the “hunger hormone” is produced in the stomach and duodenum and increases appetite by activating the orexigenic NPY/AGRP neurons through growth hormone secretagogue receptors; though ghrelin induces the “opposite” metabolic effects of leptin, ghrelin’s effects on appetite are more short-term compared to leptin and adiponectin (Gale et al., 2004; Kohno et al., 2003).

Other important gut hormones in appetite suppression that are released in response to food intake include peptide YY(PYY), glucagon-like peptide 1 (GLP1), and cholecystokinin (CCK). Like leptin, PYY suppresses appetite by inhibiting orexigenic NPY/AGRP neurons in the hypothalamus and conveying postprandial satiety (Wren & Bloom, 2007), but PYY secretion is differentially triggered depending on the macronutrient composition of a meal, with high protein meals increasing PYY levels in serum (Alhabeeb et al., 2021; Batterham et al., 2002). GLP1 instills satiety and reduces food intake by increasing insulin secretion and inhibiting glucagon secretion, thereby decreasing glucose production

(Smith & Moran, 2021). Along with gastric distension, CCK reduces food intake by providing feelings of satiety after a meal via binding with receptors on the vagus nerve instead of interacting directly with the central nervous system (Howard et al., 1996); primarily synthesized in the duodenum and jejunum, CCK is rapidly released in response to nutrients in the gut, especially fat and protein (Wren & Bloom, 2007). Secretion of CCK may be indirectly related to changes in GLP1 and PYY levels, since CCK binds to cholecystokinin receptor type A which seems to play a part in mediating the secretion of GLP1 and PYY (Alhabeeb et al., 2021).

While signaling via the central nervous system plays a key role in regulating energy intake, molecules that affect energy expenditure also impact energy balance, such as thyroid hormones that modulate basal metabolic rate and body temperature, as well as mitochondrial brown fat uncoupling protein 1 (UCP1) which induces non-shivering thermogenesis (González-Muniesa et al., 2017). One way that thyroid hormones control energy expenditure is through the stimulation of basal metabolic rate by enhancing of adenosine triphosphate (ATP) production in muscle and the maintenance of ion gradients which leads to ATP consumption (Mullur et al., 2014; Volke & Krause, 2021; F. Yu et al., 2000). Furthermore, thyroid hormones can cross the blood-brain barrier to increase sympathetic nervous system activation for inducing thermogenesis (López et al., 2013). In addition, thyroid hormones can help stimulate the expression of UCP1 which dissipates the electrochemical gradient in the mitochondrial membrane that drives ATP synthesis, and thus increase heat generation in brown adipose tissue (Cannon & Nedergaard, 2004).

Impact of Genetics and Epigenetics on Obesity

Along with environmental factors that affect energy balance, genetics play a significant role in the pathogenesis of obesity, since up to 70% of inter-individual variation in body weight may be attributed to genetic differences (Elks et al., 2012). When considering obesity severity as degrees of variation across a phenotypic trait at the population level, heritability can estimate how much genetic variation between individuals contributes to obesity phenotypic variation in a population, compared to variation of environmental factors (Falconer, 1989; Gazzaniga et al., 2016; Wray & Visscher, 2008). Prior to the advent of genetic sequencing, twin studies and studies involving closely related individuals were instrumental for measuring heritability and demonstrating the important and unique contributions of

genetics to obesity development apart from environmental factors. For example, in 1986 Stunkard studied 1,974 monozygotic and 2,097 dizygotic twin pairs and estimated the heritability for weight to be 0.78; similarly, Stunkard performed an adoption study examining 540 adult adoptees divided into four weight classes (thin, median weight, overweight, and obese) and relating the weight class of the adoptees to their biological parents and adopted parents, which demonstrated that the adoptees had body sizes more like their biological parents than their adopted parents across the whole range of body fatness, from very lean to very fat (Stunkard, 1986; Stunkard et al., 1986). The results from these studies were combined four years later in a seminal paper that also examined obesity phenotypes of identical and fraternal twins that were raised together and apart, which reported heritability estimates for obesity phenotypes to be 0.70 for men and 0.66 for women (Stunkard et al., 1990). More recently, Katzmarzyk et al. examined the heritability of BMI, skinfold thickness, and waist circumference in 327 Canadian Caucasian participants from 102 nuclear families and estimated heritability for BMI and other measures of fatness to be 0.46-0.60, while heritability estimates for measures of fat distribution (e.g. waist circumference adjusted for BMI) were in the range of 0.29-0.48 (Katzmarzyk et al., 2000). Along with heritability estimates of obesity phenotypes such as BMI and measures of central obesity from numerous other studies (Koeppen-Schomerus et al., 2001; Moll et al., 1991; Pietiläinen et al., 1999; Selby et al., 1989), these results indicate a minimum heritability of 0.4 for obesity and/or fatness.

Traditionally, heritability estimations are made using pedigree study designs relying on twin studies or studies that involve closely related individuals, but developments in genetic sequencing technology have facilitated the wide-spread application of large-scale genome-wide sequencing in "unrelated" individuals, which has enabled the use of single nucleotide polymorphisms (SNPs) as genetic markers in genome-wide association studies (GWAS) and advanced statistical methods such as mixed linear models for the calculation of heritability in a population study design (Yang et al., 2010, 2017). Heritability calculated using twin and full sibling data estimates *total* heritability which gives more precision but potentially more bias from environmental variation confounded with additive genetic variation, whereas heritability calculated using SNP data estimates the genetic variance explained by the SNPs which gives less precision but less bias (Vinkhuyzen et al., 2013), which may result in discrepancies between heritability estimates for the same trait. Referred to as the "missing heritability

problem" (Maher, 2008), multiple explanations have been suggested for this discrepancy between heritability estimates calculated for the same trait using different study designs, including (but not limited to) the presence of a large number of common variants of small effect yet to be discovered, rare variants of large effect not tagged by common SNPs during genotyping, epigenetic factors, and influences from the microbiome (Eichler et al., 2010; Manolio et al., 2009; Sandoval-Motta et al., 2017). When estimating heritability using the population (SNP) approach, the "missing heritability problem" can be partially ameliorated when phenotypic and SNP data are available for 10,000s of individuals since the population design is as efficient as the twin design with a large enough sample size (Vinkhuyzen et al., 2013). Additionally, Yang et al. proposed using a method to estimate heritability for human complex traits in unrelated individuals using whole-genome sequencing data followed by imputation to capture the variation at both common and rare genetic variants, suggesting that heritability is likely to be 30-40% for BMI (Yang et al., 2015). Although the "missing heritability problem" remains to be fully elucidated, heritability estimates for fat distribution and measures of fatness indicate that genetics have a substantial effect on obesity.

Differences in genetic architecture can lead to the development of obesity through a plethora of diverse mechanisms. In fact, types of obesity can be categorized based on the underlying genetic origins responsible for their development, specifically syndromic obesity, monogenic obesity, and common (polygenic) obesity. Approximately 20 rare syndromes caused by genetic defects or chromosomal abnormalities are currently known to be characterized by obesity, often accompanied by mental retardation or learning disabilities; at least four of these syndromes are characterized by hyperphagia that results in obesity and/or other signs of hypothalamic disorder (Delrue & Michaud, 2004), such as Prader-Wili syndrome and Single minded-1 (Sim-1) syndrome. Characterized by decreased muscle tone, short stature, and the absence or decreased function of gonads in addition to obesity, hyperphagia, and mental retardation, Prader-Willi syndrome is an autosomal-dominant disorder and is the most prevalent of syndromes associated with obesity occurring in 1 of 25,000 births (Goldstone, 2004). Most cases of Prader-Willi syndrome are caused by a paternally inherited deletion at the chromosomal region 15q11.2-q12 and occasionally by maternal uniparental disomy (González-Jiménez et al., 2012; Jiang et al., 1998). Some mechanisms proposed to cause hyperphagia in Prader-Willi syndrome include hypothalamic

impairment resulting in endocrine abnormalities, and increased secretion of ghrelin in the stomach leading to elevated stimulation of the POMC/CART and NPY neurons in the arcuate nucleus of the hypothalamus (Cummings et al., 2002; Farooqi & O'Rahilly, 2004). Sim-1 syndrome is characterized by "Prader-Willi-like" phenotypes, delayed development, and/or early-onset obesity, but instead results from deletion or disruption of the SIM1 region on chromosome 6q (Faivre, 2002; Holder et al., 2000). The suggested mechanism behind hyperphagia in Sim-1 syndrome involves dysfunction of the hypothalamic paraventricular nucleus which modulate effector neurons involved in appetite signaling (e.g. via MC4R signaling) caused by the deletion or disruption of the SIM1 gene, which encodes a transcription factor critical to neurogenesis (Holder et al., 2000; Ramachandrappa et al., 2013).

The etiology of obesity may be more complex in other syndromes such as Bardet-Biedl syndrome. Unlike Prader-Willi syndrome, Bardet-Biedl syndrome is typically an autosomal recessive disorder, and occasionally may manifest through triallelic inheritance in certain families (Beales et al., 2003). In addition to early-onset obesity and learning disabilities, Bardet-Biedl syndrome is also characterized by rod-cone dystrophy, polydactyly, hypogonadism in males, and renal abnormalities (Farooqi, 2005; Ristow, 2004). Thus far, at least 20 genes associated with Bardet-Biedl syndrome have been identified (BBS1-BBS20), all of them involved in the regulation of primary cilia function (Priya et al., 2016; Suspitsin & Imyanitov, 2016). Loss-of-function mutations in these genes result in reduced number of cilia and disruptions in the sonic hedgehog (Shh) and Wnt signaling in differentiating preadipocytes, as well as altered leptin resistance and neuroendocrine signaling from ciliated neurons to adipose tissue (D.-F. Guo & Rahmouni, 2011; Priya et al., 2016). BBS knockout mice are hyperphagic and have decreased locomotor activity, suggesting that both energy intake and expenditure are perturbed to contribute to obesity development in individuals with Bardet-Biedl syndrome (Rahmouni et al., 2008).

Monogenic forms of obesity refer to obesity without the additional symptoms that accompany syndromic obesity and are caused by mutations in single genes that commonly encode proteins involved in the regulation of appetite such as POMC, leptin, leptin receptor (LEPR), NPY, ghrelin receptor, MC3R, and MC4R, typically resulting in increased feeding behavior (Singh et al., 2017; van der Klaauw & Farooqi, 2015). Compared to common obesity, cases of monogenic obesity are relatively rare Mendelian

disorders, but phenotypes tend to be more severe (Farooqi & O'Rahilly, 2004). Interestingly, MC4R deficiency is the most common form of monogenic obesity identified thus far, present in 1-6% of obese individuals from different ethnic groups (Farooqi et al., 2003; Lubrano-Berthelier et al., 2003), though European populations exhibit higher pathogenicity of MC4R mutations than Mediterranean or Asian populations (Kublaoui & Zinn, 2006). In humans, the severity of MC4R mutations appear greater in homozygous obese individuals than in heterozygous obese individuals (Farooqi et al., 2003). In MC4R-deficient mice, the degree of hyperphagia increases as dietary fat increases (Butler & Cone, 2003), suggesting that gene-environment interactions may alleviate or worsen dysregulation of energy intake.

Identification of the genes involved in these distinct types of obesity and understanding the associated biological mechanisms that disrupt energy balance are crucial for effective obesity prevention and treatment. Identification of genes related to common obesity can be a challenge since the genetic variants of polygenic obesity can differ from one individual to another (Clément, 2006; Hinney et al., 2010). One approach used to find genes related to common obesity that does not require prior knowledge about potential gene candidates is to perform large-scale genome-wide association studies (GWAS) that test the association of millions of common genetic variants with adiposity traits, which has shown some success. Although GWAS often require a large sample size to find significant results, over 300 genetic loci associated with obesity traits have been found, where the first major breakthrough was the discovery of the FTO locus (Frayling et al., 2007; Scuteri et al., 2007). Further investigation of the association between the non-coding variants found in this locus with obesity risk suggest that the locus may regulate RPGRIP1L or IRX3-IRX5 expression, resulting in alterations in appetite, thermogenesis, adipocyte browning, and other processes related to obesity (Claussnitzer et al., 2015; J. Yang et al., 2012). In addition to the FTO locus, there are currently 1,818 genes in the mouse that have corresponding human homologs found in the GWAS catalog to be associated with an obesity trait such as BMI or waist-to-hip ratio (Buniello et al., 2019). Furthermore, Kunej et al. have created a freely available obesity database compiling data of 1,736 obesity-associated loci collected from human, cattle, rat, and mouse studies to facilitate comparisons of obesity-associated loci across different biological systems (Kunej et al., 2013). Recently, Akbari et al. performed large-scale sequencing of 640,000 human exomes to identify rare protein-coding variants with large impacts on obesity and found 16 genes significantly associated with

BMI, five of which encode brain-expressed G protein-coupled receptors (*CALCR*, *MC4R*, *GIPR*, *GPR151*, and *GPR75*) (Akbari et al., 2021). The authors then verified the therapeutic potential of targeting *GPR75* as a candidate gene for weight loss by demonstrating resistance to weight gain in *Gpr75* knockout mice.

Other important obesity candidate genes discovered or confirmed through GWAS include *SLC6A14* (Durand et al., 2004; Suviolahti et al., 2003) and genes in the uncoupling proteins (UCPs) gene family (Damcott et al., 2004; Herrmann et al., 2003; Oppert et al., 1994; Yanovski et al., 2000). SLC6A14 is highly expressed in the region of the hypothalamus where appetite signaling is regulated and encodes an amino acid transporter that modulates tryptophan availability for serotonin synthesis, which may affect feeding behavior (Suviolahti et al., 2003). The association between SLC6A14 and polygenic obesity development has been reinforced by additional studies that related SLC6A14 deficiency to obesity in mice on a high-fat diet (Sivaprakasam et al., 2021) and demonstrated that genetic variants may affect food intake and nutritional status in children (Miranda et al., 2015). Candidate genes associated with obesity in the UCP gene family include UCP1, UCP2, and UCP3, which vary in terms of function and locations of expression. While UCP2 is ubiquitously expressed in any tissue, UCP1 is expressed in brown adipose tissue and UCP3 is expressed in both brown adipose tissue and skeletal muscle; UCP1 and UCP3 mediate thermogenesis, while UCP1 and UCP2 influence energy metabolism (Gong et al., 1997; GENOI et al., 2004; Ochoa et al., 2004; Saleh et al., 2002).

Despite the success of discovering numerous highly significant associations, the genetic effect sizes of most loci discovered via GWAS on obesity traits are small; for example, all currently identified BMI-associated variants combined only explain <5% of variation in BMI (The LifeLines Cohort Study et al., 2015; Winkler et al., 2015). Given the vast differences in environmental exposure between individuals, interactions between genetic and environmental factors may explain the variations in individual body weight response; additional research using animal models is required to determine which factors interact and how these interactions impact body weight since both genetics and environmental setting can be controlled in animal model studies.

Highly responsive to both external environmental perturbations (e.g., diet and physical activity) and internal biological influences (e.g., hormones and genetics), the epigenome may partially explain how

mechanisms that govern gene-by-environment interactions function for the regulation of energy balance. The epigenome induces changes in gene function or expression without modification of DNA sequence that may be heritable and reversible; some examples of epigenetic processes include DNA methylation, histone modification, and mechanisms mediated by RNA. Because epigenetic processes are cell-, time-, and tissue-specific, investigating their particular functions in obesity directly in humans is a challenge, given the major role that the central nervous system plays in the regulation of energy balance. However, two critical developmental periods where the importance of epigenetics is well-established are the prenatal and neonatal periods in which metabolic imprinting transpires. During metabolic imprinting, programming of fetal metabolism occurs at both the genomic and epigenomic levels to affect future disease risk and health (Eriksson, 2016; Hanley et al., 2010), since both overnutrition and undernutrition during fetal development (as measured by birth weight) are associated with higher risk of obesity and higher percentage of body fat independent of BMI, respectively (Labayen et al., 2009; Yu et al., 2011). In addition to the amount of maternal food consumption during pregnancy and lactation, the type of nutrients consumed during these times has also been associated with the development of metabolic complications in adulthood (Chen et al., 2017). Considering the pivotal role that the epigenome plays in conveying intergenerational effects on obesity, expanding our understanding of epigenetic effects on energy metabolism by using animal model studies to overcome the barriers inherent to human studies could lead to the discovery of important therapeutic targets for the prevention and treatment of obesity.

Advantages of utilizing a genetically diverse mouse panel to investigate obesity development

Two challenges present in studying the effects of genetics and diet on the development of obesity in humans that can be overcome using a model organism include the heterogeneity in environmental exposures, as well as the inability to manipulate genetics. Although technological advances in sequencing have facilitated the discovery of candidate genes in humans through GWAS, model organisms such as the mouse are still vital for establishing and verifying the function of identified candidate genes and how genetic architecture and diet influence their expression (Attie et al., 2017). The high similarity in physiology, protein functions, biological pathways, and genome organization between humans and mice makes the mouse an excellent model organism for differentiating the effects of genetics compared to diet

on obesity development, since the environmental influences that affect obesity can be more easily controlled in mouse studies compared to human studies, while findings in mouse studies are still translatable due to the homology between humans and mice. In addition, it is possible to induce various types of mutations to specific genes in the mouse to test changes in expression or function in a cost-effective manner (Harms et al., 2014).

Compared to other mammalian model organisms, the lowered cost of mouse maintenance, shorter life cycle, and smaller size of the mouse allows the inclusion of many biological samples in one study, which is crucial for generating large-scale -omics and phenotyping data sets to explore the relationship between gene expression and resulting phenotypes. In fact, there are two essential facets of obesity that are logistically challenging or cost-prohibitive to study in humans but made possible to examine in the mouse: comparing -omics data derived from tissues of healthy control subjects with data from patients in a diseased state, and quantifying metabolism as energy expenditure through indirect calorimetry.

For example, the liver plays a crucial role in the development of obesity and obesity-related health complications. Not only does the liver interact with adipose tissue, the central nervous system, and hormones that regulate energy balance through numerous signaling pathways (Bell et al., 2005; González-Muniesa et al., 2017), but the liver also shapes obesity through its metabolism of dietary macronutrients including glycogenolysis, production of triglycerides, lipogenesis, and the synthesis of molecules used as building blocks for hormones such as amino acids, cholesterol, and lipoproteins (Rui, 2014; Trefts et al., 2017). Furthermore, insulin resistance in the liver leads to the disruption of appropriate carbohydrate and lipid metabolism, impairing the ability of insulin to decrease glucose output from the liver while continuing to stimulate lipogenesis, which is thought to exacerbate the severity of the health complications associated with obesity like metabolic syndrome. Naturally, alterations in hepatic gene expression from genetic or dietary effects would impact liver function and signaling, and thus impact metabolism and mechanisms involved in energy balance. Despite the importance of investigating the effects of genetics and diet on hepatic gene expression in the context of obesity, obtaining liver tissue from healthy human subjects remains challenging while obtaining liver tissue in both healthy and disease

states is much more feasible in mice, which illustrates the necessity to use the mouse as a model to study obesity.

Since obesity is caused by disruption in energy balance, quantifying changes in energy intake and expenditure due to genetics or diet and relating these changes to adiposity is vital to understanding how genetics and diet impact obesity development. In humans, total energy expenditure (TEE) is defined as the amount of heat energy used for daily functions and can be divided into three components based on what the energy is used for that vary in terms of the amount of energy required relative to TEE (Gupta et al., 2017): basal or resting energy expenditure is the amount of energy require to sustain basic cellular metabolic activity and vital functions (60-65% of TEE); diet-induced thermogenesis is the energy used for postprandial substrate metabolism (5-10% of TEE); and active energy expenditure is the energy used during physical activity (25-30% of TEE). Fundamentally, the two ways to measure energy metabolism are either to measure heat as the end product of metabolic activity (direct calorimetry) or to measure components of the metabolic process that generate heat instead of measuring heat directly (indirect calorimetry) (Speakman, 2013). Indirect calorimetry is the preferred method for estimating energy expenditure from the calculation of heat produced derived from measuring oxygen consumption and/or carbon dioxide production since measuring oxygen and carbon dioxide gases can be performed much more accurately than directly measuring heat. After measuring respiratory gases, an estimate of heat produced and energy expenditure can then be calculated using equations derived from values for the metabolism of typical carbohydrates, fats, and proteins. Furthermore, during rest or mild to moderate exercise the measured ratio of oxygen consumption to carbon dioxide production (called the respiratory exchange ratio [RER]) can be used to approximate the respiratory quotient (RQ), which indicates which macronutrient (e.g. carbohydrate or fat) is serving as the main fuel source in the body (Katch et al., 2011), where 0.7 implies pure fat oxidation and 1.0 implies pure carbohydrate oxidation (Kenney et al., 2012). Although several indirect calorimetric methods are available for estimating energy expenditure in humans such as the Douglas bag, metabolic carts, and metabolic wards, these methods can only be applied over short periods of time to small study cohorts either due to cost or difficulty in recruiting volunteers for long-term studies (Hall et al., 2015; Haugen et al., 2007; McClave & Snider, 1992; Zhao et al., 2014). In contrast, machine systems such as the CLAMS system produced by Columbus instruments, the

Promethion system by Sable systems, Inc., and the Phenomaster system produced by TSE systems, Ltd., enable the inclusion of numerous “subjects” within a mouse study for measuring numerous aspects of energy metabolism (Speakman, 2013). For example, the Phenomaster system facilitates the measurement of respiratory gases to calculate heat for the estimation of energy expenditure, food consumed to measure energy intake, and movement to approximate basal physical activity over the course of several days to quantify energy metabolism during periods of activity and rest.

Alternatively, the doubly labeled water method, in which the hydrogen and oxygen have been replaced by heavy but non-radioactive forms of each element, can also be used to measure metabolic rate over a longer period of time (from days to weeks). This technique is performed by administering a dose of doubly labeled water and measuring the elimination rates of the labeled hydrogen and oxygen over time by sampling saliva, urine, or blood where the magnitude of the difference in elimination of the isotopes is related to carbon dioxide production (Speakman, 1997, 2013). Because of the time required for the elimination of these isotopes and relative convenience of sample collection, this method has been widely used to measure metabolic rate in humans (Pontzer et al., 2021) and in mouse studies where the mice could not be housed separately, such as in studies where metabolism is measured in lactating mice (M. S. Johnson & Speakman, 2001; Speakman, 2013). Limitations of this technique include the cost of doubly labeled water and the reliance on expensive mass spectrometry equipment (Speakman, 2013).

In addition to the aforementioned benefits of using the mouse model to study obesity, the power to control and manipulate mouse genetics make the mouse indispensable to obesity research for both gene functional validation studies and discovery of genetic and molecular causes of obesity. After candidate genes for obesity have been identified through GWAS performed in humans, functional validation studies in a model organism are necessary for determining the function of the gene in the context of obesity. Typically, a reverse genetics approach is used to elucidate the function of the gene and the mechanism it employs to affect the trait of interest, where the expression of the candidate gene is disrupted followed by the study of the changes in phenotype (García-García, 2020). Traditional inbred mouse strains are excellent for such studies due to their affordability, ease of inducing mutations, well-defined phenotypes for comparison of phenotypic differences resulting from the studied mutation(s), and reproducibility of results. In contrast, forward genetics is a useful approach for determining the genetic or

molecular causes of the phenotype of interest, where models exhibiting the phenotype of interest are created from naturally occurring or induced genetic changes followed by the investigation of the genetic cause(s) that induced the morphological manifestation of the phenotype (García-García, 2020). One important advantage of forward genetics is that no prior knowledge about the identity of causal genes nor the specific type of mutation or genetic alteration responsible for the eliciting the phenotype are necessary for discovering the association between the genetic/molecular cause and the phenotype (Clark et al., 2020), which makes forward genetics an unbiased strategy for finding novel elements. Genetic screens that find phenotype-regulating genomic regions known as quantitative trait loci (QTLs) for complex traits such as obesity require a genetically diverse reference population to provide the high resolution necessary for genetic mapping and for better translatability of cross-species comparisons (Swanzey et al., 2021).

Two recently developed murine genetic reference populations well-suited for studying the effects of genetics and diet on the development of obesity using either reverse or forward genetic approaches are the Collaborative Cross (CC) and the Diversity Outbred (DO) multiparent mouse panels. The CC is a large recombinant inbred mouse population developed for systems genetics, derived from elaborate intercrosses of eight founder strains, five classically inbred mouse strains (C57BL/6J, A/J, NOD/ShiLtJ, NZO/HiLtJ, and 129S1/SvImJ) and three wild-derived strains representing three *Mus musculus* subspecies (WSB/EiJ, CAST/EiJ, and PWK/PhJ) (Aylor et al., 2011; Churchill et al., 2004; Iraqi et al., 2008; Threadgill & Churchill, 2012). CC strains were generated using a funnel breeding scheme that combined the genomes from the eight founders via outbreeding for three generations before repeated generations of sibling inbreeding (Churchill et al., 2004; Collaborative Cross Consortium, 2012). The tremendous genetic diversity of the CC population exhibits greater phenotypic diversity than other available recombinant inbred mouse panels and captures over 90% of the genetic variation in laboratory mice including 45 million segregating polymorphisms, a degree of genetic diversity that is comparable to the genetic divergence captured in most of the human population after the great expansion (Collaborative Cross Consortium, 2012; Garrigan et al., 2007; Henn et al., 2012; Keane et al., 2011; Philip et al., 2011; Roberts et al., 2007; Shorter et al., 2019; Srivastava et al., 2017). Moreover, the CC strains were designed to have equal genetic contribution and genomic randomization from all eight founder strains

normally distributed across their genomes, therefore encompassing both high genetic diversity and reproducibility at the genome level (Collaborative Cross Consortium, 2012). In comparison, the DO population was generated by randomly outcrossing CC strains, creating a population of mice with high allelic heterozygosity and accumulation of recombination events that improve genomic resolution, increases statistical power, and reduces the sample size needed for fine genetic mapping (Churchill et al., 2012; Rockman & Kruglyak, 2008). Although DO mice are genetically unique and thus not replicable at the genome level, the CC and DO founder strains can be used for the identification of replicable genotypes in regions of interest discovered in the DO population.

Since the underlying causes of obesity are vast and complex, using a systems genetics approach provides a platform for studying multidimensional data sets collectively to delineate the effects of genetics and diet on obesity development for multiple related physiological and molecular traits across multiple "treatment" groups. In the absence of genetic mapping, the integration of multiple data sets and utilization of modeling network analyses facilitate the discovery of obesity-associated genes whose expression is modulated by genetics or diet and relating those genes to molecular pathways that underlie obesity development. For example, gene expression can be correlated with phenotypes and differential gene expression analysis can be performed to identify genes modulated by genetic background or diet, followed by enrichment analysis of functional genes and pathways to bridge the effect of genetics or diet on gene expression with elicited phenotypes and the activation of biological pathways in the context of obesity (Ashburner et al., 2000; E. Y. Chen et al., 2013; Kanehisa, 2000; Ritchie et al., 2015; Subramanian et al., 2005). Another model networking approach that enables the exploration of genetic and diet effects on the expression of groups of genes is weighted gene coexpression network analysis (WGCNA), in which gene networks are constructed based on correlated expression levels (Zhang & Horvath, 2005). Following gene network construction, groups of genes with highly correlated expression patterns called "modules" are identified, their average expression levels which can then be summarized using dimensional reduction techniques, correlated with phenotypes, and used in enrichment analysis to find related biological functions or pathways, and thus elucidate the specific effects of genetics and diet on obesity.

References

Abete, I., Astrup, A., Martínez, J. A., Thorsdottir, I., & Zulet, M. A. (2010). Obesity and the metabolic syndrome: Role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. *Nutrition Reviews*, *68*(4). https://doi.org/10.1111/j.1753-4887.2010.00280.x

Abildgaard, J., Ploug, T., Al-Saoudi, E., Wagner, T., Thomsen, C., Ewertsen, C., Bzorek, M., Pedersen, B. K., Pedersen, A. T., & Lindegaard, B. (2021). Changes in abdominal subcutaneous adipose tissue phenotype following menopause is associated with increased visceral fat mass. *Scientific Reports*, *11*(1), 14750. https://doi.org/10.1038/s41598-021-94189-2

Achilike, I., Hazuda, H. P., Fowler, S. P., Aung, K., & Lorenzo, C. (2015). Predicting the development of the metabolically healthy obese phenotype. *International Journal of Obesity*, *39*(2), 228–234. https://doi.org/10.1038/ijo.2014.113

Aggarwal, A., Monsivais, P., Cook, A. J., & Drewnowski, A. (2011). Does diet cost mediate the relation between socioeconomic position and diet quality? *European Journal of Clinical Nutrition*, *65*(9), 1059–1066. https://doi.org/10.1038/ejcn.2011.72

Air, E. L., Strowski, M. Z., Benoit, S. C., Conarello, S. L., Salituro, G. M., Guan, X.-M., Liu, K., Woods, S. C., & Zhang, B. B. (2002). Small molecule insulin mimetics reduce food intake and body weight and prevent development of obesity. *Nature Medicine*, *8*(2), 179–183. https://doi.org/10.1038/nm0202-179

Akbari, P., Gilani, A., Sosina, O., Kosmicki, J. A., Khrimian, L., Fang, Y.-Y., Persaud, T., Garcia, V., Sun, D., Li, A., Mbatchou, J., Locke, A. E., Benner, C., Verweij, N., Lin, N., Hossain, S., Agostinucci, K., Pascale, J. V., Dirice, E., … Lotta, L. A. (2021). Sequencing of 640,000 exomes identifies GPR75 variants associated with protection from obesity. *Science (New York, N.Y.)*, *373*(6550), eabf8683. https://doi.org/10.1126/science.abf8683

Alberti, K. G. M. M., Eckel, R. H., Grundy, S. M., Zimmet, P. Z., Cleeman, J. I., Donato, K. A., Fruchart, J.-C., James, W. P. T., Loria, C. M., Smith, S. C., International Diabetes Federation Task Force on Epidemiology and Prevention, Hational Heart, L., and Blood Institute, American Heart Association, World Heart Federation, International Atherosclerosis Society, & International Association for the Study of Obesity. (2009). Harmonizing the metabolic syndrome: A joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention; National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. *Circulation*, *120*(16). https://doi.org/10.1161/CIRCULATIONAHA.109.192644

Alblas, M. C., Mollen, S., Wennekers, A. M., Fransen, M. L., & van den Putte, B. (2021). Consuming media, consuming food: Investigating concurrent TV viewing and eating using a 7-d time use diary survey. *Public Health Nutrition*, 1–10. https://doi.org/10.1017/S1368980021002858

Alhabeeb, H., AlFaiz, A., Kutbi, E., AlShahrani, D., Alsuhail, A., AlRajhi, S., Alotaibi, N., Alotaibi, K., AlAmri, S., Alghamdi, S., & AlJohani, N. (2021). Gut Hormones in Health and Obesity: The Upcoming Role of Short Chain Fatty Acids. *Nutrients*, *13*(2), 481. https://doi.org/10.3390/nu13020481

Apovian, C. M. (2016). Obesity: Definition, comorbidities, causes, and burden. *The American Journal of Managed Care*, *22(7 Suppl), s176–s185.*

Apovian, C. M., Aronne, L. J., Bessesen, D. H., McDonnell, M. E., Murad, M. H., Pagotto, U., Ryan, D. H., & Still, C. D. (2015). Pharmacological Management of Obesity: An Endocrine Society Clinical Practice Guideline. *The Journal of Clinical Endocrinology & Metabolism*, *100*(2), 342–362. https://doi.org/10.1210/jc.2014-3415

Appleton, S. L., Seaborn, C. J., Visvanathan, R., Hill, C. L., Gill, T. K., Taylor, A. W., Adams, R. J., & on behalf of the North West Adelaide Health Study Team. (2013). Diabetes and Cardiovascular Disease Outcomes in the Metabolically Healthy Obese Phenotype: A cohort study. *Diabetes Care*, *36*(8), 2388–2394. https://doi.org/10.2337/dc12-1971

Ashburner, M., Ball, C. A., Blake, J. A., Botstein, D., Butler, H., Cherry, J. M., Davis, A. P., Dolinski, K., Dwight, S. S., Eppig, J. T., Harris, M. A., Hill, D. P., Issel-Tarver, L., Kasarskis, A., Lewis, S., Matese, J. C., Richardson, J. E., Ringwald, M., Rubin, G. M., & Sherlock, G. (2000). Gene Ontology: Tool for the unification of biology. *Nature Genetics*, *25*(1), 25–29. https://doi.org/10.1038/75556

Attie, A. D., Churchill, G. A., & Nadeau, J. H. (2017). How mice are indispensable for understanding obesity and diabetes genetics. *Current Opinion in Endocrinology & Diabetes and Obesity*, *24*(2). https://doi.org/10.1097/med.0000000000000321

Avila, C., Holloway, A. C., Hahn, M. K., Morrison, K. M., Restivo, M., Anglin, R., & Taylor, V. H. (2015). An Overview of Links Between Obesity and Mental Health. *Current Obesity Reports*, *4*(3), 303–310. https://doi.org/10.1007/s13679-015-0164-9

Aylor, D. L., Valdar, W., Foulds-Mathes, W., Buus, R. J., Verdugo, R. A., Baric, R. S., Ferris, M. T., Frelinger, J. A., Heise, M., Frieman, M. B., Gralinski, L. E., Bell, T. A., Didion, J. D., Hua, K., Nehrenberg, D. L., Powell, C. L., Steigerwalt, J., Xie, Y., Kelada, S. N. P., … Churchill, G. A. (2011). Genetic analysis of complex traits in the emerging Collaborative Cross. *Genome Research*, *21*(8), 1213–1222. https://doi.org/10.1101/gr.111310.110

Bandy, L. K., Scarborough, P., Harrington, R. A., Rayner, M., & Jebb, S. A. (2020). Reductions in sugar sales from soft drinks in the UK from 2015 to 2018. *BMC Medicine*, *18*(1), 20. https://doi.org/10.1186/s12916-019-1477-4

Barsh, G. S., & Schwartz, M. W. (2002). Genetic approaches to studying energy balance: Perception and integration. *Nature Reviews Genetics*, *3*(8), 589–600. https://doi.org/10.1038/nrg862

Batis, C., Hernandez-Barrera, L., Barquera, S., Rivera, J. A., & Popkin, B. M. (2011). Food Acculturation Drives Dietary Differences among Mexicans, Mexican Americans, and Non-Hispanic Whites. *The Journal of Nutrition*, *141*(10), 1898–1906. https://doi.org/10.3945/jn.111.141473

Batterham, R. L., Cowley, M. A., Small, C. J., Herzog, H., Cohen, M. A., Dakin, C. L., Wren, A. M., Brynes, A. E., Low, M. J., Ghatei, M. A., Cone, R. D., & Bloom, S. R. (2002). Gut hormone PYY3-36 physiologically inhibits food intake. *Nature*, *418*(6898), 650–654. https://doi.org/10.1038/nature00887

Beales, P. L., Badano, J. L., Ross, A. J., Ansley, S. J., Hoskins, B. E., Kirsten, B., Mein, C. A., Froguel, P., Scambler, P. J., Lewis, R. A., Lupski, J. R., & Katsanis, N. (2003). Genetic interaction of BBS1 mutations with alleles at other BBS loci can result in non-Mendelian Bardet-Biedl syndrome. *American Journal of Human Genetics*, *72*(5), 1187–1199. https://doi.org/10.1086/375178

Begriche, K., Levasseur, P. R., Zhang, J., Rossi, J., Skorupa, D., Solt, L. A., Young, B., Burris, T. P., Marks, D. L., Mynatt, R. L., & Butler, A. A. (2011). Genetic Dissection of the Functions of the Melanocortin-3 Receptor, a Seven-transmembrane G-protein-coupled Receptor, Suggests Roles for Central and Peripheral Receptors in Energy Homeostasis. *Journal of Biological Chemistry*, *286*(47), 40771–40781. https://doi.org/10.1074/jbc.M111.278374

Bell, C. G., Walley, A. J., & Froguel, P. (2005). The genetics of human obesity. *Nature Reviews Genetics*, *6*(3). https://doi.org/10.1038/nrg1556

Bell, W., Coates, J., Fanzo, J., Wilson, N. L. W., & Masters, W. A. (2021). Beyond price and income: Preferences and food values in peri-urban Viet Nam. *Appetite*, *166*, 105439. https://doi.org/10.1016/j.appet.2021.105439

Berry, S. E., Valdes, A. M., Drew, D. A., Asnicar, F., Mazidi, M., Wolf, J., Capdevila, J., Hadjigeorgiou, G., Davies, R., Al Khatib, H., Bonnett, C., Ganesh, S., Bakker, E., Hart, D., Mangino, M., Merino, J., Linenberg, I., Wyatt, P., Ordovas, J. M., … Spector, T. D. (2020). Human postprandial responses to food and potential for precision nutrition. *Nature Medicine*, *26*(6). https://doi.org/10.1038/s41591-020-0934-0

Bes-Rastrollo, M., Basterra-Gortari, F. J., Sánchez-Villegas, A., Marti, A., Martínez, J. A., & Martínez-González, M. A. (2010). A prospective study of eating away-from-home meals and weight gain in a Mediterranean population: The SUN (Seguimiento Universidad de Navarra) cohort. *Public Health Nutrition*, *13*(9), 1356–1363. https://doi.org/10.1017/S1368980009992783

Bhupathiraju, S. N., & Hu, F. B. (2016). Epidemiology of Obesity and Diabetes and Their Cardiovascular Complications. *Circulation Research*, *118*(11), 1723–1735. https://doi.org/10.1161/CIRCRESAHA.115.306825

Bhutani, S., Schoeller, D. A., Walsh, M. C., & McWilliams, C. (2018). Frequency of Eating Out at Both Fast-Food and Sit-Down Restaurants Was Associated With High Body Mass Index in Non-Large Metropolitan Communities in Midwest. *American Journal of Health Promotion*, *32*(1), 75–83. https://doi.org/10.1177/0890117116660772

Bray, G. A., Frühbeck, G., Ryan, D. H., & Wilding, J. P. H. (2016). Management of obesity. *The Lancet*, *387*(10031), 1947–1956. https://doi.org/10.1016/S0140-6736(16)00271-3

Bray, G. A., & Siri-Tarino, P. W. (2016). The Role of Macronutrient Content in the Diet for Weight Management. *Endocrinology and Metabolism Clinics of North America*, *45*(3), 581–604. https://doi.org/10.1016/j.ecl.2016.04.009

Buniello, A., MacArthur, J. A. L., Cerezo, M., Harris, L. W., Hayhurst, J., Malangone, C., McMahon, A., Morales, J., Mountjoy, E., Sollis, E., Suveges, D., Vrousgou, O., Whetzel, P. L., Amode, R., Guillen, J. A., Riat, H. S., Trevanion, S. J., Hall, P., Junkins, H., … Parkinson, H. (2019). The NHGRI-EBI GWAS Catalog of published genome-wide association studies, targeted arrays and summary statistics 2019. *Nucleic Acids Research*, *47*(D1), D1005–D1012. https://doi.org/10.1093/nar/gky1120

Burke, L. E., Wang, J., & Sevick, M. A. (2011). Self-Monitoring in Weight Loss: A Systematic Review of the Literature. *Journal of the American Dietetic Association*, *111*(1), 92–102. https://doi.org/10.1016/j.jada.2010.10.008

Butler, A. A., & Cone, R. D. (2003). Knockout studies defining different roles for melanocortin receptors in energy homeostasis. *Annals of the New York Academy of Sciences*, *994*, 240–245. https://doi.org/10.1111/j.1749-6632.2003.tb03186.x

Callaway, E. (2012). Soapy taste of coriander linked to genetic variants. *Nature*, nature.2012.11398. https://doi.org/10.1038/nature.2012.11398

Caner, N., & Evgin, D. (2021). Digital risks and adolescents: The relationships between digital game addiction, emotional eating, and aggression. *International Journal of Mental Health Nursing*, inm.12912. https://doi.org/10.1111/inm.12912

Cannon, B., & Nedergaard, J. (2004). Brown adipose tissue: Function and physiological significance. *Physiological Reviews*, *84*(1), 277–359. https://doi.org/10.1152/physrev.00015.2003

Carpenter, K. M., Hasin, D. S., Allison, D. B., & Faith, M. S. (2000). Relationships between obesity and DSM-IV major depressive disorder, suicide ideation, and suicide attempts: Results from a general population study. *American Journal of Public Health*, *90*(2), 251–257. https://doi.org/10.2105/AJPH.90.2.251

Centers for Disease Control and Prevention (CDC). (2018). *Safe Routes to School (SRTS)*. https://www.cdc.gov/policy/hst/hi5/saferoutes/index.html

Cerhan, J. R., Moore, S. C., Jacobs, E. J., Kitahara, C. M., Rosenberg, P. S., Adami, H.-O., Ebbert, J. O., English, D. R., Gapstur, S. M., Giles, G. G., Horn-Ross, P. L., Park, Y., Patel, A. V., Robien, K., Weiderpass, E., Willett, W. C., Wolk, A., Zeleniuch-Jacquotte, A., Hartge, P., … Berrington de Gonzalez, A. (2014). A Pooled Analysis of Waist Circumference and Mortality in 650,000 Adults. *Mayo Clinic Proceedings*, *89*(3), 335–345. https://doi.org/10.1016/j.mayocp.2013.11.011

Chang, S.-H., Stoll, C. R. T., Song, J., Varela, J. E., Eagon, C. J., & Colditz, G. A. (2014). The Effectiveness and Risks of Bariatric Surgery: An Updated Systematic Review and Meta-analysis, 2003-2012. *JAMA Surgery*, *149*(3), 275. https://doi.org/10.1001/jamasurg.2013.3654

Chavez, M., Seeley, R. J., & Woods, S. C. (1995). A comparison between effects of intraventricular insulin and intraperitoneal lithium chloride on three measures sensitive to emetic agents. *Behavioral Neuroscience*, *109*(3), 547–550.

Chen, E. Y., Tan, C. M., Kou, Y., Duan, Q., Wang, Z., Meirelles, G. V., Clark, N. R., & Ma'ayan, A. (2013). Enrichr: Interactive and collaborative HTML5 gene list enrichment analysis tool. *BMC Bioinformatics*, *14*, 128. https://doi.org/10.1186/1471-2105-14-128

Chen, L.-T., & Hsu, Y.-W. (2020). Socio-Ecological Predictors of Frequent Bike Share Trips: Do Purposes Matter? *International Journal of Environmental Research and Public Health*, *17*(20), 7640. https://doi.org/10.3390/ijerph17207640

Chen, L.-W., Aris, I. M., Bernard, J. Y., Tint, M.-T., Colega, M., Gluckman, P. D., Tan, K. H., Shek, L. P.-C., Chong, Y.-S., Yap, F., Godfrey, K. M., van Dam, R. M., Chong, M. F.-F., & Lee, Y. S. (2017). Associations of maternal macronutrient intake during pregnancy with infant BMI peak characteristics and childhood BMI. *The American Journal of Clinical Nutrition*, *105*(3), 705–713. https://doi.org/10.3945/ajcn.116.148270

Churchill, G. A., Airey, D. C., Allayee, H., Angel, J. M., Attie, A. D., Beatty, J., Beavis, W. D., Belknap, J. K., Bennett, B., Berrettini, W., Bleich, A., Bogue, M., Broman, K. W., Buck, K. J., Buckler, E., Burmeister, M., Chesler, E. J., Cheverud, J. M., Clapcote, S., … Complex Trait Consortium. (2004). The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nature Genetics*, *36*(11). https://doi.org/10.1038/ng1104-1133

Churchill, G. A., Gatti, D. M., Munger, S. C., & Svenson, K. L. (2012). The diversity outbred mouse population. *Mammalian Genome*, *23*(9–10), 713–718. https://doi.org/10.1007/s00335-012-9414-2
Clark, J. F., Dinsmore, C. J., & Soriano, P. (2020). A most formidable arsenal: Genetic technologies for building a better mouse. *Genes & Development*, *34*(19–20), 1256–1286. https://doi.org/10.1101/gad.342089.120
Claussnitzer, M., Dankel, S. N., Kim, K.-H., Quon, G., Meuleman, W., Haugen, C., Glunk, V., Sousa, I. S., Beaudry, J. L., Puviindran, V., Abdennur, N. A., Liu, J., Svensson, P.-A., Hsu, Y.-H., Drucker, D. J., Mellgren, G., Hui, C.-C., Hauner, H., & Kellis, M. (2015). FTO Obesity Variant Circuitry and Adipocyte Browning in Humans. *New England Journal of Medicine*, *373*(10). https://doi.org/10.1056/nejmoa1502214
Clément, K. (2006). Genetics of human obesity. *Comptes Rendus Biologies*, *329*(8), 608–622. https://doi.org/10.1016/j.crvi.2005.10.009
Colchero, M. A., Rivera-Dommarco, J., Popkin, B. M., & Ng, S. W. (2017). In Mexico, Evidence Of Sustained Consumer Response Two Years After Implementing A Sugar-Sweetened Beverage Tax. *Health Affairs*, *36*(3), 564–571. https://doi.org/10.1377/hlthaff.2016.1231
Collaborative Cross Consortium. (2012). The Genome Architecture of the Collaborative Cross Mouse Genetic Reference Population. *Genetics*, *190*(2). https://doi.org/10.1534/genetics.111.132639
Cornier, M.-A., Marshall, J. A., Hill, J. O., Maahs, D. M., & Eckel, R. H. (2011). Prevention of Overweight/Obesity as a Strategy to Optimize Cardiovascular Health. *Circulation*, *124*(7), 840–850. https://doi.org/10.1161/CIRCULATIONAHA.110.968461
Courcoulas, A. P. (2013). Weight Change and Health Outcomes at 3 Years After Bariatric Surgery Among Individuals With Severe Obesity. *JAMA*. https://doi.org/10.1001/jama.2013.280928
Cummings, D. E., Clement, K., Purnell, J. Q., Vaisse, C., Foster, K. E., Frayo, R. S., Schwartz, M. W., Basdevant, A., & Weigle, D. S. (2002). Elevated plasma ghrelin levels in Prader Willi syndrome. *Nature Medicine*, *8*(7), 643–644. https://doi.org/10.1038/nm0702-643
D'Agostino, G., & Diano, S. (2010). Alpha-melanocyte stimulating hormone: Production and degradation. *Journal of Molecular Medicine (Berlin, Germany)*, *88*(12), 1195–1201. https://doi.org/10.1007/s00109-010-0651-0
Damcott, C. M., Feingold, E., Moffett, S. P., Barmada, M. M., Marshall, J. A., Hamman, R. F., & Ferrell, R. E. (2004). Genetic variation in uncoupling protein 3 is associated with dietary intake and body composition in females. *Metabolism: Clinical and Experimental*, *53*(4), 458–464. https://doi.org/10.1016/j.metabol.2003.11.019
Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P., & Schaefer, E. J. (2005). Comparison of the Atkins, Ornish, Weight Watchers, and Zone Diets for Weight Loss and Heart Disease Risk Reduction. *JAMA*, *293*(1). https://doi.org/10.1001/jama.293.1.43
Darmon, N., & Drewnowski, A. (2015). Contribution of food prices and diet cost to socioeconomic disparities in diet quality and health: A systematic review and analysis. *Nutrition Reviews*, *73*(10), 643–660. https://doi.org/10.1093/nutrit/nuv027
de Heredia, F. P., Gómez-Martínez, S., & Marcos, A. (2012). Obesity, inflammation and the immune system. *Proceedings of the Nutrition Society*, *71*(2), 332–338. https://doi.org/10.1017/S0029665112000092
Delrue, M.-A., & Michaud, J. (2004). Fat chance: Genetic syndromes with obesity: Fat chance: genetic syndromes with obesity. *Clinical Genetics*, *66*(2), 83–93. https://doi.org/10.1111/j.0009-9163.2004.00300.x
Després, J.-P. (2012). Body Fat Distribution and Risk of Cardiovascular Disease: An Update. *Circulation*, *126*(10), 1301–1313. https://doi.org/10.1161/CIRCULATIONAHA.111.067264
Després, J.-P., & Lemieux, I. (2006). Abdominal obesity and metabolic syndrome. *Nature*, *444*(7121), 881–887. https://doi.org/10.1038/nature05488
Dobbs, R., Sawers, C., Thompson, F., Manyika, J., Woetzel, J., Child, P., McKenna, S., & Spatharou, A. (2014). *How the world could better fight obesity*. McKinsey Global Institute. https://www.mckinsey.com/industries/healthcare-systems-and-services/our-insights/how-the-world-could-better-fight-obesity
Dobson, R., Burgess, M. I., Sprung, V. S., Irwin, A., Hamer, M., Jones, J., Daousi, C., Adams, V., Kemp, G. J., Shojaee-Moradie, F., Umpleby, M., & Cuthbertson, D. J. (2015). Metabolically healthy and unhealthy obesity: Differential effects on myocardial function according to metabolic syndrome, rather than obesity. *International Journal of Obesity*, *40*(1). https://doi.org/10.1038/ijo.2015.151

Dong, B., Wang, Z., Wang, H.-J., & Ma, J. (2015). Associations between adiposity indicators and elevated blood pressure among Chinese children and adolescents. *Journal of Human Hypertension*, *29*(4), 236–240. https://doi.org/10.1038/jhh.2014.95

Dong, C., Li, W.-D., Li, D., & Price, R. A. (2006). Extreme obesity is associated with attempted suicides: Results from a family study. *International Journal of Obesity*, *30*(2), 388–390. https://doi.org/10.1038/sj.ijo.0803119

Donnelly, J. E., Blair, S. N., Jakicic, J. M., Manore, M. M., Rankin, J. W., & Smith, B. K. (2009). Appropriate Physical Activity Intervention Strategies for Weight Loss and Prevention of Weight Regain for Adults: *Medicine & Science in Sports & Exercise*, *41*(2), 459–471. https://doi.org/10.1249/MSS.0b013e3181949333

Drewnowski, A. (2018). Nutrient density: Addressing the challenge of obesity. *British Journal of Nutrition*, *120*(s1), S8–S14. https://doi.org/10.1017/S0007114517002240

Duffy, V. B., Hayes, J. E., Davidson, A. C., Kidd, J. R., Kidd, K. K., & Bartoshuk, L. M. (2010). Vegetable Intake in College-Aged Adults Is Explained by Oral Sensory Phenotypes and TAS2R38 Genotype. *Chemosensory Perception*, *3*(3–4), 137–148. https://doi.org/10.1007/s12078-010-9079-8

Durand, E., Boutin, P., Meyre, D., Charles, M. A., Clement, K., Dina, C., & Froguel, P. (2004). Polymorphisms in the Amino Acid Transporter Solute Carrier Family 6 (Neurotransmitter Transporter) Member 14 Gene Contribute to Polygenic Obesity in French Caucasians. *Diabetes*, *53*(9), 2483–2486. https://doi.org/10.2337/diabetes.53.9.2483

Duren, D. L., Sherwood, R. J., Czerwinski, S. A., Lee, M., Choh, A. C., Siervogel, R. M., & Chumlea, Wm. C. (2008). Body Composition Methods: Comparisons and Interpretation. *Journal of Diabetes Science and Technology*, *2*(6), 1139–1146. https://doi.org/10.1177/193229680800200623

Ebbeling, C. B., Swain, J. F., Feldman, H. A., Wong, W. W., Hachey, D. L., Garcia-Lago, E., & Ludwig, D. S. (2012). Effects of Dietary Composition on Energy Expenditure During Weight-Loss Maintenance. *JAMA*, *307*(24). https://doi.org/10.1001/jama.2012.6607

Eichler, E. E., Flint, J., Gibson, G., Kong, A., Leal, S. M., Moore, J. H., & Nadeau, J. H. (2010). Missing heritability and strategies for finding the underlying causes of complex disease. *Nature Reviews Genetics*, *11*(6), 446–450. https://doi.org/10.1038/nrg2809

Elks, C. E., den Hoed, M., Zhao, J. H., Sharp, S. J., Wareham, N. J., Loos, R. J. F., & Ong, K. K. (2012). Variability in the Heritability of Body Mass Index: A Systematic Review and Meta-Regression. *Frontiers in Endocrinology*, *3*. https://doi.org/10.3389/fendo.2012.00029

Elshahat, S., O'Rorke, M., & Adlakha, D. (2020). Built environment correlates of physical activity in low- and middle-income countries: A systematic review. *PLOS ONE*, *15*(3), e0230454. https://doi.org/10.1371/journal.pone.0230454

Enright, G., Gyani, A., Raadsma, S., Allman-Farinelli, M., Rissel, C., Innes-Hughes, C., Lukeis, S., Rodgers, A., & Redfern, J. (2016). Evaluating factors influencing the delivery and outcomes of an incentive-based behaviour change strategy targeting child obesity: Protocol for a qualitative process and impact evaluation. *BMJ Open*, *6*(12), e012536. https://doi.org/10.1136/bmjopen-2016-012536

Eriksson, J. G. (2016). Developmental Origins of Health and Disease – from a small body size at birth to epigenetics. *Annals of Medicine*, *48*(6), 456–467. https://doi.org/10.1080/07853890.2016.1193786

Eriksson, L., Esberg, A., Haworth, S., Holgerson, P. L., & Johansson, I. (2019). Allelic Variation in Taste Genes Is Associated with Taste and Diet Preferences and Dental Caries. *Nutrients*, *11*(7), 1491. https://doi.org/10.3390/nu11071491

Fabbrini, E., Magkos, F., Mohammed, B. S., Pietka, T., Abumrad, N. A., Patterson, B. W., Okunade, A., & Klein, S. (2009). Intrahepatic fat, not visceral fat, is linked with metabolic complications of obesity. *Proceedings of the National Academy of Sciences*, *106*(36), 15430–15435. https://doi.org/10.1073/pnas.0904944106

Faivre, L. (2002). Deletion of the SIM1 gene (6q16.2) in a patient with a Prader-Willi-like phenotype. *Journal of Medical Genetics*, *39*(8), 594–596. https://doi.org/10.1136/jmg.39.8.594

Falconer, D. S. (1989). *Introduction to Quantitative Genetics*. Longman, Scientific & Technical ; New York.

Farooqi, I. S. (2005). Genetic and hereditary aspects of childhood obesity. *Best Practice & Research Clinical Endocrinology & Metabolism*, *19*(3), 359–374. https://doi.org/10.1016/j.beem.2005.04.004

Farooqi, I. S., Keogh, J. M., Yeo, G. S. H., Lank, E. J., Cheetham, T., & O'Rahilly, S. (2003). Clinical spectrum of obesity and mutations in the melanocortin 4 receptor gene. *The New England Journal of Medicine*, *348*(12), 1085–1095. https://doi.org/10.1056/NEJMoa022050

Farooqi, I. S., & O'Rahilly, S. (2004). Monogenic human obesity syndromes. *Recent Progress in Hormone Research*, *59*, 409–424. https://doi.org/10.1210/rp.59.1.409

Feeney, E., O'Brien, S., Scannell, A., Markey, A., & Gibney, E. R. (2011). Genetic variation in taste perception: Does it have a role in healthy eating? *Proceedings of the Nutrition Society*, *70*(1), 135–143. https://doi.org/10.1017/S0029665110003976

Ferguson, L. R., De Caterina, R., Görman, U., Allayee, H., Kohlmeier, M., Prasad, C., Choi, M. S., Curi, R., de Luis, D. A., Gil, Á., Kang, J. X., Martin, R. L., Milagro, F. I., Nicoletti, C. F., Nonino, C. B., Ordovas, J. M., Parslow, V. R., Portillo, M. P., Santos, J. L., … Martinez, J. A. (2016). Guide and Position of the International Society of Nutrigenetics/Nutrigenomics on Personalised Nutrition: Part 1 - Fields of Precision Nutrition. *Lifestyle Genomics*, *9*(1), 12–27. https://doi.org/10.1159/000445350

Finkelstein, E. A., Khavjou, O. A., Thompson, H., Trogdon, J. G., Pan, L., Sherry, B., & Dietz, W. (2012). Obesity and Severe Obesity Forecasts Through 2030. *American Journal of Preventive Medicine*, *42*(6), 563–570. https://doi.org/10.1016/j.amepre.2011.10.026

Finkelstein, E. A., Trogdon, J. G., Cohen, J. W., & Dietz, W. (2009). Annual Medical Spending Attributable To Obesity: Payer-And Service-Specific Estimates: Amid calls for health reform, real cost savings are more likely to be achieved through reducing obesity and related risk factors. *Health Affairs*, *28*(Supplement 1), w822–w831. https://doi.org/10.1377/hlthaff.28.5.w822

Flier, J. S., Harris, M., & Hollenberg, A. N. (2000). Leptin, nutrition, and the thyroid: The why, the wherefore, and the wiring. *Journal of Clinical Investigation*, *105*(7), 859–861. https://doi.org/10.1172/JCI9725

Fosbøl, M. Ø., & Zerahn, B. (2015). Contemporary methods of body composition measurement. *Clinical Physiology and Functional Imaging*, *35*(2), 81–97. https://doi.org/10.1111/cpf.12152

Frayling, T. M., Timpson, N. J., Weedon, M. N., Zeggini, E., Freathy, R. M., Lindgren, C. M., Perry, J. R. B., Elliott, K. S., Lango, H., Rayner, N. W., Shields, B., Harries, L. W., Barrett, J. C., Ellard, S., Groves, C. J., Knight, B., Patch, A.-M., Ness, A. R., Ebrahim, S., … McCarthy, M. I. (2007). A Common Variant in the FTO Gene Is Associated with Body Mass Index and Predisposes to Childhood and Adult Obesity. *Science*, *316*(5826), 889–894. https://doi.org/10.1126/science.1141634

Friedman, J. M., & Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. *Nature*, *395*(6704). https://doi.org/10.1038/27376

Fulton, M., & Srinivasan, V. N. (2021). Obesity, Stigma And Discrimination. *In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing*. https://www.ncbi.nlm.nih.gov/books/NBK554571/#

Gale, S. M., Daniel Castracane, V., & Christos, S. (2004). Energy Homeostasis, Obesity and Eating Disorders: Recent Advances in Endocrinology. *Obesity and Metabolism*, *1*(1), 39. https://doi.org/10.14341/2071-8713-5181

García-García, M. J. (2020). A History of Mouse Genetics: From Fancy Mice to Mutations in Every Gene. In A. Liu (Ed.), *Animal Models of Human Birth Defects* (Vol. 1236, pp. 1–38). Springer Singapore. https://doi.org/10.1007/978-981-15-2389-2_1

Gardner, C. D., Trepanowski, J. F., Del Gobbo, L. C., Hauser, M. E., Rigdon, J., Ioannidis, J. P. A., Desai, M., & King, A. C. (2018). Effect of Low-Fat vs Low-Carbohydrate Diet on 12-Month Weight Loss in Overweight Adults and the Association With Genotype Pattern or Insulin Secretion. *JAMA*, *319*(7). https://doi.org/10.1001/jama.2018.0245

Garfield, A. S., Lam, D. D., Marston, O. J., Przydzial, M. J., & Heisler, L. K. (2009). Role of central melanocortin pathways in energy homeostasis. *Trends in Endocrinology & Metabolism*, *20*(5), 203–215. https://doi.org/10.1016/j.tem.2009.02.002

Garrigan, D., Kingan, S. B., Pilkington, M. M., Wilder, J. A., Cox, M. P., Soodyall, H., Strassmann, B., Destro-Bisol, G., de Knijff, P., Novelletto, A., Friedlaender, J., & Hammer, M. F. (2007). Inferring Human Population Sizes, Divergence Times and Rates of Gene Flow From Mitochondrial, X and Y Chromosome Resequencing Data. *Genetics*, *177*(4), 2195–2207. https://doi.org/10.1534/genetics.107.077495

Gazzaniga, M., Heatherton, T., & Halpern, D. (2016). *Psychological science* (5th edition, International Student edition). W. W. Norton.

Goldstone, A. P. (2004). Prader-Willi syndrome: Advances in genetics, pathophysiology and treatment. *Trends in Endocrinology & Metabolism*, *15*(1), 12–20. https://doi.org/10.1016/j.tem.2003.11.003

Gong, D.-W., He, Y., Karas, M., & Reitman, M. (1997). Uncoupling Protein-3 Is a Mediator of Thermogenesis Regulated by Thyroid Hormone, β3-Adrenergic Agonists, and Leptin. *Journal of Biological Chemistry*, *272*(39), 24129–24132. https://doi.org/10.1074/jbc.272.39.24129

Goni, L., Cuervo, M., Milagro, F. I., & Martínez, J. A. (2015). Future Perspectives of Personalized Weight Loss Interventions Based on Nutrigenetic, Epigenetic, and Metagenomic Data. *The Journal of Nutrition*, *146*(4), 905S-912S. https://doi.org/10.3945/jn.115.218354

González-Jiménez, E., Aguilar Cordero, M. J., Padilla López, C. A., & García García, I. (2012). Monogenic human obesity: Role of the leptin-melanocortin system in the regulation of food intake and body weight in humans. *Anales Del Sistema Sanitario De Navarra*, *35*(2), 285–293. https://doi.org/10.4321/s1137-66272012000200010

González-Morales, R., Canto-Osorio, F., Stern, D., Sánchez-Romero, L. M., Torres-Ibarra, L., Hernández-López, R., Rivera-Paredez, B., Vidaña-Pérez, D., Ramírez-Palacios, P., Salmerón, J., Popkin, B. M., & Barrientos-Gutiérrez, T. (2020). Soft drink intake is associated with weight gain, regardless of physical activity levels: The health workers cohort study. *International Journal of Behavioral Nutrition and Physical Activity*, *17*(1), 60. https://doi.org/10.1186/s12966-020-00963-2

González-Muniesa, P., Garcia-Gerique, L., Quintero, P., Arriaza, S., Lopez-Pascual, A., & Martinez, J. A. (2016). Effects of Hyperoxia on Oxygen-Related Inflammation with a Focus on Obesity. *Oxidative Medicine and Cellular Longevity*, *2016*, 1–11. https://doi.org/10.1155/2016/8957827

González-Muniesa, P., Mártinez-González, M.-A., Hu, F. B., Després, J.-P., Matsuzawa, Y., Loos, R. J. F., Moreno, L. A., Bray, G. A., & Martinez, J. A. (2017). Obesity. *Nature Reviews Disease Primers*, *3*. https://doi.org/10.1038/nrdp.2017.34

Graham, C. A.-M., Pilic, L., Mcgrigor, E., Brown, M., Easton, I. J., Kean, J. N., Sarel, V., Wehliye, Y., Davis, N., Hares, N., Barac, D., King, A., & Mavrommatis, Y. (2021). The Associations Between Bitter and Fat Taste Sensitivity, and Dietary Fat Intake: Are They Impacted by Genetic Predisposition? *Chemical Senses*, *46*, bjab029. https://doi.org/10.1093/chemse/bjab029

Grupo de Estudio Navarro de Obesidad Infantil (GENOI), Ochoa, M. C., Marti, A., Azcona, C., Chueca, M., Oyarzábal, M., Pelach, R., Patiño, A., Moreno-Aliaga, M. J., Martínez-González, M. A., & Martínez, J. A. (2004). Gene–gene interaction between PPARγ2 and ADRβ3 increases obesity risk in children and adolescents. *International Journal of Obesity*, *28*(S3), S37–S41. https://doi.org/10.1038/sj.ijo.0802803

Guo, D.-F., & Rahmouni, K. (2011). Molecular basis of the obesity associated with Bardet-Biedl syndrome. *Trends in Endocrinology and Metabolism: TEM*, *22*(7), 286–293. https://doi.org/10.1016/j.tem.2011.02.009

Guo, F., Moellering, D. R., & Garvey, W. T. (2014). The progression of cardiometabolic disease: Validation of a new cardiometabolic disease staging system applicable to obesity: CMDS Risk Staging System. *Obesity*, *22*(1), 110–118. https://doi.org/10.1002/oby.20585

Gupta, R. D., Ramachandran, R., Venkatesan, P., Anoop, S., Joseph, M., & Thomas, N. (2017). Indirect Calorimetry: From Bench to Bedside. *Indian Journal of Endocrinology and Metabolism*, *21*(4), 594–599. https://doi.org/10.4103/ijem.IJEM_484_16

Hall, K. D., Bemis, T., Brychta, R., Chen, K. Y., Courville, A., Crayner, E. J., Goodwin, S., Guo, J., Howard, L., Knuth, N. D., Miller, B. V., Prado, C. M., Siervo, M., Skarulis, M. C., Walter, M., Walter, P. J., & Yannai, L. (2015). Calorie for Calorie, Dietary Fat Restriction Results in More Body Fat Loss than Carbohydrate Restriction in People with Obesity. *Cell Metabolism*, *22*(3), 427–436. https://doi.org/10.1016/j.cmet.2015.07.021

Hammarstedt, A., Gogg, S., Hedjazifar, S., Nerstedt, A., & Smith, U. (2018). Impaired Adipogenesis and Dysfunctional Adipose Tissue in Human Hypertrophic Obesity. *Physiological Reviews*, *98*(4), 1911–1941. https://doi.org/10.1152/physrev.00034.2017

Hanley, B., Dijane, J., Fewtrell, M., Grynberg, A., Hummel, S., Junien, C., Koletzko, B., Lewis, S., Renz, H., Symonds, M., Gros, M., Harthoorn, L., Mace, K., Samuels, F., & van Der Beek, E. M. (2010). Metabolic imprinting, programming and epigenetics – a review of present priorities and future opportunities. *British Journal of Nutrition*, *104*(S1), S1–S25. https://doi.org/10.1017/S0007114510003338

Harms, D. W., Quadros, R. M., Seruggia, D., Ohtsuka, M., Takahashi, G., Montoliu, L., & Gurumurthy, C. B. (2014). Mouse Genome Editing Using the CRISPR/Cas System. *Current Protocols in Human Genetics*, *83*, 15.7.1-27. https://doi.org/10.1002/0471142905.hg1507s83

Haugen, H. A., Chan, L.-N., & Li, F. (2007). Indirect calorimetry: A practical guide for clinicians. *Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition*, *22*(4), 377–388. https://doi.org/10.1177/0115426507022004377

Heilbronn, K., Smith, R., & Ravussin, E. (2003). The Insulin-sensitizing Role of the Fat Derived Hormone Adiponectin. *Current Pharmaceutical Design*, *9*(17), 1411–1418. https://doi.org/10.2174/1381612033454766

Henn, B. M., Cavalli-Sforza, L. L., & Feldman, M. W. (2012). The great human expansion. *Proceedings of the National Academy of Sciences*, *109*(44), 17758–17764. https://doi.org/10.1073/pnas.1212380109

Hernández-Conde, M., Llop, E., Fernández-Carrillo, C., Perelló, C., López-Gómez, M., Abad, J., Martínez-Porras, J. L., Fernández-Puga, N., & Calleja, J. L. (2019). Visceral fat is associated with cirrhotic portal vein thrombosis. *Expert Review of Gastroenterology & Hepatology*, *13*(10), 1017–1022. https://doi.org/10.1080/17474124.2019.1651644

Herrmann, S.-M., Wang, J.-G., Staessen, J. A., Kertmen, E., Schmidt-Petersen, K., Zidek, W., Paul, M., & Brand, E. (2003). Uncoupling protein 1 and 3 polymorphisms are associated with waist-to-hip ratio. *Journal of Molecular Medicine (Berlin, Germany)*, *81*(5), 327–332. https://doi.org/10.1007/s00109-003-0431-1

Heymsfield, S. B., Ebbeling, C. B., Zheng, J., Pietrobelli, A., Strauss, B. J., Silva, A. M., & Ludwig, D. S. (2015). Multi-component molecular-level body composition reference methods: Evolving concepts and future directions: Body composition reference methods. *Obesity Reviews*, *16*(4), 282–294. https://doi.org/10.1111/obr.12261

Hill, D. C., Moss, R. H., Sykes-Muskett, B., Conner, M., & O'Connor, D. B. (2018). Stress and eating behaviors in children and adolescents: Systematic review and meta-analysis. *Appetite*, *123*, 14–22. https://doi.org/10.1016/j.appet.2017.11.109

Hill, J. O., Wyatt, H. R., & Peters, J. C. (2012). Energy Balance and Obesity. *Circulation*, *126*(1). https://doi.org/10.1161/circulationaha.111.087213

Hinney, A., Vogel, C. I. G., & Hebebrand, J. (2010). From monogenic to polygenic obesity: Recent advances. *European Child & Adolescent Psychiatry*, *19*(3), 297–310. https://doi.org/10.1007/s00787-010-0096-6

Hiuge-Shimizu, A., Kishida, K., Funahashi, T., Ishizaka, Y., Oka, R., Okada, M., Suzuki, S., Takaya, N., Nakagawa, T., Fukui, T., Fukuda, H., Watanabe, N., Yoshizumi, T., Nakamura, T., Matsuzawa, Y., Yamakado, M., & Shimomura, I. (2012). Absolute value of visceral fat area measured on computed tomography scans and obesity-related cardiovascular risk factors in large-scale Japanese general population (the VACATION-J study). *Annals of Medicine*, *44*(1), 82–92. https://doi.org/10.3109/07853890.2010.526138

Holder, J. L., Butte, N. F., & Zinn, A. R. (2000). Profound obesity associated with a balanced translocation that disrupts the SIM1 gene. *Human Molecular Genetics*, *9*(1), 101–108. https://doi.org/10.1093/hmg/9.1.101

Howard, A. D., Feighner, S. D., Cully, D. F., Arena, J. P., Liberator, P. A., Rosenblum, C. I., Hamelin, M., Hreniuk, D. L., Palyha, O. C., Anderson, J., Paress, P. S., Diaz, C., Chou, M., Liu, K. K., McKee, K. K., Pong, S. S., Chaung, L. Y., Elbrecht, A., Dashkevicz, M., … Van der Ploeg, L. H. (1996). A receptor in pituitary and hypothalamus that functions in growth hormone release. *Science (New York, N.Y.)*, *273*(5277), 974–977. https://doi.org/10.1126/science.273.5277.974

Hu, F. B. (2008). *Obesity Epidemiology*. Oxford University Press.

Ibrahim, M. M. (2010). Subcutaneous and visceral adipose tissue: Structural and functional differences. *Obesity Reviews*, *11*(1), 11–18. https://doi.org/10.1111/j.1467-789X.2009.00623.x

Inge, T. H., Courcoulas, A. P., Jenkins, T. M., Michalsky, M. P., Helmrath, M. A., Brandt, M. L., Harmon, C. M., Zeller, M. H., Chen, M. K., Xanthakos, S. A., Horlick, M., & Buncher, C. R. (2016). Weight Loss and Health Status 3 Years after Bariatric Surgery in Adolescents. *New England Journal of Medicine*, *374*(2), 113–123. https://doi.org/10.1056/NEJMoa1506699

Iraqi, F. A., Churchill, G., & Mott, R. (2008). The Collaborative Cross, developing a resource for mammalian systems genetics: A status report of the Wellcome Trust cohort. *Mammalian Genome*, *19*(6). https://doi.org/10.1007/s00335-008-9113-1

Islam, Md. S., Sujan, Md. S. H., Tasnim, R., Ferdous, Most. Z., Masud, J. H. B., Kundu, S., Mosaddek, A. S. Md., Choudhuri, M. S. K., Kircaburun, K., & Griffiths, M. D. (2020). Problematic internet use among young and adult population in Bangladesh: Correlates with lifestyle and online activities during the COVID-19 pandemic. *Addictive Behaviors Reports*, *12*, 100311. https://doi.org/10.1016/j.abrep.2020.100311

Jackson, P. J., Douglas, N. R., Chai, B., Binkley, J., Sidow, A., Barsh, G. S., & Millhauser, G. L. (2006). Structural and Molecular Evolutionary Analysis of Agouti and Agouti-Related Proteins. *Chemistry & Biology*, *13*(12), 1297–1305. https://doi.org/10.1016/j.chembiol.2006.10.006

Jakicic, J. M. (2008). Effect of Exercise on 24-Month Weight Loss Maintenance in Overweight Women. *Archives of Internal Medicine*, *168*(14), 1550. https://doi.org/10.1001/archinte.168.14.1550

Jáuregui, A., Pratt, M., Lamadrid-Figueroa, H., Hernández, B., Rivera, J. A., & Salvo, D. (2016). Perceived Neighborhood Environment and Physical Activity. *American Journal of Preventive Medicine*, *51*(2), 271–279. https://doi.org/10.1016/j.amepre.2016.03.026

Javed, A., Jumean, M., Murad, M. H., Okorodudu, D., Kumar, S., Somers, V. K., Sochor, O., & Lopez-Jimenez, F. (2015). Diagnostic performance of body mass index to identify obesity as defined by body adiposity in children and adolescents: A systematic review and meta-analysis: Diagnostic performance of BMI to identify obesity. *Pediatric Obesity*, *10*(3), 234–244. https://doi.org/10.1111/ijpo.242

Jiang, Y., Tsai, T.-F., Bressler, J., & Beaudet, A. L. (1998). Imprinting in Angelman and Prader-Willi syndromes. *Current Opinion in Genetics & Development*, *8*(3), 334–342. https://doi.org/10.1016/S0959-437X(98)80091-9

Johnson, M. S., & Speakman, J. R. (2001). Limits to sustained energy intake. V. Effect of cold-exposure during lactation in Mus musculus. *The Journal of Experimental Biology*, *204*(Pt 11), 1967–1977.

Johnston, B. C., Kanters, S., Bandayrel, K., Wu, P., Naji, F., Siemieniuk, R. A., Ball, G. D. C., Busse, J. W., Thorlund, K., Guyatt, G., Jansen, J. P., & Mills, E. J. (2014). Comparison of Weight Loss Among Named Diet Programs in Overweight and Obese Adults. *JAMA*, *312*(9). https://doi.org/10.1001/jama.2014.10397

Juul, F., Martinez-Steele, E., Parekh, N., Monteiro, C. A., & Chang, V. W. (2018). Ultra-processed food consumption and excess weight among US adults. *British Journal of Nutrition*, *120*(1), 90–100. https://doi.org/10.1017/S0007114518001046

Kanehisa, M. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, *28*(1), 27–30. https://doi.org/10.1093/nar/28.1.27

Karpe, F., & Pinnick, K. E. (2015). Biology of upper-body and lower-body adipose tissue—Link to whole-body phenotypes. *Nature Reviews Endocrinology*, *11*(2), 90–100. https://doi.org/10.1038/nrendo.2014.185

Katch, V. L., McArdle, W. D., Katch, F. I., & McArdle, W. D. (2011). *Essentials of exercise physiology* (4th ed). Wolters Kluwer/Lippincott Williams & Wilkins Health.

Katzmarzyk, P. T., Malina, R. M., Pérusse, L., Rice, T., Province, M. A., Rao, D. C., & Bouchard, C. (2000). Familial resemblance in fatness and fat distribution. *American Journal of Human Biology*, *12*(3), 395–404. https://doi.org/10.1002/(SICI)1520-6300(200005/06)12:3<395::AID-AJHB10>3.0.CO;2-J

Keane, T. M., Goodstadt, L., Danecek, P., White, M. A., Wong, K., Yalcin, B., Heger, A., Agam, A., Slater, G., Goodson, M., Furlotte, N. A., Eskin, E., Nellåker, C., Whitley, H., Cleak, J., Janowitz, D., Hernandez-Pliego, P., Edwards, A., Belgard, T. G., … Adams, D. J. (2011). Mouse genomic variation and its effect on phenotypes and gene regulation. *Nature*, *477*(7364), 289–294. https://doi.org/10.1038/nature10413

Kegler, M. C., Hermstad, A., & Haardörfer, R. (2021). Home food environment and associations with weight and diet among U.S. adults: A cross-sectional study. *BMC Public Health*, *21*(1), 1032. https://doi.org/10.1186/s12889-021-11102-2

Kelley, C. P., Sbrocco, G., & Sbrocco, T. (2016). Behavioral Modification for the Management of Obesity. *Primary Care: Clinics in Office Practice*, *43*(1), 159–175. https://doi.org/10.1016/j.pop.2015.10.004

Kelly, T., Yang, W., Chen, C.-S., Reynolds, K., & He, J. (2008). Global burden of obesity in 2005 and projections to 2030. *International Journal of Obesity*, *32*(9), 1431–1437. https://doi.org/10.1038/ijo.2008.102

Kenney, W. L., Wilmore, J. H., Costill, D. L., & Wilmore, J. H. (2012). *Physiology of sport and exercise* (5th ed). Human Kinetics.

Kim, H. J., Oh, S. Y., Choi, D.-W., & Park, E.-C. (2019). The Association between Eating-Out Rate and BMI in Korea. *International Journal of Environmental Research and Public Health*, *16*(17), 3186. https://doi.org/10.3390/ijerph16173186

Knaapila, A., Hwang, L.-D., Lysenko, A., Duke, F. F., Fesi, B., Khoshnevisan, A., James, R. S., Wysocki, C. J., Rhyu, M., Tordoff, M. G., Bachmanov, A. A., Mura, E., Nagai, H., & Reed, D. R. (2012). Genetic Analysis of Chemosensory Traits in Human Twins. *Chemical Senses*, *37*(9), 869–881. https://doi.org/10.1093/chemse/bjs070

Koehler, K., Latshaw, M., Matte, T., Kass, D., Frumkin, H., Fox, M., Hobbs, B. F., Wills-Karp, M., & Burke, T. A. (2018). Building Healthy Community Environments: A Public Health Approach. *Public Health Reports*, *133*(1_suppl), 35S-43S. https://doi.org/10.1177/0033354918798809

Koeppen-Schomerus, G., Wardle, J., & Plomin, R. (2001). A genetic analysis of weight and overweight in 4-year-old twin pairs. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, *25*(6), 838–844. https://doi.org/10.1038/sj.ijo.0801589

Kohno, D., Gao, H.-Z., Muroya, S., Kikuyama, S., & Yada, T. (2003). Ghrelin Directly Interacts With Neuropeptide-Y-Containing Neurons in the Rat Arcuate Nucleus: Ca2+ Signaling via Protein Kinase A and N-Type Channel-Dependent Mechanisms and Cross-Talk With Leptin and Orexin. *Diabetes*, *52*(4), 948–956. https://doi.org/10.2337/diabetes.52.4.948

Kotani, K., Tokunaga, K., Fujioka, S., Kobatake, T., Keno, Y., Yoshida, S., Shimomura, I., Tarui, S., & Matsuzawa, Y. (1994). Sexual dimorphism of age-related changes in whole-body fat distribution in the obese. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, *18*(4), 207–202.

Kublaoui, B. M., & Zinn, A. R. (2006). MC4R Mutations—Weight before Screening! *The Journal of Clinical Endocrinology & Metabolism*, *91*(5), 1671–1672. https://doi.org/10.1210/jc.2006-0546

Kunej, T., Skok, D. J., Zorc, M., Ogrinc, A., Michal, J. J., Kovac, M., & Jiang, Z. (2013). Obesity Gene Atlas in Mammals. *Journal of Genomics*, *1*(Dec 1). https://doi.org/10.7150/jgen.3996

Labayen, I., Ruiz, J. R., Vicente-Rodriguez, G., Turck, D., Rodriguez, G., Meirhaeghe, A., Molnar, D., Sjostrom, M., Castillo, M. J., Gottrand, F., Moreno, L. A., & on behalf of the Healthy Lifestyle in Europe by Nutrition in Adolescence (HELENA) Study Group. (2009). Early Life Programming of Abdominal Adiposity in Adolescents: The HELENA Study. *Diabetes Care*, *32*(11), 2120–2122. https://doi.org/10.2337/dc09-0983

Larsen, T. M., Dalskov, S.-M., van Baak, M., Jebb, S. A., Papadaki, A., Pfeiffer, A. F. H., Martinez, J. A., Handjieva-Darlenska, T., Kunešová, M., Pihlsgård, M., Stender, S., Holst, C., Saris, W. H. M., & Astrup, A. (2010). Diets with High or Low Protein Content and Glycemic Index for Weight-Loss Maintenance. *New England Journal of Medicine*, *363*(22). https://doi.org/10.1056/nejmoa1007137

Leng, J., Lui, F., Narang, B., Puebla, L., González, J., Lynch, K., & Gany, F. (2021). Developing a Culturally Responsive Lifestyle Intervention for Overweight/Obese U.S. Mexicans. *Journal of Community Health*. https://doi.org/10.1007/s10900-021-01016-w

Lima, J. P. M., Costa, S. A., Brandão, T. R. S., & Rocha, A. (2021). Food Consumption Determinants and Barriers for Healthy Eating at the Workplace—A University Setting. *Foods*, *10*(4), 695. https://doi.org/10.3390/foods10040695

López, M., Alvarez, C. V., Nogueiras, R., & Diéguez, C. (2013). Energy balance regulation by thyroid hormones at central level. *Trends in Molecular Medicine*, *19*(7), 418–427. https://doi.org/10.1016/j.molmed.2013.04.004

Lu, D., Willard, D., Patel, I. R., Kadwell, S., Overton, L., Kost, T., Luther, M., Chen, W., Woychik, R. P., Wilkison, W. O., & Cone, R. D. (1994). Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. *Nature*, *371*(6500). https://doi.org/10.1038/371799a0

Lubrano-Berthelier, C., Durand, E., Dubern, B., Shapiro, A., Dazin, P., Weill, J., Ferron, C., Froguel, P., & Vaisse, C. (2003). Intracellular retention is a common characteristic of childhood obesity-associated MC4R mutations. *Human Molecular Genetics*, *12*(2), 145–153. https://doi.org/10.1093/hmg/ddg016

Ludwig, D. S. (2016). Lifespan Weighed Down by Diet. *JAMA*, *315*(21), 2269. https://doi.org/10.1001/jama.2016.3829

Maher, B. (2008). Personal genomes: The case of the missing heritability. *Nature*, *456*(7218), 18–21. https://doi.org/10.1038/456018a

Malik, V. S., Willet, W. C., & Hu, F. B. (2020). Nearly a decade on—Trends, risk factors and policy implications in global obesity. *Nature Reviews Endocrinology*, *16*(11), 615–616. https://doi.org/10.1038/s41574-020-00411-y

Malik, V. S., Willett, W. C., & Hu, F. B. (2013). Global obesity: Trends, risk factors and policy implications. *Nature Reviews Endocrinology*, *9*(1), 13–27. https://doi.org/10.1038/nrendo.2012.199

Manolio, T. A., Collins, F. S., Cox, N. J., Goldstein, D. B., Hindorff, L. A., Hunter, D. J., McCarthy, M. I., Ramos, E. M., Cardon, L. R., Chakravarti, A., Cho, J. H., Guttmacher, A. E., Kong, A., Kruglyak, L., Mardis, E., Rotimi, C. N., Slatkin, M., Valle, D., Whittemore, A. S., … Visscher, P. M. (2009). Finding the missing heritability of complex diseases. *Nature*, *461*(7265), 747–753. https://doi.org/10.1038/nature08494

Marieb, E. N., & Hoehn, K. (2008). *Anatomy & physiology* (3rd ed). Pearson/Benjamin Cummings.

Martinez, J. A., Navas-Carretero, S., Saris, W. H. M., & Astrup, A. (2014). Personalized weight loss strategies—The role of macronutrient distribution. *Nature Reviews Endocrinology*, *10*(12), 749–760. https://doi.org/10.1038/nrendo.2014.175

Martinez, K. B., Pierre, J. F., & Chang, E. B. (2016). The Gut Microbiota. *Gastroenterology Clinics of North America*, *45*(4). https://doi.org/10.1016/j.gtc.2016.07.001

Martinho, V. J. P. D., Bartkiene, E., Djekic, I., Tarcea, M., Barić, I. C., Černelič-Bizjak, M., Szűcs, V., Sarcona, A., El-Kenawy, A., Ferreira, V., Klava, D., Korzeniowska, M., Vittadini, E., Leal, M., Bolhuis, D., Papageorgiou, M., & Guiné, R. P. F. (2021). Determinants of economic motivations for food choice: Insights for the understanding of consumer behaviour. *International Journal of Food Sciences and Nutrition*, 1–13. https://doi.org/10.1080/09637486.2021.1939659

Matsuzaki, M., Sánchez, B. N., Acosta, M. E., Botkin, J., & Sanchez-Vaznaugh, E. V. (2020). Food environment near schools and body weight—A systematic review of associations by race/ethnicity, gender, grade, and socio-economic factors. *Obesity Reviews*, *21*(4). https://doi.org/10.1111/obr.12997

Matsuzawa, Y. (2006). The metabolic syndrome and adipocytokines. *FEBS Letters*, *580*(12), 2917–2921. https://doi.org/10.1016/j.febslet.2006.04.028

Matthews, C. E., Carlson, S. A., Saint-Maurice, P. F., Patel, S., Salerno, E., Loftfield, E., Troiano, R. P., Fulton, J. E., Sampson, J. N., Tribby, C., Keadle, S., & Berrigan, D. (2021). Sedentary Behavior in United States Adults: Fall 2019. *Medicine & Science in Sports & Exercise*, *Publish Ahead of Print*. https://doi.org/10.1249/MSS.0000000000002751

McClave, S. A., & Snider, H. L. (1992). Use of indirect calorimetry in clinical nutrition. *Nutrition in Clinical Practice: Official Publication of the American Society for Parenteral and Enteral Nutrition*, *7*(5), 207–221. https://doi.org/10.1177/0115426592007005207

Minokoshi, Y., Kim, Y.-B., Peroni, O. D., Fryer, L. G. D., Müller, C., Carling, D., & Kahn, B. B. (2002). Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. *Nature*, *415*(6869), 339–343. https://doi.org/10.1038/415339a

Miranda, R. C. K., Vetter, S. B., Genro, J. P., Campagnolo, P. D. B., Mattevi, V. S., Vitolo, M. R., & Almeida, S. (2015). SLC6A14 and 5-HTR2C polymorphisms are associated with food intake and nutritional status in children. *Clinical Biochemistry*, *48*(18), 1277–1282. https://doi.org/10.1016/j.clinbiochem.2015.07.003

Moll, P. P., Burns, T. L., & Lauer, R. M. (1991). The genetic and environmental sources of body mass index variability: The Muscatine Ponderosity Family Study. *American Journal of Human Genetics*, *49*(6), 1243–1255.

Mongraw-Chaffin, M., Foster, M. C., Kalyani, R. R., Vaidya, D., Burke, G. L., Woodward, M., & Anderson, C. A. M. (2016). Obesity Severity and Duration Are Associated With Incident Metabolic Syndrome: Evidence Against Metabolically Healthy Obesity From the Multi-Ethnic Study of Atherosclerosis. *The Journal of Clinical Endocrinology & Metabolism*, *101*(11), 4117–4124. https://doi.org/10.1210/jc.2016-2460

Monsivais, P., Aggarwal, A., & Drewnowski, A. (2012). Are socio-economic disparities in diet quality explained by diet cost? *Journal of Epidemiology and Community Health*, *66*(6), 530–535. https://doi.org/10.1136/jech.2010.122333

Mougharbel, F., Valois, D. D., Lamb, M., Buchholz, A., Obeid, N., Flament, M., & Goldfield, G. S. (2020). Mediating role of disordered eating in the relationship between screen time and BMI in

adolescents: Longitudinal findings from the Research on Eating and Adolescent Lifestyles (REAL) study. *Public Health Nutrition*, *23*(18), 3336–3345. https://doi.org/10.1017/S136898002000049X
Moussa, O., Arhi, C., Ziprin, P., Darzi, A., Khan, O., & Purkayastha, S. (2019). Fate of the metabolically healthy obese—is this term a misnomer? A study from the Clinical Practice Research Datalink. *International Journal of Obesity*, *43*(5), 1093–1101. https://doi.org/10.1038/s41366-018-0096-z
Mozaffarian, D. (2016a). Dietary and Policy Priorities for Cardiovascular Disease, Diabetes, and Obesity: A Comprehensive Review. *Circulation*, *133*(2), 187–225. https://doi.org/10.1161/CIRCULATIONAHA.115.018585
Mozaffarian, D. (2016b). Food and weight gain: Time to end our fear of fat. *The Lancet Diabetes & Endocrinology*, *4*(8), 633–635. https://doi.org/10.1016/S2213-8587(16)30106-1
Mozaffarian, D., Hao, T., Rimm, E. B., Willett, W. C., & Hu, F. B. (2011). Changes in Diet and Lifestyle and Long-Term Weight Gain in Women and Men. *New England Journal of Medicine*, *364*(25), 2392–2404. https://doi.org/10.1056/NEJMoa1014296
Mullur, R., Liu, Y.-Y., & Brent, G. A. (2014). Thyroid hormone regulation of metabolism. *Physiological Reviews*, *94*(2), 355–382. https://doi.org/10.1152/physrev.00030.2013
Mylona, E. K., Shehadeh, F., Fleury, E., Kalligeros, M., & Mylonakis, E. (2020). Neighborhood-Level Analysis on the Impact of Accessibility to Fast Food and Open Green Spaces on the Prevalence of Obesity. *The American Journal of Medicine*, *133*(3), 340-346.e1. https://doi.org/10.1016/j.amjmed.2019.08.024
Nago, E. S., Lachat, C. K., Dossa, R. A. M., & Kolsteren, P. W. (2014). Association of Out-of-Home Eating with Anthropometric Changes: A Systematic Review of Prospective Studies. *Critical Reviews in Food Science and Nutrition*, *54*(9), 1103–1116. https://doi.org/10.1080/10408398.2011.627095
Naik, R. D., Choksi, Y. A., & Vaezi, M. F. (2016). Consequences of bariatric surgery on oesophageal function in health and disease. *Nature Reviews Gastroenterology & Hepatology*, *13*(2), 111–119. https://doi.org/10.1038/nrgastro.2015.202
NCD Risk Factor Collaboration (NCD-RisC). (2016). Trends in adult body-mass index in 200 countries from 1975 to 2014: A pooled analysis of 1698 population-based measurement studies with 19·2 million participants. *The Lancet*, *387*(10026), 1377–1396. https://doi.org/10.1016/S0140-6736(16)30054-X
NCD Risk Factor Collaboration (NCD-RisC). (2019). Rising rural body-mass index is the main driver of the global obesity epidemic in adults. *Nature*, *569*(7755), 260–264. https://doi.org/10.1038/s41586-019-1171-x
Neeland, I. J., Turer, A. T., Ayers, C. R., Berry, J. D., Rohatgi, A., Das, S. R., Khera, A., Vega, G. L., McGuire, D. K., Grundy, S. M., & de Lemos, J. A. (2015). Body Fat Distribution and Incident Cardiovascular Disease in Obese Adults. *Journal of the American College of Cardiology*, *65*(19), 2150–2151. https://doi.org/10.1016/j.jacc.2015.01.061
Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E. C., Biryukov, S., Abbafati, C., Abera, S. F., Abraham, J. P., Abu-Rmeileh, N. M. E., Achoki, T., AlBuhairan, F. S., Alemu, Z. A., Alfonso, R., Ali, M. K., Ali, R., Guzman, N. A., … Gakidou, E. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, *384*(9945), 766–781. https://doi.org/10.1016/S0140-6736(14)60460-8
Nicholls, R., Perry, L., Duffield, C., Gallagher, R., & Pierce, H. (2017). Barriers and facilitators to healthy eating for nurses in the workplace: An integrative review. *Journal of Advanced Nursing*, *73*(5), 1051–1065. https://doi.org/10.1111/jan.13185
Nordestgaard, B. G., Palmer, T. M., Benn, M., Zacho, J., Tybjærg-Hansen, A., Davey Smith, G., & Timpson, N. J. (2012). The Effect of Elevated Body Mass Index on Ischemic Heart Disease Risk: Causal Estimates from a Mendelian Randomisation Approach. *PLoS Medicine*, *9*(5), e1001212. https://doi.org/10.1371/journal.pmed.1001212
Ochoa, M. del C., Martí, A., & Martínez, J. A. (2004). Obesity studies in candidate genes. *Medicina Clinica*, *122*(14), 542–551. https://doi.org/10.1016/s0025-7753(04)74300-6
Olza, J., Gil-Campos, M., Leis, R., Rupérez, A. I., Tojo, R., Cañete, R., Gil, Á., & Aguilera, C. M. (2013). Influence of variants in the NPY gene on obesity and metabolic syndrome features in Spanish children. *Peptides*, *45*, 22–27. https://doi.org/10.1016/j.peptides.2013.04.007

Oppert, J. M., Vohl, M. C., Chagnon, M., Dionne, F. T., Cassard-Doulcier, A. M., Ricquier, D., Pérusse, L., & Bouchard, C. (1994). DNA polymorphism in the uncoupling protein (UCP) gene and human body fat. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, *18*(8), 526–531.

Ouchi, N., Parker, J. L., Lugus, J. J., & Walsh, K. (2011). Adipokines in inflammation and metabolic disease. *Nature Reviews Immunology*, *11*(2), 85–97. https://doi.org/10.1038/nri2921

Oussaada, S. M., van Galen, K. A., Cooiman, M. I., Kleinendorst, L., Hazebroek, E. J., van Haelst, M. M., ter Horst, K. W., & Serlie, M. J. (2019). The pathogenesis of obesity. *Metabolism*, *92*(Mar). https://doi.org/10.1016/j.metabol.2018.12.012

Pan, R., & Chen, Y. (2021). Fat biology and metabolic balance: On the significance of sex. *Molecular and Cellular Endocrinology*, *533*, 111336. https://doi.org/10.1016/j.mce.2021.111336

Parida, S., Siddharth, S., & Sharma, D. (2019). Adiponectin, Obesity, and Cancer: Clash of the Bigwigs in Health and Disease. *International Journal of Molecular Sciences*, *20*(10), 2519. https://doi.org/10.3390/ijms20102519

Peppa, M., Koliaki, C., Papaefstathiou, A., Garoflos, E., Katsilambros, N., Raptis, S. A., Hadjidakis, D. I., & Dimitriadis, G. D. (2013). Body composition determinants of metabolic phenotypes of obesity in nonobese and obese postmenopausal women. *Obesity (Silver Spring, Md.)*, *21*(9). https://doi.org/10.1002/oby.20227

Philip, V. M., Sokoloff, G., Ackert-Bicknell, C. L., Striz, M., Branstetter, L., Beckmann, M. A., Spence, J. S., Jackson, B. L., Galloway, L. D., Barker, P., Wymore, A. M., Hunsicker, P. R., Durtschi, D. C., Shaw, G. S., Shinpock, S., Manly, K. F., Miller, D. R., Donohue, K. D., Culiat, C. T., … Chesler, E. J. (2011). Genetic analysis in the Collaborative Cross breeding population. *Genome Research*, *21*(8). https://doi.org/10.1101/gr.113886.110

Pietiläinen, K. H., Kaprio, J., Rissanen, A., Winter, T., Rimpelä, A., Viken, R. J., & Rose, R. J. (1999). Distribution and heritability of BMI in Finnish adolescents aged 16y and 17y: A study of 4884 twins and 2509 singletons. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity*, *23*(2), 107–115. https://doi.org/10.1038/sj.ijo.0800767

Pontzer, H., Yamada, Y., Sagayama, H., Ainslie, P. N., Andersen, L. F., Anderson, L. J., Arab, L., Baddou, I., Bedu-Addo, K., Blaak, E. E., Blanc, S., Bonomi, A. G., Bouten, C. V. C., Bovet, P., Buchowski, M. S., Butte, N. F., Camps, S. G., Close, G. L., Cooper, J. A., … IAEA DLW Database Consortium§. (2021). Daily energy expenditure through the human life course. *Science*, *373*(6556), 808–812. https://doi.org/10.1126/science.abe5017

Pritchard, L., Turnbull, A., & White, A. (2002). Pro-opiomelanocortin processing in the hypothalamus: Impact on melanocortin signalling and obesity. *Journal of Endocrinology*, *172*(3), 411–421. https://doi.org/10.1677/joe.0.1720411

Priya, S., Nampoothiri, S., Sen, P., & Sripriya, S. (2016). Bardet-Biedl syndrome: Genetics, molecular pathophysiology, and disease management. *Indian Journal of Ophthalmology*, *64*(9), 620–627. https://doi.org/10.4103/0301-4738.194328

Puhl, R., & Brownell, K. D. (2001). Bias, Discrimination, and Obesity. *Obesity Research*, *9*(12), 788–805. https://doi.org/10.1038/oby.2001.108

Puhl, R. M., & Heuer, C. A. (2010). Obesity Stigma: Important Considerations for Public Health. *American Journal of Public Health*, *100*(6), 1019–1028. https://doi.org/10.2105/AJPH.2009.159491

Puhl, R., & Suh, Y. (2015). Health Consequences of Weight Stigma: Implications for Obesity Prevention and Treatment. *Current Obesity Reports*, *4*(2), 182–190. https://doi.org/10.1007/s13679-015-0153-z

Rahmouni, K., Fath, M. A., Seo, S., Thedens, D. R., Berry, C. J., Weiss, R., Nishimura, D. Y., & Sheffield, V. C. (2008). Leptin resistance contributes to obesity and hypertension in mouse models of Bardet-Biedl syndrome. *The Journal of Clinical Investigation*, *118*(4), 1458–1467. https://doi.org/10.1172/JCI32357

Ramachandrappa, S., Raimondo, A., Cali, A. M. G., Keogh, J. M., Henning, E., Saeed, S., Thompson, A., Garg, S., Bochukova, E. G., Brage, S., Trowse, V., Wheeler, E., Sullivan, A. E., Dattani, M., Clayton, P. E., Datta, V., Datta, V., Bruning, J. B., Wareham, N. J., … Farooqi, I. S. (2013). Rare variants in single-minded 1 (SIM1) are associated with severe obesity. *The Journal of Clinical Investigation*, *123*(7), 3042–3050. https://doi.org/10.1172/JCI68016

Razquin, C., Martinez, J. A., Martinez-Gonzalez, M. A., Mitjavila, M. T., Estruch, R., & Marti, A. (2009). A 3 years follow-up of a Mediterranean diet rich in virgin olive oil is associated with high plasma antioxidant capacity and reduced body weight gain. *European Journal of Clinical Nutrition*, *63*(12), 1387–1393. https://doi.org/10.1038/ejcn.2009.106

Rees-Punia, E., Hathaway, E. D., & Gay, J. L. (2018). Crime, perceived safety, and physical activity: A meta-analysis. *Preventive Medicine*, *111*, 307–313. https://doi.org/10.1016/j.ypmed.2017.11.017

Renehan, A. G., Soerjomataram, I., Tyson, M., Egger, M., Zwahlen, M., Coebergh, J. W., & Buchan, I. (2010). Incident cancer burden attributable to excess body mass index in 30 European countries. *International Journal of Cancer*, *126*(3), 692–702. https://doi.org/10.1002/ijc.24803

Richardson, A. S., Arsenault, J. E., Cates, S. C., & Muth, M. K. (2015). Perceived stress, unhealthy eating behaviors, and severe obesity in low-income women. *Nutrition Journal*, *14*(1), 122. https://doi.org/10.1186/s12937-015-0110-4

Risso, D., Sainz, E., Morini, G., Tofanelli, S., & Drayna, D. (2018). Taste Perception of Antidesma bunius Fruit and Its Relationships to Bitter Taste Receptor Gene Haplotypes. *Chemical Senses*, *43*(7), 463–468. https://doi.org/10.1093/chemse/bjy037

Ristow, M. (2004). Neurodegenerative disorders associated with diabetes mellitus. *Journal of Molecular Medicine (Berlin, Germany)*, *82*(8), 510–529. https://doi.org/10.1007/s00109-004-0552-1

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7). https://doi.org/10.1093/nar/gkv007

Roberts, A., Pardo-Manuel de Villena, F., Wang, W., McMillan, L., & Threadgill, D. W. (2007). The polymorphism architecture of mouse genetic resources elucidated using genome-wide resequencing data: Implications for QTL discovery and systems genetics. *Mammalian Genome: Official Journal of the International Mammalian Genome Society*, *18*(6–7), 473–481. https://doi.org/10.1007/s00335-007-9045-1

Robinson, E., Almiron-Roig, E., Rutters, F., de Graaf, C., Forde, C. G., Tudur Smith, C., Nolan, S. J., & Jebb, S. A. (2014). A systematic review and meta-analysis examining the effect of eating rate on energy intake and hunger. *The American Journal of Clinical Nutrition*, *100*(1), 123–151. https://doi.org/10.3945/ajcn.113.081745

Rockman, M. V., & Kruglyak, L. (2008). Breeding Designs for Recombinant Inbred Advanced Intercross Lines. *Genetics*, *179*(2), 1069–1078. https://doi.org/10.1534/genetics.107.083873

Romieu, I., Dossus, L., Barquera, S., Blottière, H. M., Franks, P. W., Gunter, M., Hwalla, N., Hursting, S. D., Leitzmann, M., Margetts, B., Nishida, C., Potischman, N., Seidell, J., Stepien, M., Wang, Y., Westerterp, K., Winichagoon, P., Wiseman, M., & Willett, W. C. (2017). Energy balance and obesity: What are the main drivers? *Cancer Causes & Control*, *28*(3). https://doi.org/10.1007/s10552-017-0869-z

Rosen, E. D., & Spiegelman, B. M. (2014). What We Talk About When We Talk About Fat. *Cell*, *156*(1–2), 20–44. https://doi.org/10.1016/j.cell.2013.12.012

Rosito, G. A., Massaro, J. M., Hoffmann, U., Ruberg, F. L., Mahabadi, A. A., Vasan, R. S., O'Donnell, C. J., & Fox, C. S. (2008). Pericardial Fat, Visceral Abdominal Fat, Cardiovascular Disease Risk Factors, and Vascular Calcification in a Community-Based Sample: The Framingham Heart Study. *Circulation*, *117*(5), 605–613. https://doi.org/10.1161/CIRCULATIONAHA.107.743062

Ross, R., Berentzen, T., Bradshaw, A. J., Janssen, I., Kahn, H. S., Katzmarzyk, P. T., Kuk, J. L., Seidell, J. C., Snijder, M. B., Sørensen, T. I. A., & Després, J.-P. (2008). Does the relationship between waist circumference, morbidity and mortality depend on measurement protocol for waist circumference? *Obesity Reviews*, *9*(4), 312–325. https://doi.org/10.1111/j.1467-789X.2007.00411.x

Roudnitzky, N., Behrens, M., Engel, A., Kohl, S., Thalmann, S., Hübner, S., Lossow, K., Wooding, S. P., & Meyerhof, W. (2015). Receptor Polymorphism and Genomic Structure Interact to Shape Bitter Taste Perception. *PLOS Genetics*, *11*(9), e1005530. https://doi.org/10.1371/journal.pgen.1005530

Rui, L. (2014). Energy Metabolism in the Liver. In R. Terjung (Ed.), *Comprehensive Physiology* (pp. 177–197). John Wiley & Sons, Inc. https://doi.org/10.1002/cphy.c130024

Ruiz-Castell, M., Samouda, H., Bocquet, V., Fagherazzi, G., Stranges, S., & Huiart, L. (2021). Estimated visceral adiposity is associated with risk of cardiometabolic conditions in a population based study. *Scientific Reports*, *11*(1), 9121. https://doi.org/10.1038/s41598-021-88587-9

Sacks, H., & Symonds, M. E. (2013). Anatomical Locations of Human Brown Adipose Tissue: Functional Relevance and Implications in Obesity and Type 2 Diabetes. *Diabetes*, *62*(6), 1783–1790. https://doi.org/10.2337/db12-1430

Saelens, B. E., Vernez Moudon, A., Kang, B., Hurvitz, P. M., & Zhou, C. (2014). Relation Between Higher Physical Activity and Public Transit Use. *American Journal of Public Health*, *104*(5), 854–859. https://doi.org/10.2105/AJPH.2013.301696

Saleh, M. C., Wheeler, M. B., & Chan, C. B. (2002). Uncoupling protein-2: Evidence for its function as a metabolic regulator. *Diabetologia*, *45*(2), 174–187. https://doi.org/10.1007/s00125-001-0737-x

Sanchez-Vaznaugh, E. V., Weverka, A., Matsuzaki, M., & Sánchez, B. N. (2019). Changes in Fast Food Outlet Availability Near Schools: Unequal Patterns by Income, Race/Ethnicity, and Urbanicity. *American Journal of Preventive Medicine*, *57*(3), 338–345. https://doi.org/10.1016/j.amepre.2019.04.023

Sandoval-Motta, S., Aldana, M., Martínez-Romero, E., & Frank, A. (2017). The Human Microbiome and the Missing Heritability Problem. *Frontiers in Genetics*, *8*, 80. https://doi.org/10.3389/fgene.2017.00080

Sarwer, D. B., & Polonsky, H. M. (2016). The Psychosocial Burden of Obesity. *Endocrinology and Metabolism Clinics of North America*, *45*(3), 677–688. https://doi.org/10.1016/j.ecl.2016.04.016

Savva, S., Lamnisos, D., & Kafatos, A. (2013). Predicting cardiometabolic risk: Waist-to-height ratio or BMI. A meta-analysis. *Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy*, 403. https://doi.org/10.2147/DMSO.S34220

Schauer, P. R., Bhatt, D. L., Kirwan, J. P., Wolski, K., Brethauer, S. A., Navaneethan, S. D., Aminian, A., Pothier, C. E., Kim, E. S. H., Nissen, S. E., & Kashyap, S. R. (2014). Bariatric Surgery versus Intensive Medical Therapy for Diabetes—3-Year Outcomes. *New England Journal of Medicine*, *370*(21), 2002–2013. https://doi.org/10.1056/NEJMoa1401329

Schulze, M. B. (2019). Metabolic health in normal-weight and obese individuals. *Diabetologia*, *62*(4). https://doi.org/10.1007/s00125-018-4787-8

Schwartz, M. B., Chambliss, H. O., Brownell, K. D., Blair, S. N., & Billington, C. (2003). Weight Bias among Health Professionals Specializing in Obesity. *Obesity Research*, *11*(9), 1033–1039. https://doi.org/10.1038/oby.2003.142

Schwingshackl, L., Hoffmann, G., Kalle-Uhlmann, T., Arregui, M., Buijsse, B., & Boeing, H. (2015). Fruit and Vegetable Consumption and Changes in Anthropometric Variables in Adult Populations: A Systematic Review and Meta-Analysis of Prospective Cohort Studies. *PLOS ONE*, *10*(10), e0140846. https://doi.org/10.1371/journal.pone.0140846

Scuteri, A., Sanna, S., Chen, W.-M., Uda, M., Albai, G., Strait, J., Najjar, S., Nagaraja, R., Orrú, M., Usala, G., Dei, M., Lai, S., Maschio, A., Busonero, F., Mulas, A., Ehret, G. B., Fink, A. A., Weder, A. B., Cooper, R. S., … Abecasis, G. R. (2007). Genome-Wide Association Scan Shows Genetic Variants in the FTO Gene Are Associated with Obesity-Related Traits. *PLoS Genetics*, *3*(7). https://doi.org/10.1371/journal.pgen.0030115

Seabolt, L. A., Welch, E. B., & Silver, H. J. (2015). Imaging methods for analyzing body composition in human obesity and cardiometabolic disease: Imaging methods for analyzing body composition. *Annals of the New York Academy of Sciences*, *1353*(1), 41–59. https://doi.org/10.1111/nyas.12842

Seacat, J. D., Dougal, S. C., & Roy, D. (2016). A daily diary assessment of female weight stigmatization. *Journal of Health Psychology*, *21*(2), 228–240. https://doi.org/10.1177/1359105314525067

Selby, J. V., Newman, B., Quesenberry, C. P., Fabsitz, R. R., King, M. C., & Meaney, F. J. (1989). Evidence of genetic influence on central body fat in middle-aged twins. *Human Biology*, *61*(2), 179–194.

Sellayah, D., Cagampang, F. R., & Cox, R. D. (2014). On the Evolutionary Origins of Obesity: A New Hypothesis. *Endocrinology*, *155*(5), 1573–1588. https://doi.org/10.1210/en.2013-2103

Shahnazaryan, U., Wójcik, M., Bednarczuk, T., & Kuryłowicz, A. (2019). Role of Obesogens in the Pathogenesis of Obesity. *Medicina*, *55*(9). https://doi.org/10.3390/medicina55090515

Shorter, J. R., Najarian, M. L., Bell, T. A., Blanchard, M., Ferris, M. T., Hock, P., Kashfeen, A., Kirchoff, K. E., Linnertz, C. L., Sigmon, J. S., Miller, D. R., McMillan, L., & Pardo-Manuel de Villena, F. (2019). Whole Genome Sequencing and Progress Toward Full Inbreeding of the Mouse Collaborative Cross Population. *G3, Genes, Genomes, Genetics*, *9*(5). https://doi.org/10.1534/g3.119.400039

Shulman, G. I. (2014). Ectopic Fat in Insulin Resistance, Dyslipidemia, and Cardiometabolic Disease. *New England Journal of Medicine*, *371*(12), 1131–1141. https://doi.org/10.1056/NEJMra1011035
Silver, L. D., Ng, S. W., Ryan-Ibarra, S., Taillie, L. S., Induni, M., Miles, D. R., Poti, J. M., & Popkin, B. M. (2017). Changes in prices, sales, consumer spending, and beverage consumption one year after a tax on sugar-sweetened beverages in Berkeley, California, US: A before-and-after study. *PLOS Medicine*, *14*(4), e1002283. https://doi.org/10.1371/journal.pmed.1002283
Singh, R. K., Kumar, P., & Mahalingam, K. (2017). Molecular genetics of human obesity: A comprehensive review. *Comptes Rendus Biologies*, *340*(2). https://doi.org/10.1016/j.crvi.2016.11.007
Sivaprakasam, S., Sikder, M. O. F., Ramalingam, L., Kaur, G., Dufour, J. M., Moustaid-Moussa, N., Wachtel, M. S., & Ganapathy, V. (2021). SLC6A14 deficiency is linked to obesity, fatty liver, and metabolic syndrome but only under conditions of a high-fat diet. *Biochimica Et Biophysica Acta. Molecular Basis of Disease*, *1867*(5), 166087. https://doi.org/10.1016/j.bbadis.2021.166087
Sjöström, L. (2013). Review of the key results from the Swedish Obese Subjects (SOS) trial—A prospective controlled intervention study of bariatric surgery. *Journal of Internal Medicine*, *273*(3), 219–234. https://doi.org/10.1111/joim.12012
Smith, J. D., Hou, T., Ludwig, D. S., Rimm, E. B., Willett, W., Hu, F. B., & Mozaffarian, D. (2015). Changes in intake of protein foods, carbohydrate amount and quality, and long-term weight change: Results from 3 prospective cohorts. *The American Journal of Clinical Nutrition*, *101*(6), 1216–1224. https://doi.org/10.3945/ajcn.114.100867
Smith, J. L., Estus, S., Lennie, T. A., Moser, D. K., Chung, M. L., & Mudd-Martin, G. (2020). TAS2R38 PAV Haplotype Predicts Vegetable Consumption in Community-Dwelling Caucasian Adults at Risk for Cardiovascular Disease. *Biological Research For Nursing*, *22*(3), 326–333. https://doi.org/10.1177/1099800420913935
Smith, K. R., & Moran, T. H. (2021). Gastrointestinal peptides in eating-related disorders. *Physiology & Behavior*, *238*, 113456. https://doi.org/10.1016/j.physbeh.2021.113456
Solas, M., Milagro, F. I., Martínez-Urbistondo, D., Ramirez, M. J., & Martínez, J. A. (2016). Precision Obesity Treatments Including Pharmacogenetic and Nutrigenetic Approaches. *Trends in Pharmacological Sciences*, *37*(7), 575–593. https://doi.org/10.1016/j.tips.2016.04.008
Speakman, J. R. (1997). *Doubly labelled water: Theory and practice* (1. ed). Chapman & Hall.
Speakman, J. R. (2013). Measuring energy metabolism in the mouse—Theoretical, practical, and analytical considerations. *Frontiers in Physiology*, *4*, 34. https://doi.org/10.3389/fphys.2013.00034
Speakman, J. R. (2018). Obesity and thermoregulation. *Handbook of Clinical Neurology*, *Jun*(1;10(6):679-689). https://doi.org/10.1016/b978-0-444-63912-7.00026-6
Spiegelman, B. M., & Flier, J. S. (2001). Obesity and the Regulation of Energy Balance. *Cell*, *104*(4), 531–543. https://doi.org/10.1016/S0092-8674(01)00240-9
Srivastava, A., Morgan, A. P., Najarian, M. L., Sarsani, V. K., Sigmon, J. S., Shorter, J. R., Kashfeen, A., McMullan, R. C., Williams, L. H., Giusti-Rodríguez, P., Ferris, M. T., Sullivan, P., Hock, P., Miller, D. R., Bell, T. A., McMillan, L., Churchill, G. A., & de Villena, F. P.-M. (2017). Genomes of the Mouse Collaborative Cross. *Genetics*, *206*(2). https://doi.org/10.1534/genetics.116.198838
Stefan, N., Häring, H.-U., Hu, F. B., & Schulze, M. B. (2013). Metabolically healthy obesity: Epidemiology, mechanisms, and clinical implications. *The Lancet Diabetes & Endocrinology*, *1*(2), 152–162. https://doi.org/10.1016/S2213-8587(13)70062-7
Stunkard, A. J. (1986). A Twin Study of Human Obesity. *JAMA: The Journal of the American Medical Association*, *256*(1), 51. https://doi.org/10.1001/jama.1986.03380010055024
Stunkard, A. J., Harris, J. R., Pedersen, N. L., & McClearn, G. E. (1990). The Body-Mass Index of Twins Who Have Been Reared Apart. *New England Journal of Medicine*, *322*(21), 1483–1487. https://doi.org/10.1056/NEJM199005243222102
Stunkard, A. J., Sørensen, T. I. A., Hanis, C., Teasdale, T. W., Chakraborty, R., Schull, W. J., & Schulsinger, F. (1986). An Adoption Study of Human Obesity. *New England Journal of Medicine*, *314*(4). https://doi.org/10.1056/nejm198601233140401
Sturm, R. (2002). The Effects Of Obesity, Smoking, And Drinking On Medical Problems And Costs. *Health Affairs*, *21*(2), 245–253. https://doi.org/10.1377/hlthaff.21.2.245
Subramanian, A., Tamayo, P., Mootha, V. K., Mukherjee, S., Ebert, B. L., Gillette, M. A., Paulovich, A., Pomeroy, S. L., Golub, T. R., Lander, E. S., & Mesirov, J. P. (2005). Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles.

Proceedings of the National Academy of Sciences, *102*(43), 15545–15550. https://doi.org/10.1073/pnas.0506580102
Suspitsin, E. N., & Imyanitov, E. N. (2016). Bardet-Biedl Syndrome. *Molecular Syndromology*, *7*(2), 62–71. https://doi.org/10.1159/000445491
Suviolahti, E., Oksanen, L. J., Öhman, M., Cantor, R. M., Ridderstråle, M., Tuomi, T., Kaprio, J., Rissanen, A., Mustajoki, P., Jousilahti, P., Vartiainen, E., Silander, K., Kilpikari, R., Salomaa, V., Groop, L., Kontula, K., Peltonen, L., & Pajukanta, P. (2003). The SLC6A14 gene shows evidence of association with obesity. *Journal of Clinical Investigation*, *112*(11), 1762–1772. https://doi.org/10.1172/JCI200317491
Swanzey, E., O'Connor, C., & Reinholdt, L. G. (2021). Mouse Genetic Reference Populations: Cellular Platforms for Integrative Systems Genetics. *Trends in Genetics*, *37*(3), 251–265. https://doi.org/10.1016/j.tig.2020.09.007
Teixeira, T. F., Alves, R. D., Moreira, A. P. B., & Peluzio, M. d. C. G. (2015). Main characteristics of metabolically obese normal weight and metabolically healthy obese phenotypes. *Nutrition Reviews*, *73*(3), 175–190. https://doi.org/10.1093/nutrit/nuu007
Thanassoulis, G., Massaro, J. M., Hoffmann, U., Mahabadi, A. A., Vasan, R. S., O'Donnell, C. J., & Fox, C. S. (2010). Prevalence, Distribution, and Risk Factor Correlates of High Pericardial and Intrathoracic Fat Depots in the Framingham Heart Study. *Circulation: Cardiovascular Imaging*, *3*(5), 559–566. https://doi.org/10.1161/CIRCIMAGING.110.956706
The LifeLines Cohort Study, The ADIPOGen Consortium, The AGEN-BMI Working Group, The CARDIOGRAMplusC4D Consortium, The CKDGen Consortium, The GLGC, The ICBP, The MAGIC Investigators, The MuTHER Consortium, The MIGen Consortium, The PAGE Consortium, The ReproGen Consortium, The GENIE Consortium, The International Endogene Consortium, Locke, A. E., Kahali, B., Berndt, S. I., Justice, A. E., Pers, T. H., … Speliotes, E. K. (2015). Genetic studies of body mass index yield new insights for obesity biology. *Nature*, *518*(7538), 197–206. https://doi.org/10.1038/nature14177
Thomas, E. L., Parkinson, J. R., Frost, G. S., Goldstone, A. P., Doré, C. J., McCarthy, J. P., Collins, A. L., Fitzpatrick, J. A., Durighel, G., Taylor-Robinson, S. D., & Bell, J. D. (2012). The Missing Risk: MRI and MRS Phenotyping of Abdominal Adiposity and Ectopic Fat. *Obesity*, *20*(1), 76–87. https://doi.org/10.1038/oby.2011.142
Threadgill, D. W., & Churchill, G. A. (2012). Ten Years of the Collaborative Cross. *Genetics*, *190*(2). https://doi.org/10.1534/genetics.111.138032
Tobias, D. K., Chen, M., Manson, J. E., Ludwig, D. S., Willett, W., & Hu, F. B. (2015). Effect of low-fat diet interventions versus other diet interventions on long-term weight change in adults: A systematic review and meta-analysis. *The Lancet Diabetes & Endocrinology*, *3*(12), 968–979. https://doi.org/10.1016/S2213-8587(15)00367-8
Trefts, E., Gannon, M., & Wasserman, D. H. (2017). The liver. *Current Biology*, *27*(21). https://doi.org/10.1016/j.cub.2017.09.019
United States Department of Agriculture (USDA). (2021). *History of the Dietary Guidelines*. Dietary Guidelines for Americans. https://www.dietaryguidelines.gov/about-dietary-guidelines/history-dietary-guidelines
U.S. Department of Agriculture (USDA) and U.S. Department of Health and Human Services (HHS). (2020). *Dietary Guidelines for Americans, 2020-2025* (9th Edition). DietaryGuidelines.gov
van der Klaauw, A. A., & Farooqi, I. S. (2015). The Hunger Genes: Pathways to Obesity. *Cell*, *161*(1), 119–132. https://doi.org/10.1016/j.cell.2015.03.008
Vinkhuyzen, A. A. E., Wray, N. R., Yang, J., Goddard, M. E., & Visscher, P. M. (2013). Estimation and partition of heritability in human populations using whole-genome analysis methods. *Annual Review of Genetics*, *47*, 75–95. https://doi.org/10.1146/annurev-genet-111212-133258
Volke, L., & Krause, K. (2021). Effect of Thyroid Hormones on Adipose Tissue Flexibility. *European Thyroid Journal*, *10*(1), 1–9. https://doi.org/10.1159/000508483
Wang, Y. C., McPherson, K., Marsh, T., Gortmaker, S. L., & Brown, M. (2011). Health and economic burden of the projected obesity trends in the USA and the UK. *The Lancet*, *378*(9793), 815–825. https://doi.org/10.1016/S0140-6736(11)60814-3
Williams, E. P., Mesidor, M., Winters, K., Dubbert, P. M., & Wyatt, S. B. (2015). Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. *Current Obesity Reports*, *4*(3). https://doi.org/10.1007/s13679-015-0169-4

Winkler, T. W., Justice, A. E., Graff, M., Barata, L., Feitosa, M. F., Chu, S., Czajkowski, J., Esko, T., Fall, T., Kilpeläinen, T. O., Lu, Y., Mägi, R., Mihailov, E., Pers, T. H., Rüeger, S., Teumer, A., Ehret, G. B., Ferreira, T., Heard-Costa, N. L., … Loos, R. J. F. (2015). The Influence of Age and Sex on Genetic Associations with Adult Body Size and Shape: A Large-Scale Genome-Wide Interaction Study. *PLOS Genetics*, *11*(10), e1005378. https://doi.org/10.1371/journal.pgen.1005378

World Health Organization (WHO). (2010). *Global recommendations on physical activity for health. ...* World Health Organization.

World Health Organization [WHO]. (2021). *Obesity and overweight Fact Sheet*. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight

Wray, N. R., & Visscher, P. M. (2008). Estimating Trait Heritability. *Nature Education*, *1*((1):29). https://www.nature.com/scitable/topicpage/estimating-trait-heritability-46889/

Wren, A. M., & Bloom, S. R. (2007). Gut Hormones and Appetite Control. *Gastroenterology*, *132*(6), 2116–2130. https://doi.org/10.1053/j.gastro.2007.03.048

Wu, J., Boström, P., Sparks, L. M., Ye, L., Choi, J. H., Giang, A.-H., Khandekar, M., Virtanen, K. A., Nuutila, P., Schaart, G., Huang, K., Tu, H., van Marken Lichtenbelt, W. D., Hoeks, J., Enerbäck, S., Schrauwen, P., & Spiegelman, B. M. (2012). Beige Adipocytes Are a Distinct Type of Thermogenic Fat Cell in Mouse and Human. *Cell*, *150*(2), 366–376. https://doi.org/10.1016/j.cell.2012.05.016

Wu, T., Gao, X., Chen, M., & van Dam, R. M. (2009). Long-term effectiveness of diet-plus-exercise interventions vs. diet-only interventions for weight loss: A meta-analysis. *Obesity Reviews*, *10*(3), 313–323. https://doi.org/10.1111/j.1467-789X.2008.00547.x

Xu, B., Goulding, E. H., Zang, K., Cepoi, D., Cone, R. D., Jones, K. R., Tecott, L. H., & Reichardt, L. F. (2003). Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nature Neuroscience*, *6*(7), 736–742. https://doi.org/10.1038/nn1073

Yang, J., Bakshi, A., Zhu, Z., Hemani, G., Vinkhuyzen, A. A. E., Lee, S. H., Robinson, M. R., Perry, J. R. B., Nolte, I. M., van Vliet-Ostaptchouk, J. V., Snieder, H., LifeLines Cohort Study, Esko, T., Milani, L., Mägi, R., Metspalu, A., Hamsten, A., Magnusson, P. K. E., Pedersen, N. L., … Visscher, P. M. (2015). Genetic variance estimation with imputed variants finds negligible missing heritability for human height and body mass index. *Nature Genetics*, *47*(10), 1114–1120. https://doi.org/10.1038/ng.3390

Yang, J., Benyamin, B., McEvoy, B. P., Gordon, S., Henders, A. K., Nyholt, D. R., Madden, P. A., Heath, A. C., Martin, N. G., Montgomery, G. W., Goddard, M. E., & Visscher, P. M. (2010). Common SNPs explain a large proportion of the heritability for human height. *Nature Genetics*, *42*(7), 565–569. https://doi.org/10.1038/ng.608

Yang, J., Loos, R. J. F., Powell, J. E., Medland, S. E., Speliotes, E. K., Chasman, D. I., Rose, L. M., Thorleifsson, G., Steinthorsdottir, V., Mägi, R., Waite, L., Vernon Smith, A., Yerges-Armstrong, L. M., Monda, K. L., Hadley, D., Mahajan, A., Li, G., Kapur, K., Vitart, V., … Visscher, P. M. (2012). FTO genotype is associated with phenotypic variability of body mass index. *Nature*, *490*(7419), 267–272. https://doi.org/10.1038/nature11401

Yang, J., Zeng, J., Goddard, M. E., Wray, N. R., & Visscher, P. M. (2017). Concepts, estimation and interpretation of SNP-based heritability. *Nature Genetics*, *49*(9), 1304–1310. https://doi.org/10.1038/ng.3941

Yang, Y.-K., Ollmann, M. M., Wilson, B. D., Dickinson, C., Yamada, T., Barsh, G. S., & Gantz, I. (1997). Effects of Recombinant Agouti-Signaling Protein on Melanocortin Action. *Molecular Endocrinology*, *11*(3), 274–280. https://doi.org/10.1210/mend.11.3.9898

Yanovski, J. A., Diament, A. L., Sovik, K. N., Nguyen, T. T., Li, H., Sebring, N. G., & Warden, C. H. (2000). Associations between uncoupling protein 2, body composition, and resting energy expenditure in lean and obese African American, white, and Asian children. *The American Journal of Clinical Nutrition*, *71*(6), 1405–1420. https://doi.org/10.1093/ajcn/71.6.1405

Yu, F., Göthe, S., Wikström, L., Forrest, D., Vennström, B., & Larsson, L. (2000). Effects of thyroid hormone receptor gene disruption on myosin isoform expression in mouse skeletal muscles. *American Journal of Physiology. Regulatory, Integrative and Comparative Physiology*, *278*(6), R1545-1554. https://doi.org/10.1152/ajpregu.2000.278.6.R1545

Yu, Z. B., Han, S. P., Zhu, G. Z., Zhu, C., Wang, X. J., Cao, X. G., & Guo, X. R. (2011). Birth weight and subsequent risk of obesity: A systematic review and meta-analysis: Birth weight in relation to obesity. *Obesity Reviews*, *12*(7), 525–542. https://doi.org/10.1111/j.1467-789X.2011.00867.x

Zhang, B., & Horvath, S. (2005). A General Framework for Weighted Gene Co-Expression Network Analysis. *Statistical Applications in Genetics and Molecular Biology*, *4*(1). https://doi.org/10.2202/1544-6115.1128

Zhao, D., Xian, X., Terrera, M., Krishnan, R., Miller, D., Bridgeman, D., Tao, K., Zhang, L., Tsow, F., Forzani, E. S., & Tao, N. (2014). A pocket-sized metabolic analyzer for assessment of resting energy expenditure. *Clinical Nutrition (Edinburgh, Scotland)*, *33*(2), 341–347. https://doi.org/10.1016/j.clnu.2013.06.001

Chapter 2:

Genetic Background Shapes Phenotypic Response to Diet for Adiposity in the Collaborative Cross

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Abstract

Defined as chronic excessive accumulation of adiposity, obesity results from long-term imbalance between energy intake and expenditure. The mechanisms behind how caloric imbalance occurs are complex and influenced by numerous biological and environmental factors, especially genetics, and diet. Population-based diet recommendations have had limited success partly due to the wide variation in physiological responses across individuals when they consume the same diet. Thus, it is necessary to broaden our understanding of how individual genetics and diet interact relative to the development of obesity for improving weight loss treatment. To determine how consumption of diets with different macronutrient composition alter adiposity and other obesity-related traits in a genetically diverse population, we analyzed body composition, metabolic rate, clinical blood chemistries, and circulating metabolites in 22 strains of mice from the Collaborative Cross (CC), a highly diverse recombinant inbred mouse population, before and after 8 weeks of feeding either a high protein or high fat high sucrose diet. At both baseline and post-diet, adiposity and other obesity-related traits exhibited a broad range of phenotypic variation based on CC strain; diet-induced changes in adiposity and other traits also depended largely on CC strain. In addition to estimating heritability at baseline, we also quantified the effect size of diet for each trait, which varied by trait and experimental diet. Our findings identified CC strains prone to developing obesity, demonstrate the genotypic and phenotypic diversity of the CC for studying complex traits, and highlight the importance of accounting for genetic differences when making dietary recommendations.

Introduction

Obesity is a complex disease characterized by excessive adipose tissue accumulation and has become one of the leading preventable causes of death in both developed and developing countries (Bell et al., 2005; Friedman, 2015; WHO, 2015). Fundamentally, obesity results from a chronic imbalance between energy intake and expenditure (Hill et al., 2012; Romieu et al., 2017; Swift et al., 2018; Oussaada et al., 2019). This imbalance is caused by numerous biological factors including: genetics (Bell et al., 2005; Singh et al., 2017; Loos, 2018), metabolism (Timper and Brüning, 2017; Speakman, 2018; Fernández-Verdejo et al., 2019), and the gut microbiome (John and Mullin, 2016; Martinez et al., 2016; Torres-Fuentes et al., 2017), as well as environmental factors such as chemical exposure (Janesick and Blumberg, 2016; Heindel and Blumberg, 2019; Shahnazaryan et al., 2019) and diet, particularly in the context of overfeeding relative to physical activity levels (Sims, 1976; Danforth, 1985; Schmidt et al., 2012; Cuthbertson et al., 2017; Creasy et al., 2018).

Identification of the underlying genes predisposing an individual to obesity has been a very active area of investigation. Large-scale human genome-wide association studies (GWAS) that test the association of millions of genetic variants with adiposity, body mass index, and waist-to-hip ratio have identified >300 genetic loci for obesity traits, such as the *FTO, TMEM18, CADM2,* and *LYPLAL1* loci, among others (Loos et al., 2008; González-Muniesa et al., 2017; Loos, 2018). Complementing approaches in humans, studies in mice have provided fundamental insights into the genetic regulation of adiposity and susceptibility to obesity (Coleman and Hummel, 1974; Lu et al., 1994; Carroll et al., 2004; Attie et al., 2017). For example, the genes that encode leptin and leptin receptor which arose as spontaneous deficiency mutations in *ob/ob* and *db/db* obese mice respectively (Ingalls et al., 1950; Hummel et al., 1966) were shown to regulate satiety after gene cloning was possible (Zhang et al., 1994; Tartaglia et al., 1995). Similarly, the link between the *FTO* gene and obesity was first reported in mice prior to the identification of this gene's association with obesity in humans (Fischer et al., 2008). The similar biology between humans and mice in terms of physiology, morphology, and genetics, and the ability to manipulate the mouse genome has aided our understanding of the underlying mechanisms affecting energy balance and obesity (Robinson et al., 2000; Pomp et al., 2008).

Similarly, diet is among the most studied environmental factors, as it remains an important and potentially successful focus of public health interventions (Wilborn et al., 2005; Eknoyan, 2006; Makris and Foster, 2011). One of the difficulties identifying the optimal dietary recommendation for a population is the inter-individual variation observed in response to diet (Berry et al., 2020). At a certain level there may be no “perfect” diet that works universally across populations to mitigate obesity (Dansinger et al., 2005; Johnston et al., 2014). Thus, in spite of the successes of GWAS and dietary intervention studies, there still remains practical public health challenges for understanding and preventing obesity. Animal models often solve some of the challenges by limiting confounding environmental influences to gain a more complete understanding of the etiology of obesity. Studies performed using inbred mouse strains suggest that phenotypic response to diet occurs in a strain-dependent manner (West et al., 1992, 1995; Barrington et al., 2017). Understanding the interaction of genetics and diet offers insight into how “precision nutrition” could improve and refine our dietary recommendations.

In order to broaden our understanding of how genetics and diet impact obesity at both the individual and population levels in a genetically diverse population, we analyzed how consumption of diets with different macronutrient compositions altered adiposity and other physiological traits in 22 strains of mice from the Collaborative Cross (CC), a large recombinant inbred mouse population generated from elaborate intercrosses of C57BL/6J, A/J, NOD/ShiLtJ, NZO/HiLtJ, 129S1/SvImJ, WSB/EiJ, CAST/EiJ, and PWK/PhJ, mouse strains (Churchill et al., 2004; Iraqi et al., 2008; Threadgill and Churchill, 2012). The tremendous genetic diversity of the CC population (Philip et al., 2011; Collaborative Cross Consortium, 2012; Srivastava et al., 2017; Shorter et al., 2019) facilitates the discernment between effects caused by diet from effects caused by genetic variation when measuring differences and changes in adiposity and other metabolic traits across multiple genetic “replicates” in each strain, thereby increasing power, reproducibility, and relevance to obesity in humans (Mathes et al., 2011). Following a 2-week acclimation period on standard synthetic diet (AIN-76A) to determine baseline phenotypes, mice between 8 and 11 weeks of age were randomized and put on experimental diets (high fat high sucrose or high protein) for 8 weeks, followed by analysis of body composition, metabolic rate, clinical blood chemistries, and circulating metabolites to assess the effect of diet on each trait since diets with higher

protein, low glycemic index, and lower fat content may assist in maintaining weight loss compared to diets with higher carbohydrate content (Abete et al., 2010; Larsen et al., 2010; Hu et al., 2018; Myrmel et al., 2019; San- Cristobal et al., 2020). While both genetics and diet interact to influence adiposity and other phenotypes, health outcomes were more strongly impacted by genetic effects than diet. Furthermore, the effect of diet on each trait varied depending on CC strain, indicating that genetics determine how a particular diet may affect body composition.

Materials and Methods

Animals and Husbandry

Female mice from 22 CC strains were obtained in 2016 from University of North Carolina's Systems Genetics Core Facility (Welsh et al., 2012) (total n = 204, Figure 1-1). All strains used are listed in Supplementary Table 1. Mice were then acclimated for 2 weeks on standard synthetic diet (AIN-76A), housed three mice per cage at 22◦ C with non-irradiated pine bedding, and provided free access to sterile water in a climate-controlled facility under a 12-h light/dark cycle. Mice were put on experimental diets between 8 and 11 weeks of age after the 2-week acclimation period, randomized into different cages by experimental diet (Figure S1-1, Supplementary Table 1), and housed under the same conditions. After randomization, mice were challenged on their respective diets for 8 weeks, and analysis of body composition, metabolic rate, and physical activity were performed at the UNC Animal Metabolism Phenotyping Core post diet challenge (methods for analysis of body composition, metabolic rate, and physical activity described below) followed by necropsy and tissue collection. Because only a limited number of mice were available at one time, experiments spanning 11 weeks (2 weeks of acclimation, 8 weeks of diet challenge, post-diet phenotype assessments) for each "batch" were performed in 7 batches, where each batch contained about 33 mice on average, except for batch 6 which contained 14 mice. All mice were maintained on their respective experimental diets for the remainder of the study using protocols in accordance with the University of North Carolina Institution Animal Care and Use Committee guidelines. All maintenance protocols and experimental procedures were approved by the IACUC at University of North Carolina (UNC) Chapel Hill (IACUC Protocol Number: 13-103).

Diets

During the 2-week acclimation period, mice were maintained on the defined synthetic diet containing 20.8% kcal protein, 67.7% kcal carbohydrate, and 11.5% kcal fat, referred to as AIN-76A in this study (D10001, Research Diets, New Brunswick, NJ; Supplementary Table 2) until 8–11 weeks of age to account for differences due to variable components of standard chow. Subsequently, one sibling from each of the 102 sibling trios was randomly assigned to each experimental diet (Supplementary Table 1). One hundred and two mice were transferred to a synthetic high fat high sucrose diet (HS) containing 16.8% kcal protein, 51.4% kcal carbohydrate, and 31.8% kcal fat, and 102 mice were placed on a high protein diet (HP) containing 40% kcal protein, 40% kcal carbohydrate and 20% kcal fat (D12266B and D12083101, respectively, Supplementary Table 2; Research Diets, New Brunswick, NJ).

Body Composition and Weight

Body composition (lean and fat mass) was assessed in all cohorts during the first week of the acclimation phase to establish baseline phenotypes, as well as after 8 weeks of the experimental diet challenge using the Echo MRI-130 Body Composition Analyzer (EchoMRI, Houston, TX, USA). Body fat and lean mass percentages were calculated by dividing fat mass by scale weight and dividing lean mass by scale weight, respectively.

Metabolic Rate and Activity

Mice were placed into individual indirect calorimetry cages (Phenomaster, TSE SYSTEMS, Chesterfield, MO) the week immediately following the 8 weeks of the experimental diet challenge for ~3 days and two nights (~48h) to obtain O_2 consumption and CO_2 production, activity, and feed and water consumption measurements. After an 8-h acclimation period, data were collected for two complete 12-h night cycles and one complete 12-h day cycle every 42 min (Figure S1-2). Basal activity was measured in three dimensions (x, y, and z) as breaks in the two infrared light beam frames that surrounded each cage. Rearing was detected by beam breaks in the z axis and total physical activity was defined as the sum of beam breaks in all three axes in counts. Feed and water were available *ad libitum* and consumption was measured by weighing sensors that held containers for feed and water, respectively, and recorded the

amount of feed or water consumed. Spilled feed and water were caught by extended attachments on the feed and water containers suspended from the weighing sensors, so spilled feed and water were not recorded as consumed.

Heat production calculations were performed two ways by the TSE software (LabMaster) using O_2 consumption and CO_2 production measurements: (1) for the computation of total body weight (kcal/h/kg), and (2) for the computation of an exponent lean body mass assigned to total body weight (kcal/h/kg). From the exported raw data, energy consumption was calculated by multiplying feed consumption measurement (in grams) by the calorie (kcal) content per gram feed for each diet (Supplementary Table 2). Protein, carbohydrate, and fat consumption were calculated by multiplying the feed consumption measurement (in grams) by macronutrient content (in grams) per total gram of feed. For example, the average protein consumption for mice on the high protein diet was calculated by multiplying the measured feed consumed (g) by (40.6 g protein/90.3 g feed total).

Individual and combined diurnal means were calculated for each metabolic measurement using data collected at time points between the start and end times of the day cycle. Likewise, individual and combined nocturnal means were calculated for each metabolic measurement using data collected at time points between the start and end times of the night cycle (Figure S1-2). Means for each measure were also calculated by date, e.g., mean of feed consumption for both light and dark cycles on the second day of the experiment.

Biological Samples Collection

Tail clippings and blood samples were collected immediately before putting mice on experimental diets to establish baseline values. To collect tail clippings, tail tips were cleaned with 70% ethanol, and up to 5 mm of the tail tips were excised with sterile scissors and placed in 2 ml screw-cap tubes. After 8 weeks on experimental diets, mice were anesthetized via isoflurane inhalation and euthanized using cervical dislocation during the necropsy following a 4-h fast. Blood, kidney, liver, subcutaneous and gonadal fat, and cecum samples were collected (Figure 1-1). Blood samples were collected via retro-orbital bleed with heparinized capillary tubes into EDTA tubes, placed on ice, and centrifuged at 6,000 rpm for 10 min at 4◦ C for plasma collection. Plasma was then transferred to 1.5 ml Eppendorf tubes. Tissues were placed in 2

ml screw-cap tubes and snap frozen in liquid nitrogen. All plasma, frozen tissues, and previously collected samples were stored at −80◦ C. Additional gonadal fat, kidney, and liver tissues were fixed in capped glass vials containing 10% formalin and stored at room temperature.

Plasma Clinical Chemistries

Cholesterol, triglyceride (TG), glucose, albumin, creatinine, urea, aspartate transaminase (AST), and alanine transaminase (ALT) levels were quantified using the Cobas Integra 400 Plus (Roche Diagnostics, Indianapolis, IN), according to manufacturer's instructions. An internal control (Human UTAK) was used

to assess run variation. Baseline and post-diet circulating insulin were measured using ultrasensitive mouse insulin ELISA (ALPCO Diagnostics, Salem, NH) per manufacturer's instructions except for the following adjustment: 15 µl of plasma sample dilutions were used in the assay and back calculations were performed to determine actual plasma concentrations. Insulin optical density (OD) was measured at 450nm using a spectrophotometric BioTek Synergy 2 plate reader (BioTek Instruments Inc, Winooski, VT). Insulin concentrations were derived from measured ODs using BioTek's Gen5 software.

Liquid Chromatography-Mass Spectrometry (LC-MS)

Baseline and post-diet circulating trimethylamine N-oxide (TMAO), choline, phosphocholine, betaine, and carnitine were quantified using liquid chromatography–mass spectrometry (LC-MS) methods described by Wang et al. (2014) with modifications. Standards ranging from 0 to 100µM of non- deuterated analytes in methanol were run in order to establish analyte standard curves. Two-fold serial dilutions of a 100µM stock solution in methanol was used to make 13 standards. 5µM of surrogate standard (SSTD) were prepared comprising of deuterated analytes in methanol. All standards were purchased from Sigma-Aldrich (St. Louis, MO). All reagent solvents were mass spectrometry grade and purchased from Fisher Scientific (Waltham, MA). Details of the protocol are contained in the data supplement (Supplementary File 1) and Supplementary Table 3.

Statistical Methods

Determining Contributors to Phenotypic Variance

All phenotype data were tested for normality using the Shapiro-Wilk test in the statistical programming language R (R Core Team, 2019). Baseline non-normal data were transformed using power transformation or rank normalization if necessary before linear fixed models were fitted using CC strain and mouse batch ("week" in Supplementary File 2) as fixed effects to test for significant CC strain effects on phenotypic variance. Post- diet non-normal data were also normalized using these methods as appropriate for fitting linear mixed models using restricted maximum likelihood (REML) to determine the significance of the effect of diet and strain/diet interactions. To test for the significance of the effect of diet underlying phenotypic variance, linear mixed model analysis of the relationship between diet and phenotypic traits was performed using R and packages *lme4* (Bates et al., 2015), *lmerTest* (Kuznetsova et al., 2017), and *car* (Fox and Weisberg, 2019) for each post-diet phenotype. For models testing diet as the main effect, fixed effects included experimental diet and mouse batch, and random effects (intercepts) included CC strain, CC strain × experimental diet, randomization cage nested within experimental diet, and baseline cage nested within CC strain. In models used to test for the significance of the effect of strain/diet interactions, linear mixed models were fit for each post-diet phenotype, which included CC strain, experimental diet, CC strain × experimental diet, and mouse batch as fixed effects, and randomization cage nested within experimental diet and baseline cage nested within CC strain as random effects (intercepts). Visual inspection of residual plots did not reveal obvious deviations from homoscedasticity or normality. P-values were obtained by implementing Satterthwaite approximations as described by Luke (2016).

Calculation of Health Scores to Estimate Overall Metabolic Health

Metabolic health scores were calculated for all mice at baseline and 9 weeks post-diet. First, Z scores were calculated for several metabolic risk factors (circulating glucose, insulin, glucose/insulin ratio, cholesterol, TG, and body fat %) measured at baseline and post-diet for each mouse; the distribution used to calculate the Z score for baseline was all baseline samples, while samples were separated by diet before calculating post- diet Z scores. Next, the Z scores for each metabolic risk factor were added together, and then multiplied by −1 so that decreased health is reflected by a lower health score.

Baseline Broad-Sense Heritability Estimates

From linear models fitted using baseline normalized data with CC strain and mouse batch as the covariates used to test for significant CC strain effects on phenotypic variance (described above), broad-sense heritability (H^2) was estimated for each phenotype by calculating the intraclass correlation (r_I) and the coefficient of genetic determination (g^2) using derived values for mean square between (MSB) strains and mean square within (MSW) strains (Festing, 1979). r_I may be interpreted as the proportion of total phenotypic variation that is accounted for by differences between strains, while g^2 accounts for the additive genetic variance that doubles during inbreeding (Festing, 1979; Falconer, 1989; Lightfoot et al., 2001), so g^2 is a more appropriate estimate for broad sense heritability in this study. However, other studies sometimes only provide one estimate or the other, so we have included both values to facilitate comparisons with other findings in the literature. r_I and g^2 were calculated using the following formulas, where n is the number of mice per strain:

$$r_i = \frac{(MSB - MSW)}{MSB + (n - 1)MSW} \qquad g^2 = \frac{(MSB - MSW)}{MSB + (2n - 1)MSW}$$

The number of mice per strain varies in this study, so *n* was calculated as:

$$n = \frac{1}{(a - 1)}\left(N - \frac{\Sigma n_i^2}{N}\right)$$

where a is the number of strains, n_i is the number of mice in the *i*th strain, and N is the total number of mice (samples) per phenotype.

Post-diet Broad-Sense Heritability Estimates

Post-diet broad-sense heritability estimates (H^2) were calculated for each trait to contrast the proportion of relative heritable variation attributed to genetics or diet, and to assess whether different diet "environments" affect heritability. Post-diet intraclass correlation (r_I) values and the coefficients of genetic determination (g^2) were calculated using the formulas above and the MSB and MSW derived from four different linear models: (1) a "full" additive model with strain, diet, and week as variables fitted with

phenotype data from both experimental diets, (2) a “partial” additive model including strain and week as variables (diet excluded) fitted with phenotype data from both experimental diets, (3) a “HP” additive model including strain and week as variables fitted with phenotype data from only mice fed the HP diet, and (4) a “HS” additive model including strain and week as variables fitted with phenotype data from only mice fed the HS diet. H^2 estimates derived from models fitted with data from all mice post-diet compare the contribution of genetics (strain) and diet overall to heritable phenotypic variance, while diet-specific H^2 estimates were calculated to discern differences in heritability affected by differences in macronutrient composition.

Quantification of Heritable Variation Attributed to Genetics, Diet, and Gene × Diet Interactions

Linear mixed models with strain, diet, and strain x diet interactions as random effects (intercepts) were fitted using all post-diet phenotype data for body fat % and obesity-related traits to quantify the relative heritable variation attributed to genetics, diet, and gene × diet interactions based on the variance of each term in the model. The approximate values for the proportion of variance for strain, diet, and interaction were calculated by dividing the variance for each term by the sum of the variance for all terms in the model (including residuals).

Quantification of Diet Effect Size

To quantify size effects of diet on each trait, Hedges’ g values for the HP diet were calculated by using the baseline-specific (AIN-76A) mean of the phenotype minus the HP-specific mean of the phenotype (M_1-M_2), and then dividing this value by the weighted pooled standard deviation (SD) for the two groups (Ellis, 2009):

$$\text{Hedges}' g = \frac{M_1 - M_2}{SD^*_{pooled}}$$

The weighted pooled SDs was calculated using the following equation where n_1 = the number of samples from mice on the AIN-76A diet and n_2 = the number of samples from mice on the HP diet:

$$SD^{*}_{pooled} = \sqrt{\frac{(n_1 - 1)SD_1^2 + (n_2 - 1)SD_2^2}{n_1 + n_2 - 2}}$$

Calculations for Hedges' g were performed using the following function from the effsize package in R (Torchiano, 2019), with pooled weighted SD, unpaired samples, removed NA entries and a 95% confidence interval, where d = phenotype measurements and f = experimental diets: cohen.d (d, f, pooled = TRUE, paired = FALSE, na.rm = TRUE, hedges.correction = TRUE, conf.level = 0.95). Corrected Hedges' g effect sizes are presented as standard deviation units so that a Hedges' g value of 1 indicates that the baseline diet and respective experimental diet differ by 1 standard deviation, a g of 2 indicates they differ by 2 standard deviations, and so on with the sign indicating the direction of change between diets. Positive Hedges' g indicates increased phenotype values post-diet compared to baseline, e.g. body fat % was increased from baseline in mice after feeding them the HP diet. Magnitude descriptions are based on the following cut-offs of |g|: negligible < 0.2 < small < 0.5 < medium < 0.8 < large. Hedges' g values were calculated for the HS diet for each trait using the same method.

To further quantify the effect size of diet, we also calculated the intraclass correlation (ICC) for diet using the mean square between (MSB) diets and mean square within (MSW) diets derived from post-diet linear models including strain, diet, and week as variables, using the following formula where n = number of mice on each diet:

$$ICC = \frac{(MSB - MSW)}{MSB + (n - 1)MSW}$$

The ICC for diet can be interpreted as the proportion of the total phenotypic variation that is accounted for by differences between diet.

Testing Significance of Phenotypic Difference Between Day and Night Cycles for Metabolic Traits

Phenotype data for metabolic traits were viewed in histograms to check for normality of the distributions, revealing skewness and non-normality. Thus, Wilcoxon signed rank tests with continuity correction were performed instead of student's t-tests using the following function from the stats package in R (R Core Team, 2019), with paired samples and a 95% confidence interval, where day = diurnal metabolic trait data

and night = corresponding nocturnal metabolic trait data: wilcox.test (day, night, paired = TRUE, conf.int = TRUE).

Additional Statistical Analyses

All statistical analyses were performed in R (R Core Team, 2019). Summary statistics were calculated for all phenotypic data, include means and standard error (SE). Spearman's correlations were performed to determine significant relationships between traits at baseline and post-diet. To ascertain the magnitude of the effect of diet behind gene x environmental effects found for each trait in our linear mixed models, Spearman's correlation analysis was performed between the F-statistic of the gene x diet interactions of our models and Hedges' effect size for both diets (|g|). Each trait was categorized as either largely affected by diet ($|g| > 0.8$) or not ($|g| < 0.8$), and significantly affected by gene x diet interactions ($p < 0.05$) or not ($p > 0.05$), followed by Chi square analysis of whether the effect size of diet and the gene x environment are significantly related for the given trait.

Results

Baseline Traits Show Extensive Phenotypic Variation Among CC Strains

Baseline values for adiposity (synonymous with body fat % in this study), clinical blood chemistries, and circulating metabolites were established to assess the degree of phenotypic variation due to genetic background of the CC strain (see Methods and Figure 1-1). Adiposity and circulating metabolic health marker levels exhibited wide ranges of phenotypic variation by CC strain (Figure 1-2) and there was a wide range of adiposity in the CC population ranging from 1.1 to 29.8% body fat, with strain CC019/TauUnc least susceptible to obesity (average body fat 4.4 ± 0.6%) and strain CC028/GeniUnc most susceptible (average body fat 23.1 ± 1.5%) (Figure 1-2A). Similarly, there was a wide range in average weight across the CC lines ranging from 12.4 ± 0.2g in strain CC019/TauUnc to 23.7 ± 1.0g in line CC011/Unc (Figure 1-2B). Within CC strains, CC040/TauUnc had the highest range of variability in adiposity (1.7–29.3%), while CC030/GeniUnc had the lowest range of variability in adiposity (7.6–12.6%). CC040/TauUnc had the highest variability in weight (11–28g), while CC019/TauUnc had the lowest variability in weight (11.1–13.2 g). Linear regression analysis was performed to assess the significance of

the effect of strain on each of the measured traits at baseline (Supplementary Table 4), and strain was found to have a significant effect on almost all traits, especially body fat % ($F = 12.44$, $p = 7.71 \times 10^{-25}$) and weight ($F = 19.39$, $p = 3.95 \times 10^{-35}$). To estimate the overall health of the mice from each CC strain, a metabolic health score was calculated using the sum of Z scores from measurements of several metabolic risk factors (circulating glucose, insulin, glucose/insulin ratio, cholesterol, TG, and body fat %). While the health score includes body fat % as one of the components, the phenotypes exhibited across CC strains for circulating analytes typically used as markers of metabolic health (circulating glucose, insulin, glucose/insulin ratio, cholesterol, TG) varied so that although one strain may have high body fat %, it may simultaneously have low levels of TG or glucose, such as CC040/TauUnc at baseline. Because metabolic health is determined by multiple phenotypes, the health score provides a way to estimate overall metabolic health for each CC strain in a way that accounts for these differences. For example, at baseline CC028/GeniUnc had the highest BF% but its health score was close to 0, so it was not exceedingly unhealthy relative to the other strains in this study despite its high BF%, since this strain's glucose, TG, and cholesterol levels were not elevated. Similar to adiposity and circulating analytes (Figures 1-2C–F), metabolic health also showed a wide range of phenotypic variation by CC strain at baseline (Figure 1-2G), where most strains with higher adiposity also appeared to have decreased metabolic health (Figures 1-2A, 1-2G), with the exception of CC028/GeniUnc.

Total Body Weight Has a Limited Effect on Increased Adiposity

To determine whether total body weight predicts susceptibility to increased adiposity, body fat % was correlated with total body weight. Although the leanest strain overall (CC019/TauUnc, average body fat 4.41 ± 0.56%) was on average also the smallest strain (12.4 ± 0.22 g) and body fat % was positively correlated with weight overall (Figure 1-3B, rho = 0.56, $p < 2.2 \times 10^{-16}$), the largest average CC strain was not necessarily the most susceptible to developing obesity (Figures 1-2A, 1-2B, Figure S1-3). For example, the CC strains with the highest average weight (23.4 ± 0.97 g in CC011/Unc, 22.4 ± 1.08 g in CC028/GeniUnc, and 22.3 ± 0.7 g in CC008/GeniUnc) did not necessarily always have the highest body fat % (15.7 ± 1.29% in CC011/Unc, 23.11 ± 1.59% in CC028/GeniUnc, and 15.00 ± 1.39% in CC008/GeniUnc).

Adiposity Exhibits Complex Associations Across Various Health Measures at Baseline

Excessive adiposity is a risk factor for metabolic dysfunction and thus we quantified the relationship between circulating plasma analyte levels and body fat % (Figure 1-3). For example, for traits associated with metabolic syndrome such as total weight, circulating glucose, insulin, TG, and cholesterol, body fat % was significantly correlated with weight (rho = 0.56, $p < 2.2 \times 10^{-16}$), insulin (rho = 0.44, $p = 8.8 \times 10^{-11}$), and TG (rho = 0.24, $p = 5.9 \times 10^{-4}$) as shown in Figures 1-3B – 1-3D, but not glucose nor cholesterol. In terms of metabolites associated with cardiovascular health, adiposity was not correlated with the risk factor TMAO but was moderately associated with circulating choline (rho = 0.190, p adj = 0.012), carnitine (rho = 0.17, p adj = 0.023), and phosphocholine (rho = 0.260, p adj = 0.001; Figure 1-3A, Supplementary Table 5).

Estimates for Broad Sense Heritability (H^2) Show the Size of Strain Effects on Phenotypic Variation at Baseline

We next calculated broad sense heritability (H^2) of traits at baseline to quantify the degree that genetic variation influences phenotypic variation compared to the variation of environmental factors. Linear regression analysis was performed to test whether strain had significant effects on phenotypic variation. Strain was a significant predictor for all traits at baseline except for circulating non-esterified fatty acids (Supplementary Table 4). Using the between- and within-strain mean square values (MSB and MSW, respectively) derived from these linear models, broad sense heritability (H^2) was estimated by calculating the intraclass correlation (r_I) and coefficient of genetic determination (g^2) which determine the proportion of phenotypic variation accounted for by differences between strain (genetic variation) (Table 1-1). Estimates of H^2 for phenotypic variation based on g^2 were 0.359–0.565. The highest and lowest estimates of H^2 were for lean mass ($g^2 = 0.565$) and circulating non-esterified fatty acids ($g^2 = 0.029$). Our assessment of H^2 demonstrates that genotypic variation accounts for a large proportion of phenotypic variation in the CC for all body composition traits and a medium proportion of phenotypic variation for traits related to 1-carbon metabolism. We note that not all analytes were highly heritable.

Genetic Background Mediates Degree of Weight Gain, Adiposity, and Metabolic Health in Response to Diet

After establishing baseline phenotype values to examine the effect of strain without the influence of diet, we investigated the effect of diet in the CC population on weight gain and metabolic health. To accomplish this, we randomized the 204 female mice from 22 CC strains to one of two diets and challenged them for 8 weeks with either a high protein (n = 102) or high fat high sucrose diet (n = 102). After 8 weeks on the experimental diets, we assessed whether phenotypic response to diet differed by genetic background (CC strain) (see Methods and Figure 1-1). MRI body composition analysis of the CC mice after 8 weeks on the diet challenge revealed that diet influenced susceptibility to adiposity in a strain-dependent manner (Figure 1-4A). Strain CC028/GeniUnc was most susceptible to increased adiposity on the high fat high sucrose (HS) diet (35.7 ± 2.0%) and strain CC019/TauUnc was least susceptible (4.68 ± 0.5%) (Figure 1-4A, Figure S1-4). CC040/TauUnc was most susceptible to increased adiposity on the high protein (HP) diet (29.7 ± 1.37%) and strain CC019/TauUnc was least susceptible (4.7 ± 0.47%). The effect of diet was highly variable across the selected strains from the CC. For example, CC028/GeniUnc and CC004/TauUnc had a 12% increase in adiposity when fed the HS diet compared to the HP diet (Figure S1-4), while CC019/TauUnc and CC063/Unc showed negligible differences in adiposity when fed different diets (0.05 and 0.54%). Comparisons of phenotypic differences between baseline and post-diet body fat % (Figure S1-5A) by strain and diet further emphasize the strain-dependent response of body fat % to diet in the CC.

Similar to adiposity, total weight, circulating analyte levels, and metabolic health score all showed phenotypic variation and different responses to diet depending on CC strain (Figure 1-4), though to a lesser degree than adiposity. As shown in Figure 1-4G, strain effects account for the inherent phenotypic variation in metabolic health illustrated by metabolic health score, as well as the varied responses to diet. Certain strains such as CC059/TauUnc and CC008/GeniUnc showed very little responses to diet in terms of their metabolic health score, while other strains showed improved metabolic health on either the HP compared to the HS diet (CC032/GeniUnc and CC004/TauUnc) or HS compared to the HP diet (CC012/GeniUnc and CC030/GeniUnc).

To ascertain whether there is a significant effect of CC strain x experimental diet interaction on adiposity and related traits, linear mixed models were fitted as appropriate for each trait using CC strain, experimental diet, and CC strain × experimental diet as covariates, followed by application of the Satterthwaite approximations for degrees of freedom for evaluating significance (Supplementary Table 6). A significant effect of CC strain × diet interactions was found for adiposity, fat mass, lean mass percentage, metabolic health score, and circulating TMAO and TG. The models for each phenotype were significant with the range of significant p-values from $p = 7.37 \times 10^{-5}$ for adiposity to $p = 0.03$ for metabolic health score ($F = 3.36$ and $F = 1.84$). There was a significant effect of strain on circulating cholesterol, glucose, and insulin (Supplementary Table 6), but no significant effect of CC strain × diet interactions which suggests that genotypic variation is largely responsible for the phenotypic variation of these traits.

To determine the magnitude of the effect of diet behind gene × environmental effects found for each trait in our linear mixed models, we performed Spearman's correlation analysis between the F-statistic of the gene x diet interactions of our models and Hedges' effect size for both diets (|g|), which demonstrated that the significance of gene x diet interactions were not significantly affected by diet for either diet (HP $p = 0.96$, HS $p = 0.74$). Furthermore, we categorized each trait as either largely affected by diet ($|g| > 0.8$) or not ($|g| < 0.8$), and significantly affected by gene × diet interactions ($p < 0.05$) or not ($p > 0.05$), followed by Chi square analysis of whether the effect size of diet and the gene x environment are significantly related. The results of the Chi square analysis ($p > 0.99$) were consistent with the results of the Spearman's correlations performed between the F-statistic of the gene x diet interactions models and Hedges' effect size for both diets (|g|) which suggest that the magnitude of the effect of diet is not a significant "driver" of gene x diet interactions.

Magnitude of Quantified Diet Effects Varies Depending on Diet Macronutrient Composition for Body Composition and Obesity-Related Traits

Because diet is an important environmental factor that affects the manifestation of phenotypes, we next investigated the relative effect size of diet on clinical traits associated with adiposity and metabolic health. To accurately quantify the effect size of diet on each phenotype, Hedges' g was calculated for each trait instead of Cohen's d because strain groups were dissimilar in sample size for various traits. The

difference in n by strain may result in unequal measures of variation between experimental groups, which needs to be adjusted for so that the standard deviation (SD) used to calculate effect size more closely reflects the SD of the population. Hedges' g uses pooled SD weighted by sample size of each group in its calculation (see Methods), which makes it a more appropriate measure of effect size when experimental groups are dissimilar in sample size compared to Cohen's d (Ellis, 2009). As shown in Table 1-2, the HS diet had large effects on circulating choline, urea and non-esterified fatty acids (NEFAS), as well as most traits associated with body composition ($|g| > 0.8$); medium effects on adiposity, glucose/insulin ratio, TMAO, and albumin ($0.8 > |g| > 0.5$); and small to negligible on all other phenotypes ($|g| < 0.5$). In contrast, the HP diet only had large effects on total weight ($|g| > 0.8$); medium effects on glucose/insulin ratio, circulating choline, TMAO, NEFAS, albumin, urea, cholesterol, and TG, as well as all phenotypes associated with body composition; and small to negligible on all other phenotypes ($|g| < 0.5$).

Post-diet values for body fat %, clinical blood chemistries, and circulating metabolites were established for each diet to assess the degree of phenotypic variation due to differences in macronutrient composition of diet (Figure 1-5). For both diets, there was a wide range of phenotypic variation within each diet for body fat % (HP = 3.6–33.9%, HS = 3.17–41.7%), total weight (HP = 14.1–35.5 g, HS = 14.1–39 g) and cholesterol (HP = 64.8–199.5 mg/dL, HS = 63–228.9 mg/dL). Means per diet for body fat % (HP = 17.95 ± 0.77%, HS = 20.31 ± 0.97%), total body weight (HP = 21.78 ± 0.42 g, HS = 24.02 ± 0.5 g), TG (HP = 104.6 ± 4.28 mg/dL, HS = 113.65 ± 4.73 mg/dL), cholesterol (HP = 115.1 ± 2.85 mg/dL, HS = 139.51 ± 3.3 mg/dL), glucose (HP = 173.45 ± 5.73 mg/dL, HS = 184.63 ± 6.42 mg/dL), and insulin (1.03 ± 0.05 ng/ml, HS = 1.17 ± 0.08 ng/ml) showed slightly elevated values for each trait on the HS diet compared to the HP diet (Supplementary Table 7), but the only significant increases in phenotype were for total weight and cholesterol ($p < 0.01$, Student's t-test), not body fat %, TG, glucose, insulin, nor metabolic health score (Figure 1-5). Relative to mice fed the HP diet, mice fed the HS diet showed a 10.6% increase in total weight (Figure 1-5B) and a 21.2% increase in cholesterol (Figure 1-5D), suggesting that macronutrient composition had a stronger effect on these traits compared to body fat %, TG, glucose, insulin, and metabolic health score.

To further assess whether diet had a significant effect on adiposity and related phenotypes, linear mixed model analysis was performed (Supplementary Table 8), which showed that experimental diet had a significant effect on all phenotypes related to body composition, except post-diet body fat % for which diet showed a suggestive effect ($F = 3.98$, $p = 0.057$). Although experimental diet alone did not have a significant effect in general on body fat %, TG, glucose, insulin, nor metabolic health score (Figure 1-5), experimental diet did have significant effects on total weight ($F = 20.0$, $p = 0.0002$) and cholesterol ($F = 43.8$, $p = 6.22 \times 10^{-7}$). Furthermore, experimental diet also had significant effects on circulating urea, betaine, TMAO, carnitine, and phosphocholine (Supplementary Table 8), indicating that diet macronutrient composition still plays an important role in terms of metabolic health.

To confirm the degree to which genetic background mediates weight gain, adiposity, and metabolic health in response to diet, additional linear mixed model analyses with strain, diet, and strain × diet interactions as all random effects were performed for each trait to estimate the relative heritable variation that can be attributed to genetics, environment (diet), and gene × environmental effects. From the results of these models, we calculated the variance for each of these terms (Supplementary Table 9) and found that a large proportion of relative phenotypic variation can be attributed to background strain for most traits, especially body fat %, total weight, and TG (> ∼49.6%). In contrast, the proportion of relative phenotypic variation that can be attributed to diet varied depending on the trait, where cholesterol, betaine, and urea/BUN were the traits that had the highest proportion of heritable variation attributed to diet (> ∼21%).

Post-diet Estimates for Broad Sense Heritability (H^2) Reaffirm the Strong Contribution of Strain Effects on Heritable Phenotypic Variation and Identify Traits With High Proportions of Heritable Phenotypic Variation Attributed to Diet

The degree to which genetics, diet, and gene x diet interactions influence phenotypic variation differs depending on the trait. To quantify the relative heritable phenotypic variation which can be attributed to genetics or diet for body fat % and obesity-related traits, we calculated heritability using the mean square between (MSB) strains and mean square within (MSW) strains derived from two different linear models for post-diet traits (a “full” additive model that includes strain, diet, and week as variables and a “partial”

model that excludes diet) and the intraclass correlation (ICC) for diet using the mean square between (MSB) diets and mean square within (MSW) diets derived from the "full" model (Methods). Heritability estimates were similar for most traits regardless of the model used ("full" vs. "partial") except for traits where the ICCs for diet were relatively high, such as total weight, cholesterol, urea/BUN, and betaine, demonstrating the robust contribution of strain to heritable variation compared to diet (Table 1-2). The relatively high diet ICCs for total weight, cholesterol, urea/BUN, and betaine suggest that diet may be responsible for a higher proportion of heritable variation for these traits compared to other traits, which is consistent with the results of our linear mixed models testing the significance of diet that also show diet as significantly affecting these traits (Supplementary Table 8). Traits with negative or close to zero diet ICCs had higher within-diet variation than between- diet variation. Interestingly, with the exception of insulin and metabolic health score, most post-diet traits had higher heritability estimates when the MSB term was used from linear models that included diet compared to the models excluding diet, suggesting that accounting for the effect of diet improved heritability estimates since either the within-strain variation was decreased and/or the between-strain variation was increased.

Diet-specific heritability was also calculated using linear models fitted only including mice fed HP or HS diet with strain and week as covariates to compare changes in heritability for each experimental diet due to "environmental" differences (Tables 1-1 and 1-2). One caveat of comparing baseline heritability and diet-specific post-diet heritability is that diet-specific post-diet heritability values were calculated using half the number of mice as the baseline heritability values, which could affect the within-strain variance component of the heritability calculations. Nonetheless, assuming that the genotypic variance is the same between diets and time points (baseline vs. post-diet), we can still identify which traits may be more strongly affected by differences in macronutrient composition. Indeed, after calculating the heritability estimates for each of the traits post- diet on the respective experimental diets, we found that the different "environments" (diets) resulted in slight alterations in heritability estimates depending on the trait. For example, the difference in macronutrient composition appears to have a bigger impact on traits such as cholesterol, insulin, and glucose with larger variation in heritability (Table 1-2), and less important to traits such as metabolic health score where heritability estimates remain consistent (baseline $g^2 = 0.21$, HP $g^2 = 0.24$, HS $g^2 = 0.24$).

Comparison of Quantified Metabolic Traits During Daytime and Nighttime Show Decreased Rates of Metabolism, Energy Intake, Utilization of Carbohydrates as a Fuel Source, and Basal Activity During Rest

Obesity is characterized by the excess accumulation of body fat, which results from chronic energy imbalance between energy intake and expenditure. Given the diverse range of body fat accumulation in response to diet across strains, we sought to elucidate the differences in metabolism between strains on each diet by using indirect calorimetry to measure the following traits related to energy balance in mice after 8 weeks on the experimental diets: (1) heat expenditure to estimate metabolism levels, (2) respiratory exchange rate (RER) to estimate substrate utilization (carbohydrate compared to fat as a source of energy), (3) food intake to estimate energy consumption, and (4) basal activity. Energy consumption was calculated by multiplying feed consumption measurement (in grams) by the calorie (kcal) content per gram feed for each diet. Similar to other phenotypes reported above, linear mixed model analysis was performed for each trait to test whether experimental diet, CC strain, and/or CC strain x experimental diet interactions had significant effects on metabolic traits.

Heat production, RER, energy intake, and basal activity phenotypes varied widely by CC strain (Figure 1-6, Figures S1-6 and S1-7, Supplementary Table 10), with phenotype measurements higher at night than day which reflected the active nocturnal behavior of mice. Wilcoxon signed rank tests performed comparing the day and night measurements for each trait confirmed the differences between light and dark cycles for all strains on both diets ($p < 2.2 \times 10^{-16}$ for all traits). Overall heat production while accounting for total weight (Heat1) was highest on average for both day and night cycles in the leanest strain, CC019/TauUnc on the HS diet (21.4 ± 0.64 and 27.6 ± 0.87 kcal/h/kg in the day and night) (Figure 1-6A, Figure S1-6A). During the day, heat production was lowest in CC030/GeniUnc on the HP diet (11.9 ± 2.86 kcal/h/kg) even though CC030/GeniUnc was relatively lean compared to other strains on the same diet (11.9 ± 1.2%, Figure 1-4A), while heat production was lowest in CC008/GeniUnc during the night on the HS diet (15.4 ± 0.30 kcal/h/kg), which was one of the fatter strains compared to other strains in the same diet (26.8 ± 1.3%, Figure 1-4A). Overall heat production while accounting for only lean mass (Heat2) (Figure 1-6B, Figure S1-6B) was highest on average for CC019/TauUnc on the HS diet during the day (7.61 ± 0.23 kcal/h/kg) and CC004/TauUnc on the HP diet at night (10.2 ± 0.56 kcal/h/kg) and lowest

in CC030/GeniUnc on the HP diet (4.35 ± 1.06 and 5.81 ± 0.94 kcal/h/kg in the day and night, respectively). In summary, variation in energy production was much larger between strains than diets, with the differences in phenotype by diet depending on the strain (Figure S1-6). Linear mixed model analysis showed that CC strain × experimental diet interactions had significant effects on energy production during both day and night, but the effect of CC strain was much stronger and may be driving the effects of CC strain × experimental diet interactions (Supplementary Table 6). In linear mixed models testing the effect of experimental diet alone, diet had a significant effect on Heat2 during the day ($F = 5.3$, $p = 0.03$) but not any of the other heat production measured (Supplementary Table 8), suggesting that diet may have a different effect on metabolism during the day for lean mass compared to non-lean mass.

Similar to energy production, the wide range of variation in substrate utilization (RER) depended on CC strain and were all higher during the night compared to the day for mice within the same strain on either diets (Figure 1-6C, Figure S1-7A). RER levels were lowest in CC030/GeniUnc during the day and CC012/GeniUnc during the night on the high protein diet (0.725 ± 0.007 and 0.76 ± 0.01) even though the adiposity of CC012/GeniUnc was twice the adiposity of CC030/GeniUnc (24.1 ± 1.8% and 11.9 ± 1.2%, Figure 1-4A), while RER levels were highest in CC071 during the day and CC019/TauUnc during the night on the HS diet (0.825 ± 0.019 and 0.943 ± 0.013) despite vastly different levels of adiposity (13.9 ± 1.5% in CC071 and 4.7 ± 0.5% in CC019/TauUnc, Figure 1-4A). Surprisingly, linear mixed model analysis revealed that both CC strain and experimental diet independently had significant effects on RER for both day and night (Supplementary Tables 6, 8), but despite CC strain having a stronger effect than diet, the effects of CC strain × diet interactions were not significant.

Our indirect calorimetry assays were also able to calculate the energy intake and activity of the mice over the 48-h test. As expected, there were significant differences between night and day cycles in both of these behaviors, as confirmed by the results of Wilcoxon signed rank tests ($p < 2.2 \times 10^{-16}$). Energy intake was lowest in the lean strain CC041/TauUnc on the HP diet for both day and night (0.579 ± 0.110 kcal/h and 2.68 ± 0.915 kcal/h) (Figure 1-6D, Figure S1-7B). Food intake was highest for strain CC024/GeniUnc during the day (16.47 ± 3.661 kcal/h) and CC019/TauUnc during the night (26.64 ± 7.301 kcal/h) on the HS diet. The energy consumption was variable depending on the diet consumed. For

example, in terms of mice on the HS diet, energy intake was highest in CC024/GeniUnc during the day (16.47 ± 3.66 kcal/h) and CC019/TauUnc at night (26.64 ± 7.30 kcal/h), and lowest in CC063/Unc during both day (2.32 ± 0.17 kcal/h) and night (4.59 ± 0.44 kcal/h). Additionally, energy intake for CC063/Unc was extremely variable on the HP diet during the day and night (39.45 ± 18.00 kcal/h and 40.42 ± 17.34 kcal/h). Because of this high variability, four types of linear mixed models were fitted for both day and night energy intake: (1) model testing for the effect of diet including CC063/Unc, (2) model testing for the effect of diet excluding CC063/Unc, (3) model testing for the effect of CC strain × diet including CC063/Unc, and (4) model testing for the effect of CC strain x diet excluding CC063/Unc. For energy intake both day and night, both experimental diet and CC strain had significant effects on energy intake regardless of whether CC063/Unc was included, but the CC strain × diet interaction did not significantly affect energy intake when CC063/Unc was excluded (Supplementary Table 11). Although we could not identify a specific error with the collection or calculation of the data for CC063/Unc, results for energy intake from CC063/Unc should be interpreted with caution.

Basal activity exhibited phenotypic variation depending on and between CC strains, but barely any difference by diet (Figure 1-6E, Figure S1-7C). Diurnal basal activity was lowest in CC030/GeniUnc on the HP diet and CC041/TauUnc on the HS diet (988.8 ± 383.1 and 1,188.2 ± 260.6 beam breaks/h), and highest in CC004/TauUnc on the HP diet and CC045/GeniUnc on the HS diet (4,328.1 ± 985.7 and 3,322.5 ± 988.8 beam breaks/h), while nocturnal basal activity was lowest in CC012/GeniUnc on the both HP and HS diets (2,304.4 ± 124.7 and 2,792.7 ± 337.7 beam breaks/h), and highest in CC004/TauUnc on HP and CC045/GeniUnc on HS diets (16,742.5 ± 1,919.9 and 11,081.9 ± 6,070.3 beam breaks/h). Linear mixed model analysis confirmed that only CC strain had a significant effect on both diurnal and nocturnal basal activity; the effects of experimental diet and CC strain x experimental diet interactions were not significant (Supplementary Tables 6, 8).

Complex Relationships Between Adiposity, Energy Intake, and Energy Expenditure Suggest an Important Role of Diet Substrate Utilization in Maintaining Energy Homeostasis

Our comprehensive phenotyping demonstrates the high variability among metabolic traits. Using the phenotyping data, Spearman's correlations between body composition and traits related to energy intake

or expenditure were performed. Although the individual phenotypes are variable between strains there are several notable results, such as the negative correlations between body fat percentage and all expenditure phenotypes except for basal activity and diurnal fat intake (rho < −0.17, p adj < 0.02). Conversely, lean mass percentage was positively correlated with all energy intake/expenditure phenotypes except for basal activity, diurnal fat intake, and diurnal carbohydrate intake (rho > 0.16, p adj < 0.025, Figure 1-7). Total body weight was significantly but negatively correlated with nocturnal protein intake, night RER, and heat expenditure (rho < −0.17, p adj < 0.01), while all energy intake phenotypes were positively correlated with RER, energy expenditure phenotypes, and basal activity (rho > 0.19, p adj < 0.008, Figure 1-7). Body fat percentage and heat production (accounting for total weight) are negatively correlated for both day and night (rho = −0.563 and rho = −0.612), stronger than the negative correlations between body fat percentage and energy intake (rho = −0.20 and rho = −0.26). These data demonstrate that the decrease in food intake as body fat percentage increases is not enough to maintain energy balance.

Coupled with average adiposity measurements, indirect calorimetry data demonstrated that energy expenditure varies tremendously between inbred strains of similar weight, specifically strains CC030/GeniUnc and CC019/TauUnc (Figure 1-8). Although mice from these two strains were close in terms of average total body weight (Figure 1-8A), the average body fat percentage of CC030/GeniUnc was more than twice the average body fat percent of CC019/TauUnc (Figure 1-8B). CC019/TauUnc stayed consistently lean across diets, while CC030/GeniUnc's highest average post-diet body fat percentage paradoxically decreased with increasing dietary fat content (Figure 1-4A). Comparing the two strains of mice on the same diet, CC019/TauUnc mice consistently consumed more calories than CC030/GeniUnc mice during both day and night (Figure 1-8C) but also consistently produced more heat than CC030/GeniUnc and importantly, produced enough heat to achieve energy balance (Figure 1-8D). In addition to CC019/TauUnc's relatively high metabolism, the difference in substrate utilization between the two strains could help to explain their different responses to diet (Figure 1-8E); during the night, the average RERs of CC019/TauUnc are 0.943 and 0.926 on the HS and HP diets, and the average RERs of CC030/GeniUnc are 0.82 and 0.798 on the HS and HP diets, implying that CC019/TauUnc mice are utilizing carbohydrates as their fuel source more than CC030/GeniUnc mice, which could suggest that

CC019/TauUnc mice are more active than CC030/GeniUnc mice. Intriguingly, substrate utilization during the light phase was quite different between strains. The average RERs of CC019/TauUnc across diets is 0.800 and the average RERs of CC030/GeniUnc are 0.746 and 0.725 on the HS and HP diets, which suggests that at rest CC030/GeniUnc mice preferentially utilize fat as an energy source more than carbohydrate as compared to CC019/TauUnc mice (Figure 1-8F).

Small but Significant Alterations in Metabolite Levels Are Associated With Diet-Driven Adiposity, but Largely Not Associated With Metabolic Phenotypes

Given the variation in diet-driven changes in adiposity, we next investigated whether there were alterations in metabolic health in corresponding fashion. We correlated body fat % after diet feeding with other traits (Figure 1-9). Broadly, body fat % is strongly correlated with total weight (Figure 1-9B; rho > 0.579, $p < 3.91 \times 10^{-10}$), and moderately correlated with insulin levels, total heat production, and total RER (Supplementary Table 12). Remarkably, the significant correlations at baseline (Figure 1-3) between body fat % and TG (rho = 0.24, p adj = 1.35×10^{-3}), carnitine (rho = 0.17, p adj = 0.036), and choline (rho = 0.19, p adj = 0.018) were no longer significant after the diet challenge (Supplementary Table 12), indicating that the metabolic effect of diet varies among clinical traits.

Spearman's correlation analysis performed between metabolic traits and other traits related to adiposity (Figure 1-9A) revealed significant correlations between metabolic health score and heat production accounting for total weight (Heat1) (rho = 0.37, p adj = 2.61×10^{-7}), heat production accounting for lean mass (Heat2) (rho = 0.32, p adj = 9.12×10^{-6}), feed intake (rho = 0.20, p adj =0.009), energy intake (rho = 0.20, p adj = 0.009), and RER (rho = 0.36, p adj = 7.08×10^{-7}) but not basal activity (p adj = 0.76). These relationships are heavily driven by the body fat % component of metabolic health score, as body fat % was also significantly correlated with these traits but in the "opposite" direction (Supplementary Table 12). Of all circulating analytes and 1-carbon metabolites, RER was moderately correlated with albumin (rho = −0.29, p adj = 3.21×10^{-4}), heat production accounting for total body weight and lean mass showed a slight negative correlation with albumin (rho = −0.19, p adj = 0.01 for both heat production estimations), and feed intake was positively correlated with betaine (rho = 0.21, p adj = 0.02) and carnitine (rho = 0.22, p adj = 9.12×10^{-3}). Other than metabolic traits, the only traits which

total basal activity showed slight correlations with were insulin (rho = 0.23, p adj = 3.31×10^{-3}) and glucose/insulin ratio (rho = −0.19, p adj = 0.02).

Discussion

With the rapid rise in the global prevalence of obesity and obesity-related diseases in the recent decades (Flegal, 2010; Ogden et al., 2016, 2018), there is a crucial need to improve our understanding of obesity. Individually, diet and genetics are known to be critical factors in the development of obesity, but our understanding of how diet and genetics interact to affect obesity remain to be fully elucidated. Taken at the individual gene level, this is a daunting task. There are hundreds of genes associated with body weight and BMI reported in the GWAS catalog and these can interact with each other and diet, increasing the complexity of obesity (Bell et al., 2005; Rankinen et al., 2006; Kunej et al., 2013; Singh et al., 2017). Thus, the complexity and heterogeneity of obesity may affect dietary recommendations, as illustrated by the lack of a universally “perfect” diet for weight loss (Dansinger et al., 2005; Johnston et al., 2014). Increasing our knowledge of how genetics and environmental factors (particularly diet) interact, the degree to which these interactions impact the development of obesity, and the mechanisms behind these effects are crucial to developing successful methods for mitigating obesity.

To investigate the degree that genetics, diet, and gene-by-diet interactions impact phenotypic variation in obesity, obesity- related traits, and metabolic traits, we performed our study on multiple strains of mice from the CC genetic reference population to overcome the limitations of human studies, especially in terms of controlling genetic background, diet, and other environmental influences. The CC is well-suited for investigating genetic vs. environmental impacts on phenotypic variation due to its high genetic diversity and ability to generate genetic “replicates” which enables increased accuracy in phenotypic measurements. Indeed, the CC has already been used to provide a genetic framework to depict the relationship between body weight and the central nervous system (Mao et al., 2015), high fat diet and fasting glucose levels (Atamni et al., 2016), and hepatic gene expression in response to impaired glucose tolerance (Atamni et al., 2019). The CC has also been used as a model to study exercise-induced paradoxical fat response (McMullan et al., 2018). The current manuscript adds to this literature by examining the dietary responses of the CC.

While previous studies have examined subsets of obesity-related traits in the CC and energy balance traits have been examined in pre-CC lines (Mathes et al., 2011), we examined the unique effect of diet in this population. In this study we sought to elucidate the relationships between genetic background, diet, adiposity, and obesity-related traits. Our comprehensive phenotyping included: body composition, circulating analyte and metabolite levels, and metabolism through indirect calorimetry, followed by the integration of all these data in common analyses. We found that in the absence of dietary perturbation, many of the traits phenotyped in this manuscript are heritable in the CC. Defined as the proportion of phenotypic variation due to genetic variation for a specific population, we calculated broad sense heritability for adiposity and other traits for mice on the synthetic chow diet at baseline to estimate the strength of genetic contribution. Traits related to body composition had moderately high broad sense heritability (g^2) at baseline ranging between 0.359 and 0.565, with the broad sense heritability estimate of total body weight at 0.499 which is higher than a previously reported estimate in the CC at 0.37 (Atamni et al., 2016). Given that H^2 estimates can vary among studies, we also calculated g^2 for baseline body fat %, lean %, and total weight using four publicly available body composition data sets (McMullan et al., 2018). The range of g^2 for these traits across the 4 data sets were between 0.268 and 0.511, similar to the estimates in this study. The average baseline g^2 for lean % in the McMullan study ($g^2 = 0.358$) and the current study ($g^2 = 0.359$) were closer than the average g^2 for weight in the McMullan study ($g^2 = 0.357$) and the current study ($g^2 = 0.499$), but the average g^2 for body fat % in the McMullan study and the current study was the same ($g^2 = 0.383$), which is close to the minimum heritability of 0.4 in humans as indicated by twin studies (Bell et al., 2005). The heritability of most circulating metabolites in the CC varied between 12 and 46%, similar to the heritability of circulating small metabolites and amino acids in humans, which has been reported to vary between 23 and 55% (Dharuri et al., 2014). Interestingly, broad sense heritability for circulating insulin (0.153) was much lower than heritability for adiposity (0.383), which implies that environmental factors such as diet or lifestyle may have a stronger effect on attenuating hyperinsulinemia than adiposity. Overall, these data suggest similar overall metabolic health parameters to those observed in humans, demonstrating that the high genetic, and phenotypic diversity of the CC make this mouse panel a suitable model for studying obesity, a trait with complex etiology.

Furthermore, we have identified which specific strains have predispositions for increased adiposity accumulation, total weight, circulating analyte levels, and metabolic phenotypes.

Like the CC, the relationship between weight and adiposity is not always uniform within humans (Hashimoto et al., 2016; Verheggen et al., 2016). While the CC mostly showed a strong positive relationship between adiposity and weight, several strains such as CC011/Unc, CC008/GeniUnc, and CC059/TauUnc that weighed more than the majority of other strains had only ∼15% body fat, compared to the fattest strains with 20–23% body fat. Similar to the relationship between weight and adiposity, the relationship between adiposity and overall metabolic health can vary within humans (Yaghootkar et al., 2014, 2016; Ding et al., 2016; Gonçalves et al., 2016; Iacobini et al., 2019). At both baseline and post-diet, significant associations between body fat % and individual markers of metabolic health were only detected consistently for body fat % and insulin, and body fat % and alanine transaminase (ALT). One possible explanation for the lack of associations obtained is the nocturnal eating pattern of mice, since the concentration of glucose and insulin fluctuates with their circadian rhythms (Jensen et al., 2013), though the number of hours that the mice were fasted prior to the blood draw could also have minor effects on the analytes measured.

By estimating the average metabolic health of each CC strain via calculation of a metabolic health score, we identified CC028/GeniUnc and CC040/TauUnc as two of the fattest strains in our study that were healthier than the leaner strains CC030/GeniUnc and CC041/TauUnc at baseline, whose body fat % were half of CC028/GeniUnc and CC040/TauUnc, mirroring the “sub-phenotypes” within obesity of metabolically “healthy” or “unhealthy” individuals found in human studies (Peppa et al., 2013; Dobson et al., 2015; Schulze, 2019). After the 8-week diet challenge, CC028/GeniUnc and CC040/TauUnc remained healthier than CC030/GeniUnc, while CC041/TauUnc was both leaner and healthier than these three strains, reflecting the strain- dependent effect of diet.

At baseline the body fat % measured in the CC mice demonstrated that the predisposition to developing obesity occurred in a strain-dependent manner; baseline body fat % also highlighted the wide phenotypic variation across strains and minor phenotypic variation within strains, which varied by trait and strain. For most traits at baseline such as total weight, TG, cholesterol, and glucose, the ranges of strain

coefficients of variation (CV%) were within ~20%; for example, the CV% of total weight for each strain was 4.91–23.2% where CC030/GeniUnc exhibited the lowest within-strain phenotypic variation (CV% = 4.91) and CC040/TauUnc exhibited the highest within-strain phenotypic variation (CV% = 23.2%). The range of strain CV% for baseline body fat % was slightly larger (13.9–44.7%), demonstrating that certain traits may be more sensitive to environmental differences such as being housed in different cages which could lead to differences in microbiome exposure, or minor genetic differences since completed CC lines are at least 98% homozygous (UNC Systems Genetics Core Facility, 2012) but not necessarily the same degree of homozygosity across individuals.

By analyzing the post-diet metabolic traits measured in these 22 CC strains together, our data recapitulates some key findings in humans by Sims (1976). As expected, metabolic rate estimated as heat production had the strongest inverse relationship with post-diet body fat %, which implies that body fat % increases as metabolic rate decreases. Body fat % was not significantly correlated with basal activity, showing that spontaneous physical activity alone did not significantly alter the degree of adiposity accumulation. Remarkably, energy intake decreased as body fat % increased; when adjusted for total body weight, this negative correlation increased in both strength and significance regardless of diet (Figure S1-8), suggesting that the body attempts to adjust energy consumption and maintain energy homeostasis when adiposity is in excess, as reflected by changes in hormone levels that regulate energy consumption such as increased leptin secretion from adipose tissue (Caro et al., 1996; Friedman and Halaas, 1998) and lower levels of the gut satiety-related peptide tyrosine-tyrosine (PYY) found in obese individuals (Simpson et al., 2011). As body fat % increases, the secretion of the satiety hormone leptin from adipocytes also increases, which would lead to a decrease in appetite and therefore a decrease in feed consumption. Because the HS diet contains 290 g of sucrose for 1,042.8 g of HS diet and the HP diet contains 113 g of sucrose for 1,000.1 g of HP diet, another potential explanation for the negative correlation between energy intake and body fat % is the glucostatic theory, which states that glucose availability and utilization in specific regions of the brain may affect the regulation of satiety perception and short- term regulation of appetite (Mayer, 1953). Thus, for two mice consuming the same grams of experimental diet, the mouse fed the HS diet would consume more sucrose than the mouse fed the HP diet, resulting in a difference in the availability of glucose for each mouse and possible differences in the

utilization of macronutrients depending on the strain (genetic effects). For example, the night RER of CC002/Unc was 0.847 on the HP diet and 0.921 on the HS diet, whereas the night RER of CC008/GeniUnc was 0.821 on the HP diet and 0.824 on the HS diet (Supplementary Table 10). Future studies using isocaloric diets with more extreme differences only in fat content or only sucrose content would help determine whether the stronger negative correlation between energy intake after correcting for total weight and body fat % of mice fed the HS diet is attributed to increased fat or sucrose content.

As accumulation of adiposity increased, RER decreased which implies increased utilization of fat as the substrate for energy expenditure since fat is in excess. RER was strongly positively correlated with heat production, illustrating that the increase in metabolic rate shifts substrate utilization toward carbohydrates and away from fat. If energy expenditure remains unchanged, the metabolic flexibility of shifting from carbohydrate utilization toward lipid utilization would compensate for the decrease in energy consumption (Farias et al., 2011; Goodpaster and Sparks, 2017). Along with the strong positive correlation between heat production and energy intake, the relationships between metabolic traits reaffirm the implication of energy balance. The consistency between the current results and Sims' results demonstrates the ability of the CC to reliably model human genotypic and phenotypic variation when studying complex traits.

After 8 weeks of feeding the CC mice either the HP or HS diet, assessing body composition in the CC revealed the strains' different responses to diet in terms of weight gain and other phenotypic changes in obesity-related traits. Consistent with the findings of Barrington et al. (2017), the strength of the effect of diet depended on the trait examined, macronutrient composition, and subject strain (genetic background). For example, certain CC strains did not respond to differences in macronutrient composition, either remaining persistently fat (CC040/TauUnc, CC063/Unc, CC001/Unc) or lean (CC019/TauUnc) regardless of experimental diet, while other strains clearly accumulated less body fat % on the HP diet compared to the HS diet (CC028/GeniUnc, CC004/TauUnc, CC006/TauUnc). Furthermore, experimental diet alone did not have a significant effect in general on circulating glucose, insulin, nor TG based on the results of the linear mixed model analysis, but certain CC strains showed drastic differences in phenotypic response to diet for these traits, such as CC036/Unc, CC002, and

CC004/TauUnc for TG; CC036/Unc and CC040/TauUnc for glucose; and CC040/TauUnc, CC004/TauUnc, CC045/GeniUnc, and CC032/GeniUnc for insulin. The different response to diet by CC strain suggests that variation in genetic architecture may contribute to differences in individual nutrient need and substrate utilization, which should be taken into account when developing weight loss strategies.

Similar to the findings in this study, a recent large-scale human study performed by Berry et al. (2020) examining postprandial metabolic response to food relative to precision nutrition highlighted large inter-individual variability when subjects were fed identical meals, and found that genetic background and environmental factors, including person-specific factors (e.g., the microbiome) and meal macronutrients, had varying degrees of influence on traits assessed. Mirroring the broad range of phenotypic response to diet in the CC, human participants in the DIETFITS Randomized Clinical Trial that were administered either a low-fat or low-carbohydrate diet also exhibited a wide range of response to diet in terms of weight loss over 12 months, regardless of their genotypes defined by three SNPs (Gardner et al., 2018). Due to the complex etiology of obesity, studies in humans endeavoring to prove direct relationships between individual SNPs and obesity have succeeded in finding associations between genetic loci and body weight (Deeb et al., 1998; Scuteri et al., 2007; Speliotes et al., 2010; Claussnitzer et al., 2015; Hägg et al., 2015), but translational application of these associations will first require further investigation into the biological function of novel obesity-associated genetic loci (Loos, 2018) and elucidation of the causes behind conflicting findings where associations between genetic loci and phenotypes were not detected (Sørensen et al., 2006; Drabsch et al., 2018; Gardner et al., 2018; Merino et al., 2018). Nevertheless, the phenotypic variation in adiposity by CC strain in this study clearly illustrate the genetic predisposition for developing obesity, concurrent with findings in humans (Stunkard et al., 1986; Bouchard and Tremblay, 1997; Viitasalo et al., 2019). Therefore, effective mitigation of obesity using personalized nutrition would ideally incorporate information regarding an individual's genetic background, behavior, environmental influences, physiological response to diet, and socioeconomic situation in addition to their genotype in terms of recommendations for alterations in diet and exercise levels (Drabsch and Holzapfel, 2019).

One caveat of our study design is that we cannot assess the effect of aging nor whether there are strain specific age-related phenotypes given the natural variation both between strains and between individuals within strains. Similar to the current study, a preprint of a pending manuscript utilizing B × D mice indicates that certain age-related phenotypes such as longevity and weight are under strong genetic regulation and are also affected by diet and gene-by-environmental interactions (Roy et al., 2019). Our diet challenge and age are confounded and we cannot assess differences in genetic susceptibility that are age dependent. Additional investigations using a modified study design could effectively assess the effect of aging on metabolic factors in CC mice.

Although basal activity levels were assessed, one limitation of this study is the lack of "true" exercise activity (e.g., wheel running), since increased weight loss in humans has been shown to be associated with increased physical activity if calorie intake is controlled (Zemel et al., 2009). Another caveat of this study is the unavailability of metabolic phenotype data for the mice at baseline (e.g., energy expenditure, feed intake, RER, basal activity), which limits the conclusions that can be made regarding the effects of diet compared to feed intake on energy balance when interpreting these data. Moreover, recent studies have found that the gut microbiota also potentially play a significant role in the development of obesity (Tilg and Kaser, 2011; Pace and Crowe, 2016; Lee et al., 2018). Further studies should be performed with multiple genetically diverse populations to determine which diets would be most effective for weight loss by individuals according to their genetic background and to examine the state of epigenetic markers and transcript expression levels in specific tissues.

References

Abete, I., Astrup, A., Martínez, J. A., Thorsdottir, I., and Zulet, M. A. (2010). Obesity and the metabolic syndrome: role of different dietary macronutrient distribution patterns and specific nutritional components on weight loss and maintenance. Nutr. Rev. 68, 214–231. doi: 10.1111/j.1753-4887.2010. 00280.x

Atamni, H. J., Abu-Toamih, M. R., Soller, M., and Iraqi, F. A. (2016). High-fat-diet induced development of increased fasting glucose levels and impaired response to intraperitoneal glucose challenge in the collaborative cross mouse genetic reference population. BMC Genet. 17:10. doi: 10.1186/s12863-015-0321-x

Atamni, H. J. A-T., Kontogianni, G., Binenbaum, I., Mott, R., Himmelbauer, H., Lehrach, H., et al. (2019). Hepatic gene expression variations in response to high-fat diet-induced impaired glucose tolerance using RNAseq analysis in collaborative cross mouse population. Mamm. Genome 30, 260–275. doi: 10.1007/s00335-019-09816-1

Attie, A. D., Churchill, G. A., and Nadeau, J. H. (2017). How mice are indispensable for understanding obesity and diabetes genetics. Curr. Opin. Endocrinol. Diabetes Obes. 24, 83–91. doi: 10.1097/MED.0000000000000321

Barrington, W. T., Wulfridge, P., Wells, A. E., Rojas, C. M., Howe, S. Y. F., Perry, A., et al. (2017). Improving metabolic health through precision dietetics in mice. Genetics 208, 399–417. doi: 10.1534/genetics.117.300536

Bates, D., Mächler, M., Bolker, B., and Walker, S. (2015). Fitting linear mixed- effects models using lme4. J. Stat. Softw. 67, 1–48. doi: 10.18637/jss.v067.i01

Bell, C. G., Walley, A. J., and Froguel, P. (2005). The genetics of human obesity. Nat. Rev. Genet. 6, 221–234. doi: 10.1038/nrg1556

Berry, S. E., Valdes, A. M., Drew, D. A., Asnicar, F., Mazidi, M., Wolf, J., et al. (2020). Human postprandial responses to food and potential for precision nutrition. Nat. Med. 26, 964–973. doi: 10.1038/s41591-020-0934-0

Bouchard, C., and Tremblay, A. (1997). Genetic influences on the response of body fat and fat distribution to positive and negative energy balances in human identical twins. J. Nutr. 127, 943S−947S. doi: 10.1093/jn/127.5.943S

Caro, J. F., Sinha, M. K., Kolaczynski, J. W., Li Zhang, P., and Considine, R. V. (1996). Leptin: the tale of an obesity gene. Diabetes 45, 1455–1462. doi: 10.2337/diab.45.11.1455

Carroll, L., Voisey, J., and Van Daal, A. (2004). Mouse models of obesity. Clin. Dermatol. 22, 345–349. doi: 10.1016/j.clindermatol.2004.01.004

Churchill, G. A., Airey, D. C., Allayee, H., Angel, J. M., Attie, A. D., Beatty, J., et al. (2004). The collaborative cross, a community resource for the genetic analysis of complex traits. Nat. Genet. 36, 1133–1137. doi: 10.1038/ng1104-1133

Claussnitzer, M., Dankel, S. N., Kim, K.-H., Quon, G., Meuleman, W., Haugen, C., et al. (2015). FTO obesity variant circuitry and adipocyte browning in humans. N. Engl. J. Med. 373, 895–907. doi: 10.1056/NEJMoa1502214

Coleman, D. L., and Hummel, K. P. (1974). Hyperinsulinemia in pre-weaning diabetes (db) mice. Diabetologia 10, 607–610. doi: 10.1007/BF01221993

Collaborative Cross Consortium (2012). The genome architecture of the collaborative cross mouse genetic reference population. Genetics 190, 389–401. doi: 10.1534/genetics.111.132639

Creasy, S. A., Rynders, C. A., Bergouignan, A., Kealey, E. H., and Bessesen, D. H. (2018). Free-living responses in energy balance to short-term overfeeding in adults differing in propensity for obesity. Obesity 26, 696–702. doi: 10.1002/oby.22121

Cuthbertson, D. J., Steele, T., Wilding, J. P., Halford, J. C., Harrold, J. A., Hamer, M., et al. (2017). What have human experimental overfeeding studies taught us about adipose tissue expansion and susceptibility to obesity and metabolic complications? Int. J. Obes. 41, 853–865. doi: 10.1038/ijo.2017.4

Danforth, E. (1985). Diet and obesity. Am. J. Clin. Nutr. 41, 1132–1145. doi: 10.1093/ajcn/41.5.1132

Dansinger, M. L., Gleason, J. A., Griffith, J. L., Selker, H. P., and Schaefer, E. J. (2005). Comparison of the atkins, ornish, weight watchers, and zone diets for weight loss and heart disease risk reduction. JAMA 293, 43–53. doi: 10.1001/jama.293.1.43

Deeb, S. S., Fajas, L., Nemoto, M., Pihlajamäki, J., Mykkänen, L., Kuusisto, J., et al. (1998). A Pro12Ala substitution in PPARgamma2 associated with decreased receptor activity, lower body mass index and improved insulin sensitivity. Nat. Genet. 20, 284–287. doi: 10.1038/3099

Dharuri, H., Demirkan, A., van Klinken, J. B., Mook-Kanamori, D. O., van Duijn, C. M., t Hoen, P. A. C., et al. (2014). Genetics of the human metabolome, what is next? Biochim. Biophys. Acta Mol. Basis Dis. 1842, 1923–1931. doi: 10.1016/j.bbadis.2014.05.030

Ding, C., Chan, Z., and Magkos, F. (2016). Lean, but not healthy. Curr. Opin. Clin. Nutr. Metab. Care 19, 408–417. doi: 10.1097/MCO.0000000000000317

Dobson, R., Burgess, M. I., Sprung, V. S., Irwin, A., Hamer, M., Jones, J., et al. (2015). Metabolically healthy and unhealthy obesity: differential effects on myocardial function according to metabolic syndrome, rather than obesity. Int. J. Obes. 40, 153–161. doi: 10.1038/ijo.2015.151

Drabsch, T., Gatzemeier, J., Pfadenhauer, L., Hauner, H., and Holzapfel, C. (2018). Associations between single nucleotide polymorphisms and total energy, carbohydrate, and fat intakes: a systematic review. Adv. Nutr. 9, 425–453. doi: 10.1093/advances/nmy024

Drabsch, T., and Holzapfel, C. (2019). A scientific perspective of personalised gene- based dietary recommendations for weight management. Nutrients 11:617. doi: 10.3390/nu11030617

Eknoyan, G. (2006). A history of obesity, or how what was good became ugly and then bad. Adv. Chronic Kidney Dis. 13, 421–427. doi: 10.1053/j.ackd.2006.07.002

Ellis, P. D. (2009). Effect Size Equations. Polyu.Edu.Hk. Available online at: https:// www.polyu.edu.hk/mm/effectsizefaqs/effect_size_equations2.html

Falconer, D. S. (1989). Introduction to Quantitative Genetics. New York, NY: Longman, Scientific & Technical.

Farias, M. M., Cuevas, A. M., and Rodriguez, F. (2011). Set-point theory and obesity. Metab. Syndr. Relat. Disord. 9, 85–89. doi: 10.1089/met.2010.0090

Fernández-Verdejo, R., Marlatt, K. L., Ravussin, E., and Galgani, J. E. (2019). Contribution of brown adipose tissue to human energy metabolism. Mol. Aspects Med. 68, 82–89. doi: 10.1016/j.mam.2019.07.003

Festing, M. F. W. (1979). Inbred Strains in Biomedical Research. London: Macmillan Education UK. doi: 10.1007/978-1-349-03816-9

Fischer, J., Emmerling, C., and Ruther, U. (2008). On the history of FTO. Obes. Facts 1, 43–44. doi: 10.1159/000113466

Flegal, K. M. (2010). Prevalence and trends in obesity among US adults, 1999–2008. JAMA 303, 235–241. doi: 10.1001/jama.2009.2014

Fox, J., and Weisberg, S. (2019). An {R} Companion to Applied Regression, 3rd Edn. Sage. Available online at: https://socialsciences.mcmaster.ca/jfox/Books/ Companion/

Friedman, H. (2015). "Behavioral medicine," in Encyclopedia of Menta Health, 2nd Edn, 158. Available online at: https://www.elsevier.com/books/encyclopedia-of-mental-health/friedman/978-0-12-397045-9

Friedman, J. M., and Halaas, J. L. (1998). Leptin and the regulation of body weight in mammals. Nature 395, 763–770. doi: 10.1038/27376

Gardner, C. D., Trepanowski, J. F., Del Gobbo, L. C., Hauser, M. E., Rigdon, J., Ioannidis, J. P. A., et al. (2018). Effect of low-fat vs low-carbohydrate diet on 12-month weight loss in overweight adults and the association with genotype pattern or insulin secretion. JAMA 319, 667–679. doi: 10.1001/jama. 2018.0245

Gonçalves, C. G., Glade, M. J., and Meguid, M. M. (2016). Metabolically healthy obese individuals: key protective factors. Nutrition 32, 14–20. doi: 10.1016/j.nut.2015.07.010

González-Muniesa, P., Mártinez-González, M.-A., Hu, F. B., Després, J.-P., Matsuzawa, Y., Loos, R. J. F., et al. (2017). Obesity. Nat. Rev. Dis. Prim. 3:17034. doi:10.1038/nrdp.2017.34

Goodpaster, B. H., and Sparks, L. M. (2017). Metabolic flexibility in health and disease. Cell Metab. 25, 1027–1036. doi: 10.1016/j.cmet.2017.04.015

Hägg, S., Ganna, A., Van Der Laan, S. W., Esko, T., Pers, T. H., Locke, A. E., et al. (2015). Gene-based meta-analysis of genome-wide association studies implicates new loci involved in obesity. Hum. Mol. Genet. 24, 6849–6860. doi: 10.1093/hmg/ddv379

Hashimoto, Y., Fukuda, T., Oyabu, C., Tanaka, M., Asano, M., Yamazaki, M., et al. (2016). Impact of low-carbohydrate diet on body composition: meta-analysis of randomized controlled studies. Obes. Rev. 17, 499–509. doi: 10.1111/obr. 12405

Heindel, J. J., and Blumberg, B. (2019). Environmental obesogens: mechanisms and controversies. Annu. Rev. Pharmacol. Toxicol. 59, 89–106. doi: 10.1146/annurev-pharmtox-010818-021304

Hill, J. O., Wyatt, H. R., and Peters, J. C. (2012). Energy balance and obesity. Circulation 126, 126–132. doi: 10.1161/CIRCULATIONAHA.111.087213

Hu, S., Wang, L., Yang, D., Li, L., Togo, J., Wu, Y., et al. (2018). Dietary fat, but not protein or carbohydrate, regulates energy intake and causes adiposity in mice. Cell Metab. 28, 415–431.e4. doi: 10.1016/j.cmet.2018.06.010

Hummel, K. P., Dickie, M. M., and Coleman, D. L. (1966). Diabetes, a new mutafton in the mouse. Science 153, 1127–1128. doi: 10.1126/science.153.3740.1127

Iacobini, C., Pugliese, G., Blasetti Fantauzzi, C., Federici, M., and Menini, S. (2019). Metabolically healthy versus metabolically unhealthy obesity. Metab. Clin. Exp. 92, 51–60. doi: 10.1016/j.metabol.2018.11.009

Ingalls, A. M., Dickie, M. M., and Snell, G. D. (1950). Obese, a new mutation in the house mouse∗. J. Heredity 41, 317–318. doi: 10.1093/oxfordjournals.jhered.a106073

Iraqi, F. A., Churchill, G., and Mott, R. (2008). The collaborative cross, developing a resource for mammalian systems genetics: a status report of the wellcome trust cohort. Mamm. Genome 19, 379–381. doi: 10.1007/s00335-008-9113-1

Janesick, A. S., and Blumberg, B. (2016). Obesogens: an emerging threat to public health. Am. J. Obstet. Gynecol. 214, 559–565. doi: 10.1016/j.ajog.2016.01.182

Jensen, T., Kiersgaard, M., Sørensen, D., and Mikkelsen, L. (2013). Fasting of mice: a review. Lab. Anim. 47, 225–240. doi: 10.1177/0023677213501659

John, G. K., and Mullin, G. E. (2016). The gut microbiome and obesity. Curr. Oncol. Rep. 18:45. doi: 10.1007/s11912-016-0528-7

Johnston, B. C., Kanters, S., Bandayrel, K., Wu, P., Naji, F., Siemieniuk, R. A., et al. (2014). Comparison of weight loss among named diet programs in overweight and obese adults. JAMA 312, 923–933. doi: 10.1001/jama.2014.10397

Kunej, T., Skok, D. J., Zorc, M., Ogrinc, A., Michal, J. J., Kovac, M., et al. (2013). Obesity gene atlas in mammals. J. Genomics 1, 145–55. doi: 10.7150/jgen.3996

Kuznetsova, A., Brockhoff, P. B., and Christensen, R. H. B. (2017). lmerTest package: tests in linear mixed effects models. J. Stat. Softw. 82, 1–26. doi: 10.18637/jss.v082.i13

Larsen, T. M., Dalskov, S.-M., van Baak, M., Jebb, S. A., Papadaki, A., Pfeiffer, A. F. H., et al. (2010). Diets with high or low protein content and glycemic index for weight-loss maintenance. N. Engl. J. Med. 363, 2102–2113. doi: 10.1056/NEJMoa1007137

Lee, P., Yacyshyn, B. R., and Yacyshyn, M. B. (2018). Gut microbiota and obesity: an opportunity to alter obesity through faecal microbiota transplant (FMT). Diabetes Obes. Metab. 21, 479–490. doi: 10.1111/dom.13561

Lightfoot, J. T., Turner, M. J., Debate, K. A., and Kleeberger, S. R. (2001). Interstrain variation in murine aerobic capacity. Med. Sci. Sports Exerc. 33, 2053–2057. doi: 10.1097/00005768-200112000-00012

Loos, R. J. (2018). The genetics of adiposity. Curr. Opin. Genet. Dev. 50, 86–95. doi: 10.1016/j.gde.2018.02.009

Loos, R. J. F., Lindgren, C. M., Li, S., Wheeler, E., Zhao, J. H., Prokopenko, I., et al. (2008). Common variants near MC4R are associated with fat mass, weight and risk of obesity. Nat. Genet. 40, 768–775. doi: 10.1038/ng.140

Lu, D., Willard, D., Patel, I. R., Kadwell, S., Overton, L., Kost, T., et al. (1994). Agouti protein is an antagonist of the melanocyte-stimulating-hormone receptor. Nature 371, 799–802. doi: 10.1038/371799a0

Luke, S. G. (2016). Evaluating significance in linear mixed-effects models in R. Behav. Res. Methods 49, 1494–1502. doi: 10.3758/s13428-016-0809-y

Makris, A., and Foster, G. D. (2011). Dietary approaches to the treatment of obesity. Psychiatr. Clin. North Am. 34, 813–827. doi: 10.1016/j.psc.2011.08.004

Mao, J.-H., Langley, S. A., Huang, Y., Hang, M., Bouchard, K. E., Celniker, S. E., et al. (2015). Identification of genetic factors that modify motor performance and body weight using collaborative cross mice. Sci. Rep. 5:16247. doi: 10.1038/srep16247

Martinez, K. B., Pierre, J. F., and Chang, E. B. (2016). The gut microbiota. Gastroenterol. Clin. North Am. 45, 601–614. doi: 10.1016/j.gtc.2016.07.001

Mathes, W. F., Aylor, D. L., Miller, D. R., Churchill, G. A., Chesler, E. J., de Villena, F. P.-M., et al. (2011). Architecture of energy balance traits in emerging lines of the collaborative cross. Am. J. Physiol. Endocrinol. Metab. 300, E1124–E1134. doi: 10.1152/ajpendo.00707.2010

Mayer, J. (1953). Glucostatic mechanism of regulation of food intake. N. Engl. J. Med. 249, 13–16. doi: 10.1056/NEJM195307022490104

McMullan, R. C., Ferris, M. T., Bell, T. A., Menachery, V. D., Baric, R. S., Hua, K., et al. (2018). CC002/Unc females are mouse models of exercise-induced paradoxical fat response. Physiol. Rep. 6:e13716. doi: 10.14814/phy2.13716

Merino, J., Dashti, H. S., Li, S. X., Sarnowski, C., Justice, A. E., Graff, M., et al. (2018). Genome-wide meta-analysis of macronutrient intake of 91,114 European ancestry participants from the cohorts for heart and aging research in genomic epidemiology consortium. Mol. Psychiatry 24, 1920–1932.

doi: 10.1038/s41380-018-0079-4

Myrmel, L. S., Fauske, K. R., Fjære, E., Bernhard, A., Liisberg, U., Hasselberg,

A. E., et al. (2019). The impact of different animal-derived protein sources on adiposity and glucose homeostasis during ad libitum feeding and energy restriction in already obese mice. Nutrients 11:1153. doi: 10.3390/nu11051153

Ogden, C. L., Carroll, M. D., Fakhouri, T. H., Hales, C. M., Fryar, C. D., Li, X., et al. (2018). Prevalence of obesity among youths by household income and education level of head of household - united states 2011–2014. MMWR Morb. Mortal. Wkly Rep. 67, 186–189. doi: 10.15585/mmwr.mm6706a3

Ogden, C. L., Carroll, M. D., Lawman, H. G., Fryar, C. D., Kruszon-Moran, D., Kit, B. K., et al. (2016). Trends in obesity prevalence among children and adolescents in the United States, 1988-1994 through 2013-2014. JAMA 315, 2292–2299. doi: 10.1001/jama.2016.6361

Oussaada, S. M., van Galen, K. A., Cooiman, M. I., Kleinendorst, L., Hazebroek, E. J., van Haelst, M. M., et al. (2019). The pathogenesis of obesity. Metab. Clin. Exp. 92, 26–36. doi: 10.1016/j.metabol.2018.12.012

Pace, L. A., and Crowe, S. E. (2016). Complex relationships between food, diet, and the microbiome. Gastroenterol. Clin. North Am. 45, 253–265. doi: 10.1016/j.gtc.2016.02.004

Peppa, M., Koliaki, C., Papaefstathiou, A., Garoflos, E., Katsilambros, N., Raptis, S. A., et al. (2013). Body composition determinants of metabolic phenotypes of obesity in nonobese and obese postmenopausal women. Obesity 21, 1807–1814. doi: 10.1002/oby.20227

Philip, V. M., Sokoloff, G., Ackert-Bicknell, C. L., Striz, M., Branstetter, L., Beckmann, M. A., et al. (2011). Genetic analysis in the collaborative cross breeding population. Genome Res. 21, 1223–1238. doi: 10.1101/gr.113 886.110

Pomp, D., Nehrenberg, D., and Estrada-Smith, D. (2008). Complex genetics of obesity in mouse models. Annu. Rev. Nutr. 28, 331–345. doi: 10.1146/annurev.nutr.27.061406.093552

R Core Team (2019). R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing. Available online at: https://www.R- project.org/

Rankinen, T., Zuberi, A., Chagnon, Y. C., Weisnagel, S. J., Argyropoulos, G., Walts, B., et al. (2006). The human obesity gene map: the 2005 update. Obesity 14, 529–644. doi: 10.1038/oby.2006.71

Robinson, S. W., Dinulescu, D. M., and Cone, R. D. (2000). Genetic models of obesity and energy balance in the mouse. Annu. Rev. Genet. 34, 687–745. doi: 10.1146/annurev.genet.34.1.687

Romieu, I., Dossus, L., Barquera, S., Blottière, H. M., Franks, P. W., Gunter, M., et al. (2017). Energy balance and obesity: what are the main drivers? Cancer Causes Control 28, 247–258. doi: 10.1007/s10552-017-0869-z

Roy, S., Sleiman, M. B., Jha, P., Williams, E. G., Ingels, J. F., Chapman, C. J., et al. (2019). Gene-by-environmental modulation of longevity and weight gain in the murine BXD family. BioRxiv. doi: 10.1101/776559

San-Cristobal, R., Navas-Carretero, S., Martínez-González, M. Á., Ordovas, J. M., and Martínez, J. A. (2020). Contribution of macronutrients to obesity: implications for precision nutrition. Nat. Rev. Endocrinol. 16, 305–320. doi: 10.1038/s41574-020-0346-8

Schmidt, S. L., Harmon, K. A., Sharp, T. A., Kealey, E. H., and Bessesen, D. H. (2012). The effects of overfeeding on spontaneous physical activity in obesity prone and obesity resistant humans. Obesity 20, 2186–2193. doi: 10.1038/oby.2012.103

Schulze, M. B. (2019). Metabolic health in normal-weight and obese individuals. Diabetologia 62, 558–566. doi: 10.1007/s00125-018-4787-8

Scuteri, A., Sanna, S., Chen, W.-M., Uda, M., Albai, G., Strait, J., et al. (2007). Genome-wide association scan shows genetic variants in the FTO gene are associated with obesity-related traits. PLoS Genet. 3:e115. doi: 10.1371/journal.pgen.0030115

Shahnazaryan, U., Wójcik, M., Bednarczuk, T., and Kuryłowicz, A. (2019). Role of obesogens in the pathogenesis of obesity. Medicina 55:515. doi: 10.3390/medicina55090515

Shorter, J. R., Najarian, M. L., Bell, T. A., Blanchard, M., Ferris, M. T., Hock, P., et al. (2019). Whole genome sequencing and progress toward full inbreeding of the mouse collaborative cross population. G3 9, 1303–1311. doi: 10.1534/g3.119.400039

Simpson, K., Parker, J., Plumer, J., and Bloom, S. (2011). CCK, PYY and PP: the control of energy balance. Handb. Exp. Pharmacol. 209, 209–230. doi: 10.1007/978-3-642-24716-3_9

Sims, E. A. H. (1976). Experimental obesity, dietary-induced thermogenesis, and their clinical implications. Clin. Endocrinol. Metab. 5, 377–395. doi: 10.1016/S0300-595X(76)80027-8

Singh, R. K., Kumar, P., and Mahalingam, K. (2017). Molecular genetics of human obesity: a comprehensive review. C. R. Biol. 340, 87–108. doi: 10.1016/j.crvi.2016.11.007

Sørensen, T. I. A., Boutin, P., Taylor, M. A., Larsen, L. H., Verdich, C., Petersen, L., et al. (2006). Genetic polymorphisms and weight loss in obesity: a randomised trial of hypo-energetic high- versus low-fat diets. PLoS Clin. Trials 1:e12. doi: 10.1371/journal.pctr.0010012

Speakman, J. R. (2018). Obesity and thermoregulation. Handb. Clin. Neurol. 10, 679–89, 431–443. doi: 10.1016/B978-0-444-63912-7.00026-6

Speliotes, E. K., Willer, C. J., Berndt, S. I., Monda, K. L., Thorleifsson, G., Jackson, A. U., et al. (2010). Association analyses of 249,796 individuals reveal 18 new loci associated with body mass index. Nat. Genet. 42, 937–948. doi: 10.1038/ng.686

Srivastava, A., Morgan, A. P., Najarian, M. L., Sarsani, V. K., Sigmon, J. S., Shorter, J. R., et al. (2017). Genomes of the mouse collaborative cross. Genetics 206, 537–556. doi: 10.1534/genetics.116.198838

Stunkard, A. J., Sørensen, T. I. A., Hanis, C., Teasdale, T. W., Chakraborty, R., Schull, W. J., et al. (1986). An adoption study of human obesity. N. Engl. J. Med. 314, 193–198. doi: 10.1056/NEJM198601233140401

Swift, D. L., McGee, J. E., Earnest, C. P., Carlisle, E., Nygard, M., and Johannsen, N. M. (2018). The effects of exercise and physical activity on weight loss and maintenance. Prog. Cardiovasc. Dis. 61, 206–213. doi: 10.1016/j.pcad.2018.07.014

Tartaglia, L. A., Dembski, M., Weng, X., Deng, N., Culpepper, J., Devos, R., et al. (1995). Identification and expression cloning of a leptin receptor, OB-R. Cell 83, 1263–1271. doi: 10.1016/0092-8674(95)90151-5

Threadgill, D. W., and Churchill, G. A. (2012). Ten years of the collaborative cross. Genetics 190, 291–294. doi: 10.1534/genetics.111.138032

Tilg, H., and Kaser, A. (2011). Gut microbiome, obesity, and metabolic dysfunction. J. Clin. Invest. 121, 2126–2132. doi: 10.1172/JCI58109

Timper, K., and Brüning, J. C. (2017). Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. Dis. Model. Mech. 10, 679–689. doi: 10.1242/dmm.026609

Torchiano, M. (2019). effsize: Efficient Effect Size Computation. R-Packages. Available online at: https://CRAN.R-project.org/package=effsize

Torres-Fuentes, C., Schellekens, H., Dinan, T. G., and Cryan, J. F. (2017). The microbiota-gut-brain axis in obesity. Lancet Gastroenterol. Hepatol. 2, 747–756. doi: 10.1016/S.2468-1253(17)30147-4

UNC Systems Genetics Core Facility (2012). Compgen Tool Suite. Csbio.Unc.Edu. Available online at: https://csbio.unc.edu/CCstatus/index.py

Verheggen, R. J. H. M., Maessen, M. F. H., Green, D. J., Hermus, A. R. M. M., Hopman, M. T. E., and Thijssen, D. H. T. (2016). A systematic review and meta-analysis on the effects of exercise training versus hypocaloric diet: distinct effects on body weight and visceral adipose tissue. Obes. Rev. 17, 664–690. doi: 10.1111/obr.12406

Viitasalo, A., Schnurr, T. M., Pitkänen, N., Hollensted, M., Nielsen, T. R. H., Pahkala, K., et al. (2019). Genetic predisposition to higher body fat yet lower cardiometabolic risk in children and adolescents. Int. J. Obes. 43, 2007–2016. doi: 10.1038/s41366-019-0414-0

Wang, Z., Levison, B. S., Hazen, J. E., Donahue, L., Li, X.-M., and Hazen, S. L. (2014). Measurement of trimethylamine-N-oxide by stable isotope dilution liquid chromatography tandem mass spectrometry. Anal. Biochem. 455, 35–40. doi: 10.1016/j.ab.2014.03.016

Welsh, C. E., Miller, D. R., Manly, K. F., Wang, J., McMillan, L., Morahan, G., et al. (2012). Status and access to the collaborative cross population. Mamm. Genome 23, 706–712. doi: 10.1007/s00335-012-9410-6

West, D. B., Boozer, C. N., Moody, D. L., and Atkinson, R. L. (1992). Dietary obesity in nine inbred mouse strains. Am. J. Physiol. Regul. Integr. Comp. Physiol. 262, R1025–R1032. doi: 10.1152/ajpregu.1992.262.6.R1025

West, D. B., Waguespack, J., and McCollister, S. (1995). Dietary obesity in the mouse: interaction of strain with diet composition. Am. J. Physiol. Regul. Integr. Comp. Physiol. 268, R658–R665. doi: 10.1152/ajpregu.1995.268.3.R658

WHO (2015). Obesity and overweight Fact sheet N◦ 311. World Health Organization. Available online at: http://www.who.int/mediacentre/factsheets/ fs311/en/

Wilborn, C., Beckham, J., Campbell, B., Harvey, T., Galbreath, M., La Bounty, P., et al. (2005). Obesity: prevalence, theories, medical consequences, management, and research directions. J. Int. Soc. Sports Nutr. 2, 4–31. doi: 10.1186/1550-2783-2-2-4

Yaghootkar, H., Lotta, L. A., Tyrrell, J., Smit, R. A. J., Jones, S. E., Donnelly, L., et al. (2016). Genetic evidence for a link between favorable adiposity and lower risk of type 2 diabetes, hypertension, and heart disease. Diabetes 65, 2448–2460. doi: 10.2337/db15-1671

Yaghootkar, H., Scott, R. A., White, C. C., Zhang, W., Speliotes, E., Munroe, P., et al. (2014). Genetic evidence for a normal-weight “metabolically obese” phenotype linking insulin resistance, hypertension, coronary artery disease, and type 2 diabetes. Diabetes 63, 4369–4377. doi: 10.2337/db14-0318

Zemel, M., Teegarden, D., Van Loan, M., Schoeller, D., Matkovic, V., Lyle, R., et al. (2009). Dairy-rich diets augment fat loss on an energy-restricted diet: a multicenter trial. Nutrients 1, 83–100. doi: 10.3390/nu1010083

Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L., and Friedman, J. M. (1994). Positional cloning of the mouse obese gene and its human homologue. Nature 372, 425–432. doi: 10.1038/372425a0

Figure 1-1. Experimental design and timeline.

Collaborative Cross (CC) mice were obtained between 6 and 9 weeks of age (n = 204) and acclimated for 2 weeks on standard synthetic diet (AIN-76A) for baseline phenotype assessment which included body composition assessment and a blood draw for quantification of circulating plasma clinical chemistries and metabolites before cage randomization and starting diet challenges on either high protein (HP) or high fat high sucrose (HS) diet between 8 and 11 weeks of age, with an average age of 9.4 weeks. For each CC strain, 4–5 mice were assigned to each experimental diet except for CC024/GeniUnc which had 2 mice assigned to each experimental diet; the number of mice from each strain assigned to each diet are shown in Supplementary Table 1. Mice were subsequently maintained on experimental diets for a total of 8 weeks, with the final phenotype assessment performed the following week (week 9) which included another body composition assessment and indirect calorimetry to measure metabolic rate and activity. Samples collected during the necropsy were blood used in the current study, kidney, liver, subcutaneous and gonadal fat, and cecum samples for additional studies.

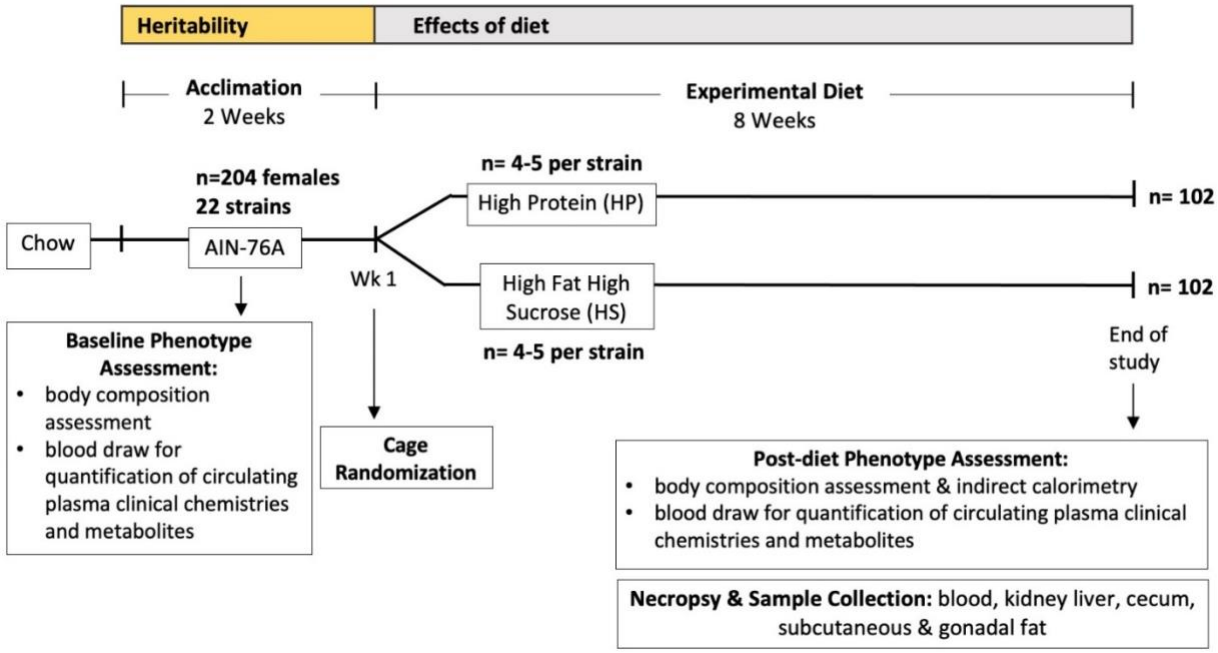


Figure 1-2. Body composition, circulating metabolic health marker levels, and metabolic health score are strain-dependent in the Collaborative Cross.

At baseline, the Collaborative Cross demonstrates phenotypic variation in a strain-dependent manner. Baseline measurements of metabolic phenotypes are shown for (A) body fat %, (B) total weight, (C) triglycerides (TG), (D) total cholesterol, (E) glucose, (F) insulin, and (G) metabolic health score by strain during the 2-week acclimation period while mice were fed the baseline diet (AIN-76A). For metabolic health score (G), strains are ordered from left to right by least healthy to most healthy. Data are mean ± SE for (A–F); data are mean for (G). For body fat % and total weight, 8-10 mice were available per strain, except for CC024/GeniUni (n = 4). For TG, cholesterol, glucose, insulin, and metabolic health score, 8–10 mice were available per strain, except for CC024/GeniUni (n = 4), and CC063/Unc (n = 6). Baseline linear models with CC strain and week as a covariate showed significant CC strain effects for all phenotypes shown ($p < 2.87 \times 10^{-6}$).

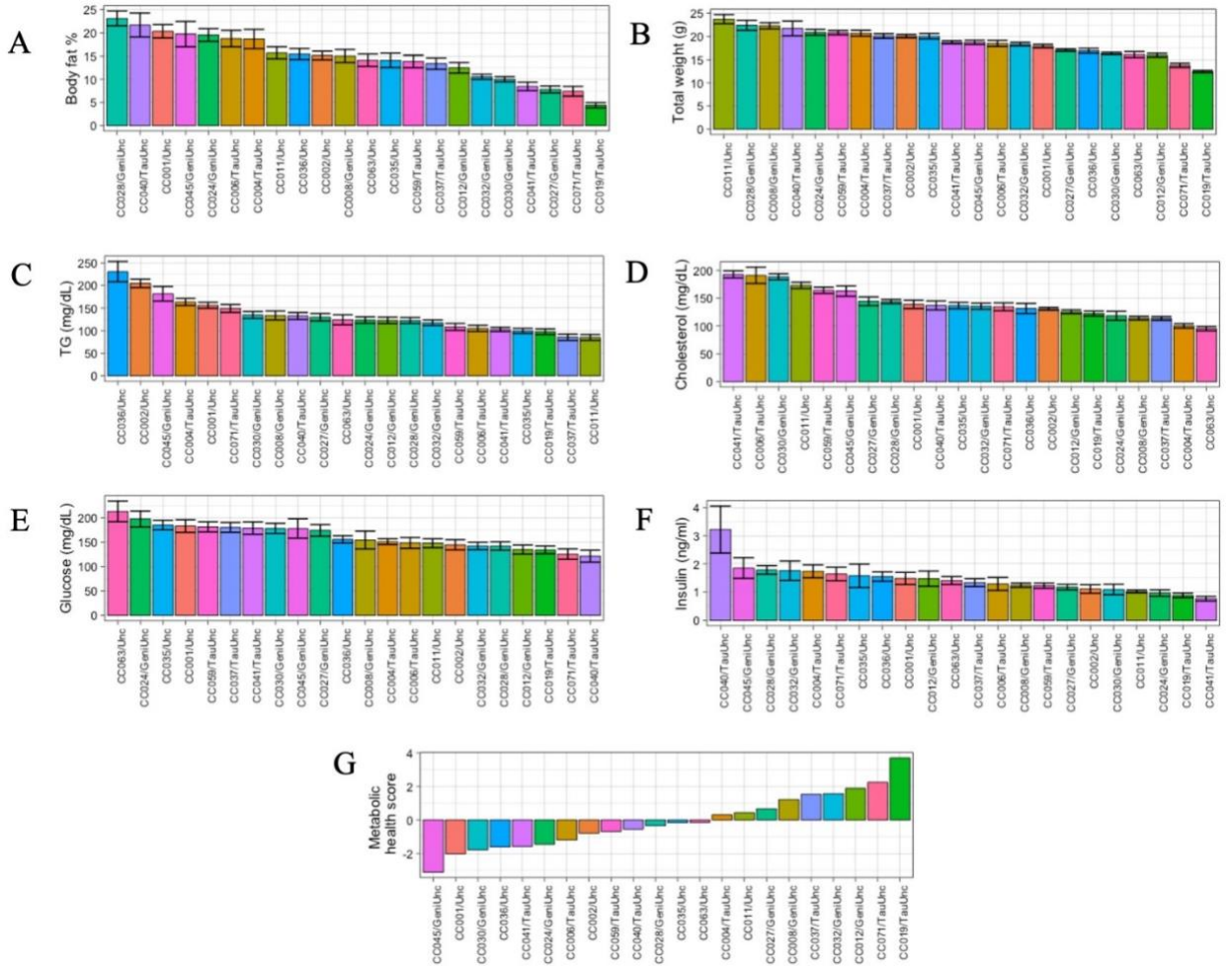
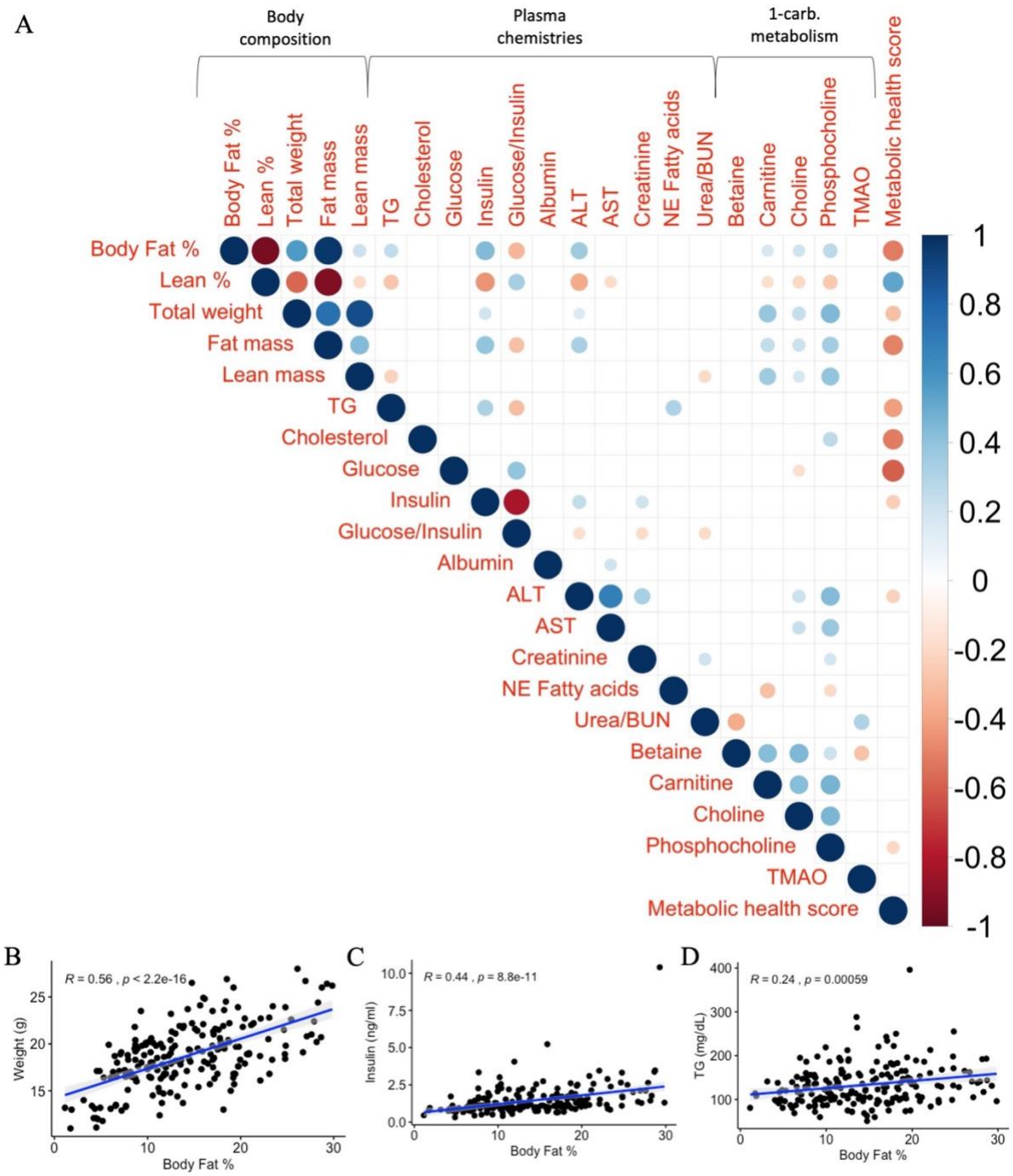


Figure 1-3. Phenotypic correlations at baseline illustrate the strength of relationships between traits without the influence of diet.

Body fat % shows the strongest relationship with weight, insulin, triglycerides (TG), and metabolic health score at baseline compared to other traits. **(A)** Spearman's correlations of baseline phenotypes with p values adjusted using the Benjamini–Hochberg method. Only significant correlations (p adj < 0.05) are shown. Scale indicates rho value. Spearman's correlations between baseline body fat % and (B) weight ($R = 0.56$, $p < 2.2 \times 10^{-16}$), (C) insulin ($R = 0.44$, $p = 8.8 \times 10^{-11}$), and (D) TG ($R = 0.24$, $p = 0.00059$) show significant correlations between body fat % and obesity-associated phenotypes. R is Spearman's rho.



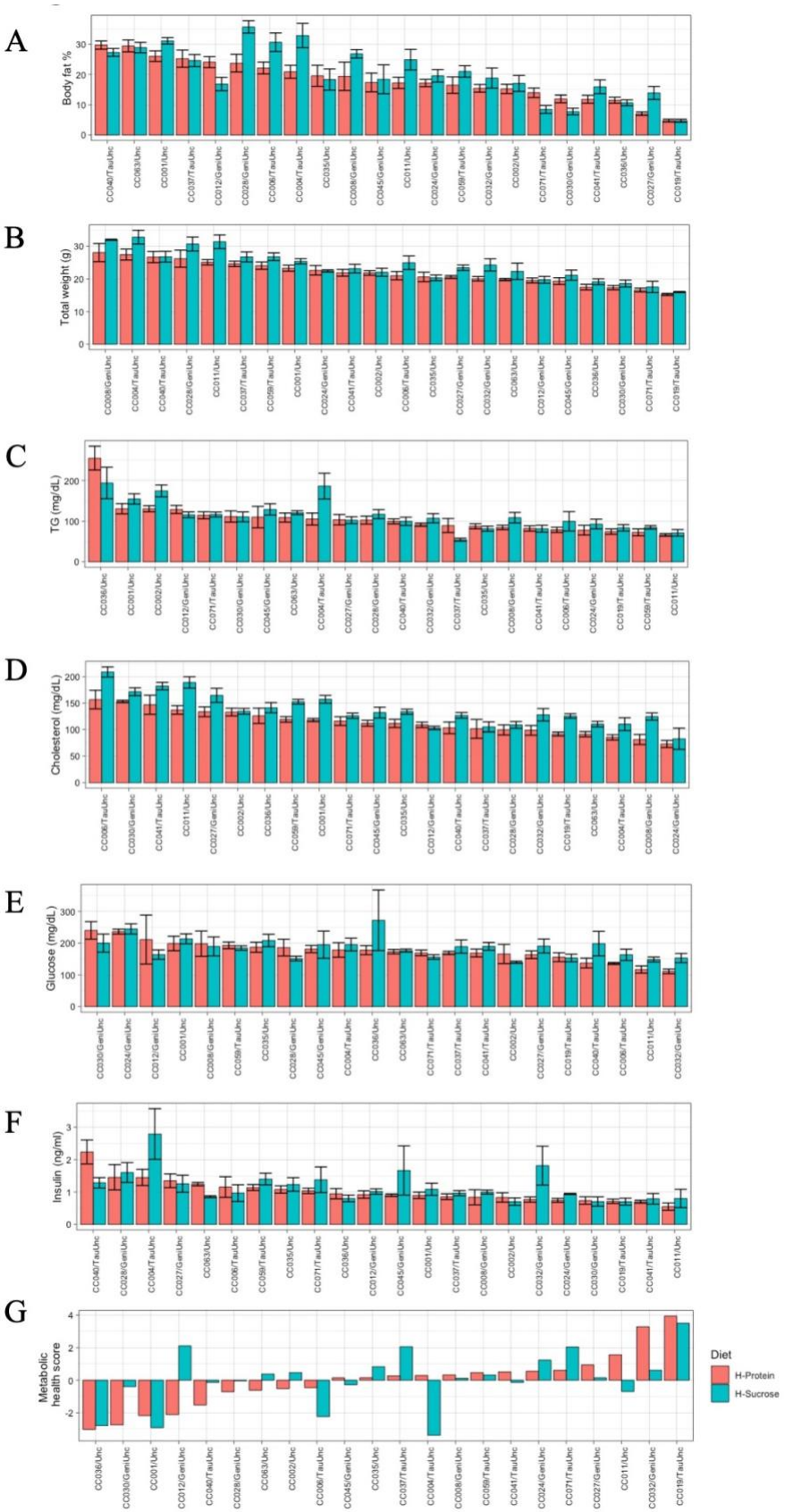


Figure 1-4. Average post-diet body fat %, total weight, circulating metabolic health marker levels, and metabolic health score by strain and diet show greater phenotypic variation by strain than diet.

Phenotypic variation showed greater dependence on CC strain than experimental diet. Post-diet measurements of metabolic phenotypes are shown by diet for **(A)** body fat %, **(B)** total weight, **(C)** triglycerides (TG), **(D)** total cholesterol, **(E)** glucose, **(F)** insulin, **(G)** metabolic health score for each CC strain. CC strains in **(A–F)** are arranged in descending order based on HP diet. CC strains for **(G)** metabolic health score are ordered left to right from least healthy to most healthy. Data are mean ± SE for **(A–F)**; data are mean for **(G)**. For body fat % and weight, there were 4–5 mice per strain per diet except for CC024/GeniUnc (*n* = 2 per diet) and CC063/Unc (*n* = 3 per diet). For TG, cholesterol, glucose, insulin, and metabolic health score, 8–10 mice were available per strain, except for CC024/GeniUni (*n* = 2 per diet), CC063/Unc (*n* = 3 per diet), and CC071/TauUnc (HP *n* = 5, HS *n* = 3). H-Protein and H-Sucrose represent the HP and HS diets, respectively.

Figure 1-5. Average post-diet body fat %, total weight, circulating metabolic health marker levels, and metabolic health score by diet.

Post-diet measurements of phenotypes are shown for **(A)** body fat %, **(B)** total weight, **(C)** triglycerides (TG), **(D)** total cholesterol, **(E)** glucose, **(F)** insulin, and **(G)** metabolic health score by diet after 8 weeks of feeding the experimental diets as indicated. Points are measurements obtained for each mouse. Linear mixed model analysis revealed that experimental diet alone did not have a significant effect in general on body fat %, TG, glucose, insulin, nor metabolic health score, but experimental diet did have significant effects on total weight ($p < 0.01$) and cholesterol ($p < 0.001$). For body fat % and weight, there were 4–5 mice per strain per diet except for CC024/GeniUnc ($n = 2$ per diet) and CC063/Unc ($n = 3$ per diet). For TG, cholesterol, glucose, insulin, and metabolic health score, 8–10 mice were available per strain, except for CC024/GeniUni ($n = 2$ per diet), CC063/Unc ($n = 3$ per diet), and CC071/TauUnc (HP $n = 5$, HS $n = 3$). H-Protein and H-Sucrose represent the HP and HS diets, respectively.

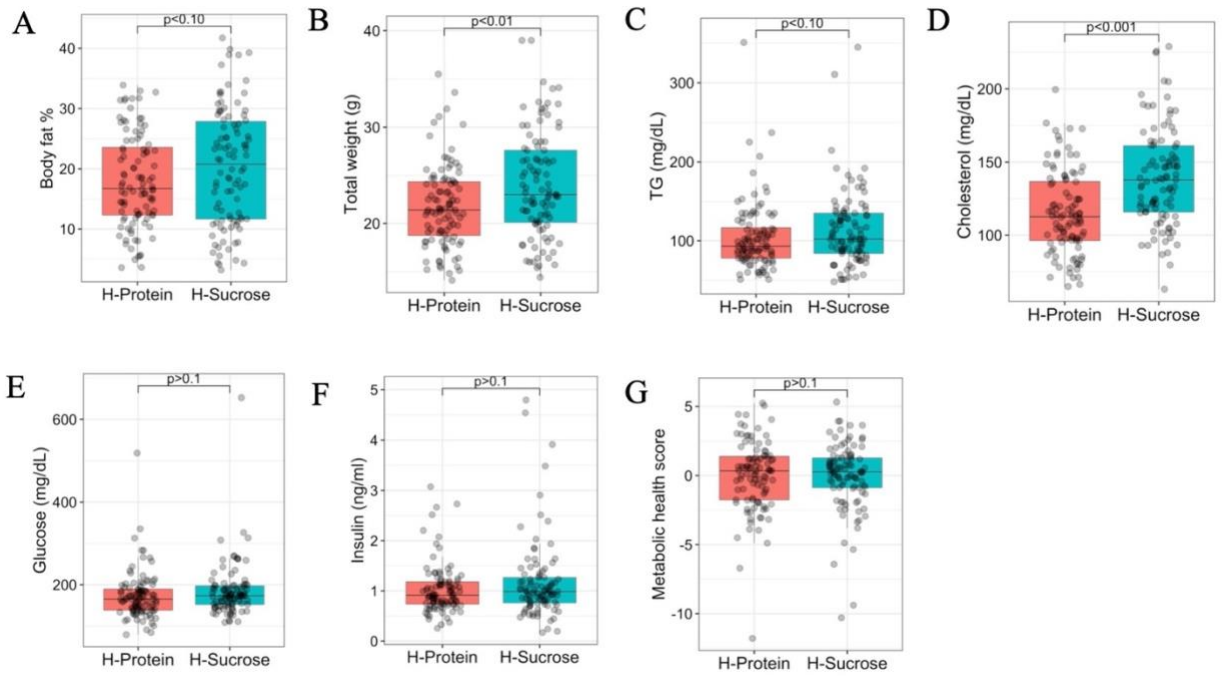
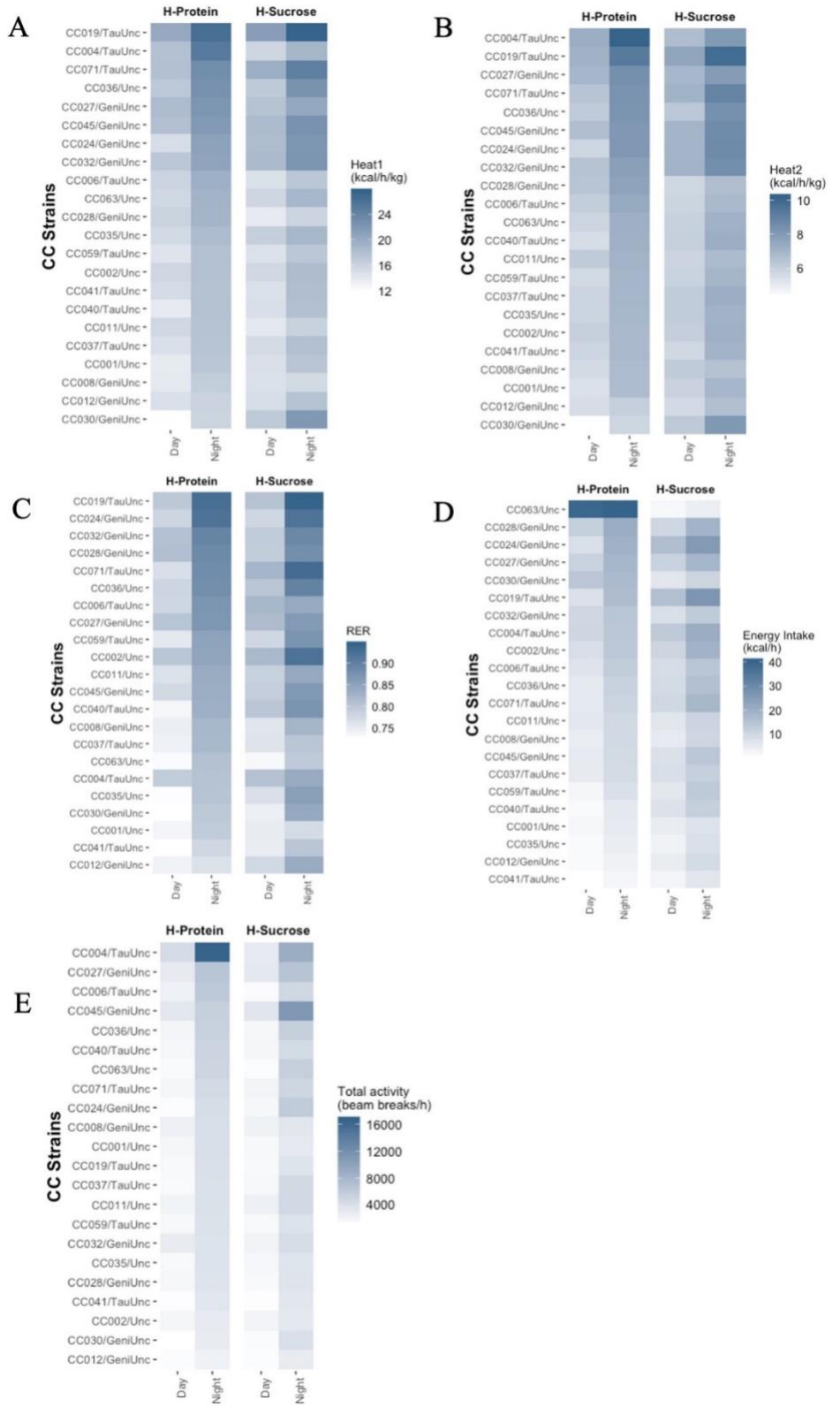


Figure 1-6. Dietary effects of heat expenditure, energy intake, RER, and activity in the Collaborative Cross.



Similar to body composition, circulating analytes, and metabolic health score, phenotypic variation of metabolic traits showed greater dependence on CC strain than experimental diet. Post-diet quantification of average (A) heat expenditure adjusted for total body mass (kcal/h/kg), (B) heat expenditure adjusted for lean mass (kcal/h/kg), (C) RER, (D) energy intake (kcal/h), and (E) total basal activity (beam breaks/h) for each CC strain on each diet shows range of variation across strains for metabolic traits. Strains are ordered in descending order by HP diet. For metabolic traits, there were 4–5 mice per strain per diet except for CC024/GeniUnc (n = 2 per diet) and CC063/Unc (n = 3 per diet). H-Protein and H-Sucrose represent the HP and HS diets, respectively.

Figure 1-7. Post-diet Spearman's correlations of indirect calorimetry phenotypes contrast the difference in relationship between body fat % and each metabolic trait depending on time.

Phenotypic correlations between body fat % and metabolic traits reveal stronger relationships between body fat % and energy expenditure than body fat % and energy intake regardless of the time of day. (A) Spearman's correlation of post-diet phenotypes assessed using indirect calorimetry with p-values adjusted using the Benjamini–Hochberg method. Only significant correlations (p adj < 0.05) are shown. Scale indicates rho value. Spearman's correlations by diet between post-diet body fat % and nocturnal (B) heat production adjusted for total body weight ($R < -0.49$, $p < 2.11 \times 10^{-7}$), (C) energy intake ($R < -0.269$, $p < 6.81 \times 10^{-3}$), (D) RER ($R < -0.471$, $p < 1.01 \times 10^{-6}$), and (E) total basal activity ($R < -0.0471$, $p > 0.419$). R is Spearman's rho. H-Protein and H-Sucrose represent the HP and HS diets, respectively.

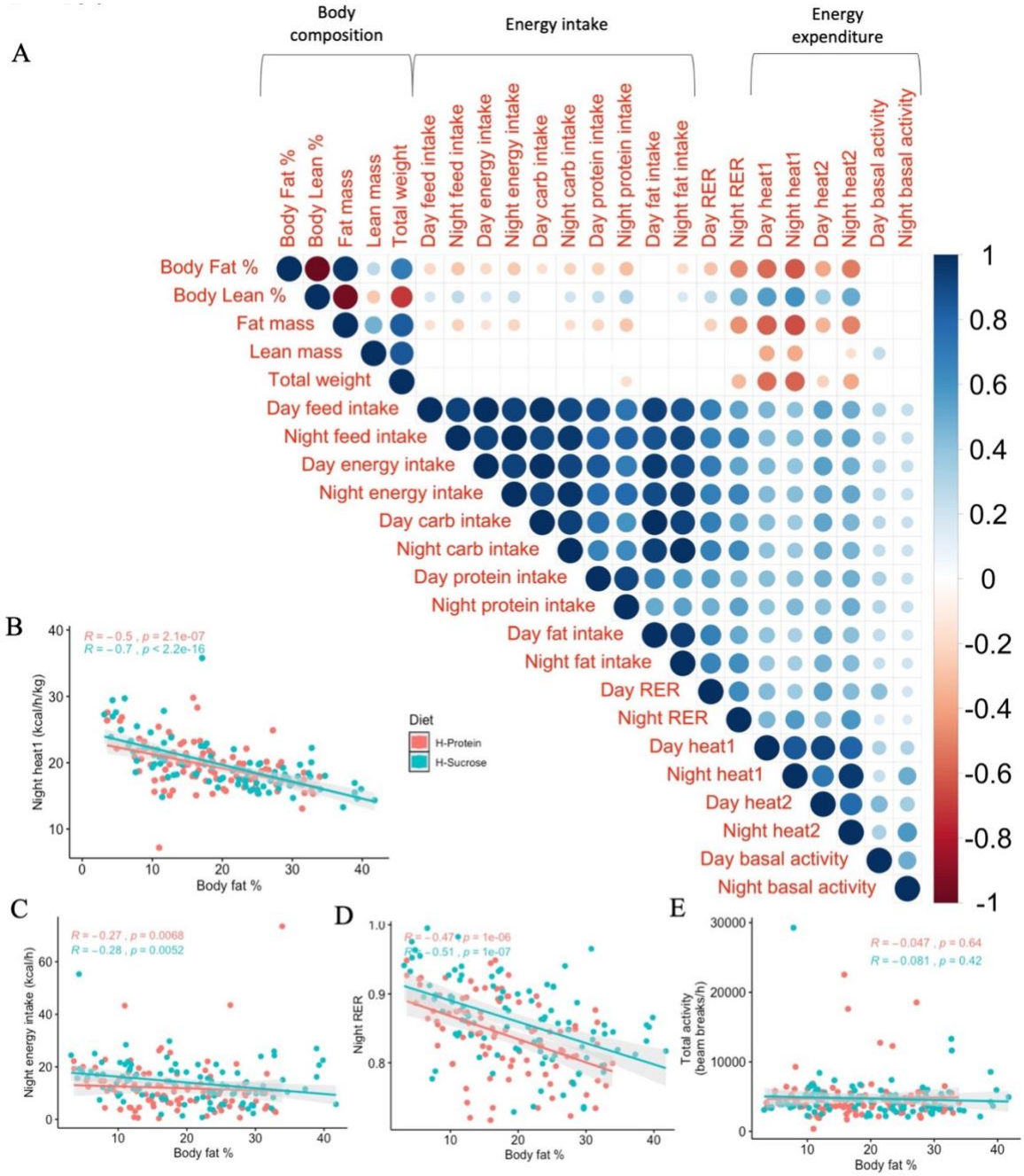


Figure 1-8. Strain-specific effects of diet on body fat %, heat expenditure, energy intake, activity, and RER.

Examination of body composition and metabolic traits of two specific CC strains suggests different methods of maintaining energy balance for each strain. Post-diet quantification of average **(A)** total weight, **(B)** body fat %, **(C)** energy intake (kcal/h), **(D)** heat expenditure adjusted for total body mass (kcal/h/kg), **(E)** RER, and **(F)** total basal activity (beam breaks/h) for strains CC019/TauUnc and CC030/GeniUnc. Data are mean ± SE calculated using data from both diets for each strain.

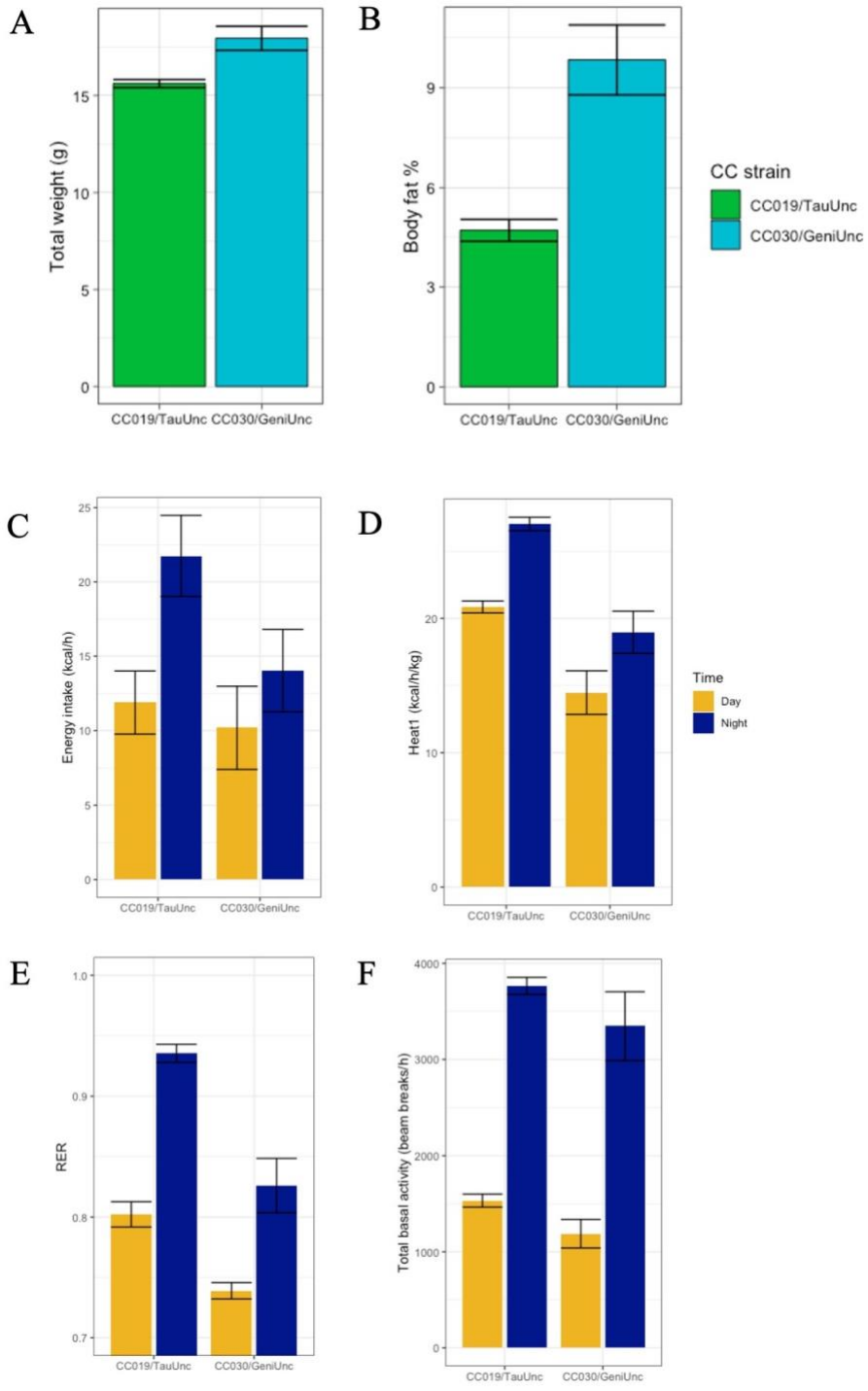


Figure 1-9. Post-diet phenotype correlations demonstrate that most relationships between traits are maintained after the diet challenge.

Relationships between body fat % and weight, insulin, and metabolic health score are still strong after the diet challenge for both diets, while the association between body fat % and triglycerides (TG) is no longer significant. (A) Spearman's correlations of post-diet phenotypes with p-values adjusted using the Benjamini–Hochberg method. Only significant correlations (p adj < 0.05) are shown. Scale indicates rho value. Spearman's correlations by diet between post-diet body fat % and (B) weight ($R > 0.579$, $p < 3.91 \times 10^{-10}$), (C) insulin ($R > 0.359$, $p < 2.91 \times 10^{-4}$), and (D) TG ($R < 0.101$, $p > 0.329$) show significant correlations between body fat % and weight, as well as body fat % and insulin, but not TG. R is Spearman's rho. H-Protein and H-Sucrose represent the HP and HS diets, respectively.

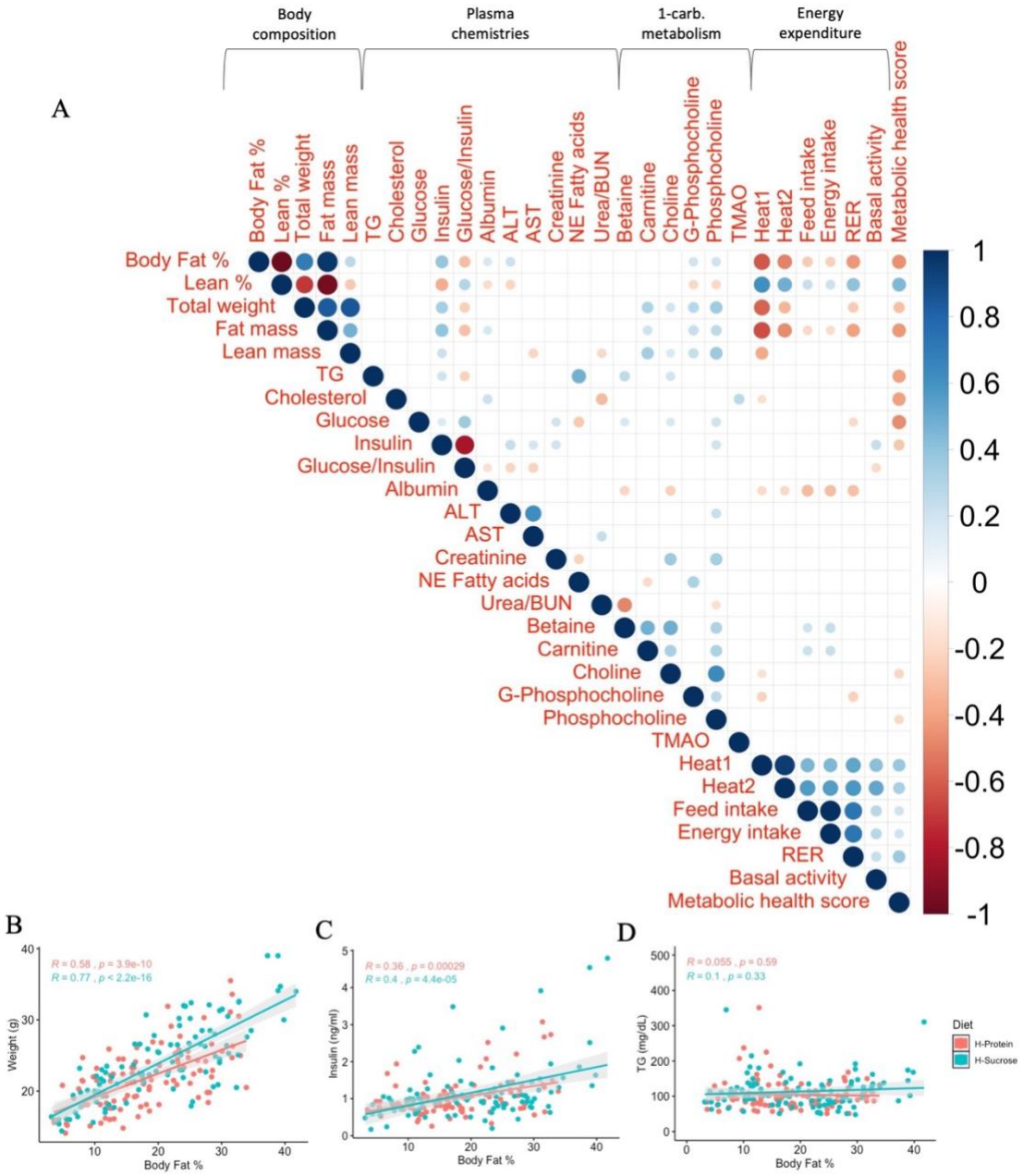


Table 1-1. Broad sense heritability for baseline traits.

Heritability estimates were calculated for traits at baseline using all mice. For each baseline trait, MSB and MSW values were derived from linear models with strain and week as covariates. Estimations of broad sense heritability were calculated for each trait represented by intraclass correlations (r_I), which may be interpreted as the proportion of total phenotypic variation that is accounted for by differences between strains, and coefficients of genetic determination (g^2), which accounts for the additive genetic variance that doubles during inbreeding. Since the CC is a recombinant inbred panel, g^2 may be a more appropriate estimate for broad sense heritability in this study. However, other studies sometimes only provide one estimate of heritability or the other, so we present both values to facilitate comparisons with other findings in the literature.

Trait	Baseline r_I	Baseline g^2
Body fat %	0.554	0.383
Lean %	0.529	0.359
Total Weight	0.666	0.499
Fat mass	0.560	0.389
Lean mass	0.722	0.565
TG	0.622	0.452
Cholesterol	0.634	0.464
Glucose	0.259	0.149
Insulin	0.266	0.153
Glucose/Insulin	0.400	0.250
Albumin	0.515	0.347
ALT	0.407	0.255
AST	0.254	0.146
Creatinine	0.213	0.119
NE Fatty acids	0.057	0.029
Urea/BUN	0.392	0.244
Betaine	0.621	0.450
Carnitine	0.398	0.249
Choline	0.329	0.197
Phosphocholine	0.419	0.265
TMAO	0.593	0.421
Metabolic health score	0.341	0.206

Table 1-2. Heritability estimations and Hedges' g diet effect sizes for post-diet traits.

Post-diet heritability estimates were calculated from linear models including strain, diet, and week as covariates [Post-diet (Full)] and from linear models that only included strain and week as covariates [Post-diet (Partial)]. Diet-specific estimations of broad sense heritability were calculated for each trait represented by intraclass correlations (r_I) and coefficients of genetic determination (g^2) for each trait using the MSB and MSW for strain derived from linear models with strain and week as covariates using only data from each experimental diet per model as indicated to assess how different diet "environments" affect heritability. Hedges' g diet effect size values for HP and HS diets as compared to the baseline diet on post-diet traits were calculated to estimate the magnitude of effect size for each diet, with the sign indicating the direction of change between diets. Positive Hedges' g indicates increased phenotype values post-diet compared to baseline, e.g., body fat % was increased from baseline in mice after feeding them the HP diet. The intraclass correlation (ICC) for diet, which is the proportion of the total phenotypic variation that is accounted for by differences between diet, was calculated to compare the proportion of phenotypic variation attributed to diet in general or genetics.

Trait	Post-diet r_I (Full)	Post-diet r_I (Partial)	HP r_I	HS r_I	Post-diet g^2 (Full)	Post-diet g^2 (Partial)	HP g^2	HS g^2	HP Hedges' g	HS Hedges' g	ICC of diet
Body fat %	0.626	0.613	0.679	0.710	0.456	0.442	0.514	0.551	0.510	0.754	0.085
Lean %	0.619	0.598	0.679	0.671	0.449	0.426	0.514	0.505	-0.557	-0.810	0.133
Total Weight	0.670	0.631	0.672	0.681	0.503	0.461	0.506	0.517	0.834	1.136	0.229
Fat mass	0.677	0.659	0.694	0.669	0.512	0.491	0.531	0.502	0.662	0.940	0.121
Lean mass	0.729	0.709	0.744	0.704	0.573	0.549	0.592	0.543	0.714	0.969	0.143
Triglycerides	0.547	0.543	0.464	0.639	0.377	0.372	0.302	0.469	-0.618	-0.423	0.027
Cholesterol	0.587	0.502	0.504	0.704	0.416	0.335	0.337	0.544	-0.798	-0.124	0.416
Glucose	0.244	0.239	0.351	0.125	0.139	0.136	0.213	0.067	0.318	0.426	0.033
Insulin	0.291	0.324	0.447	0.204	0.170	0.193	0.287	0.113	-0.439	-0.393	0.006
Glucose/Insulin	0.303	0.304	0.433	0.202	0.178	0.179	0.276	0.112	0.640	0.688	-0.010
Albumin	0.361	0.361	0.271	0.495	0.220	0.220	0.157	0.329	-0.712	-0.619	-0.002
ALT	0.222	0.223	0.281	0.248	0.125	0.125	0.164	0.141	-0.268	-0.337	-0.005
AST	0.219	0.216	0.234	0.180	0.123	0.121	0.132	0.099	-0.210	-0.326	0.018
Creatinine	0.175	0.172	0.174	0.114	0.096	0.094	0.095	0.061	-0.497	-0.364	0.025
NE Fatty acids	0.137	0.135	0.043	0.198	0.073	0.072	0.022	0.110	-0.794	-0.967	0.016
Urea/BUN	0.535	0.348	0.490	0.533	0.366	0.210	0.324	0.364	0.546	-0.800	0.610
Betaine	0.470	0.390	0.530	0.427	0.307	0.242	0.361	0.272	-0.331	0.473	0.366
Carnitine	0.424	0.411	0.491	0.321	0.269	0.258	0.326	0.191	-0.035	0.263	0.081
Choline	0.258	0.256	0.123	0.319	0.148	0.147	0.066	0.190	-0.781	-0.841	0.016
Phosphocholine	0.270	0.264	0.215	0.190	0.156	0.152	0.120	0.105	-0.417	-0.258	0.045
TMAO	0.363	0.344	0.436	0.332	0.221	0.208	0.279	0.199	-0.674	-0.622	0.107
Metabolic Health Score	0.328	0.329	0.390	0.391	0.196	0.197	0.242	0.243	-0.041	0.026	-0.010

Supplementary Material

Additional supplementary material for this chapter can be found online at: https://www.frontiersin.org/articles/10.3389/fgene. 2020.615012/full#supplementary-material

All supplementary figures are included in this dissertation below for ease of reading. Unfortunately, no supplementary tables were shown in the dissertation due the dissertation format size constraints but can be found in Supplementary File 2. A brief description of the supplementary files as follows:

Supplementary File 1. All supplementary figures and methods.

Supplementary File 2. All supplementary tables.

Supplementary File 3. R code, data required for generating baseline figures and calculating baseline H^2 estimates, and a README file with brief descriptions of all contents within Supplementary File 3.

Supplementary File 4. R code, data required for generating post-diet figures and calculating post-diet H^2 estimates, and a README file with brief descriptions of all contents within Supplementary File 4.

Figure S1-1. Mouse randomization procedure

During the 2-week acclimation phase, all mice were fed the AIN-76A synthetic chow diet and housed by strain, one strain per cage. Prior to the experimental diet challenge, the mice were randomly assigned to either the high fat high sucrose (HS) or high protein (HP) diet, and subsequently the strain siblings were moved to new cages according to the assigned experimental diet. Mice were housed according to experimental diet for the duration of the 8-week diet challenge.

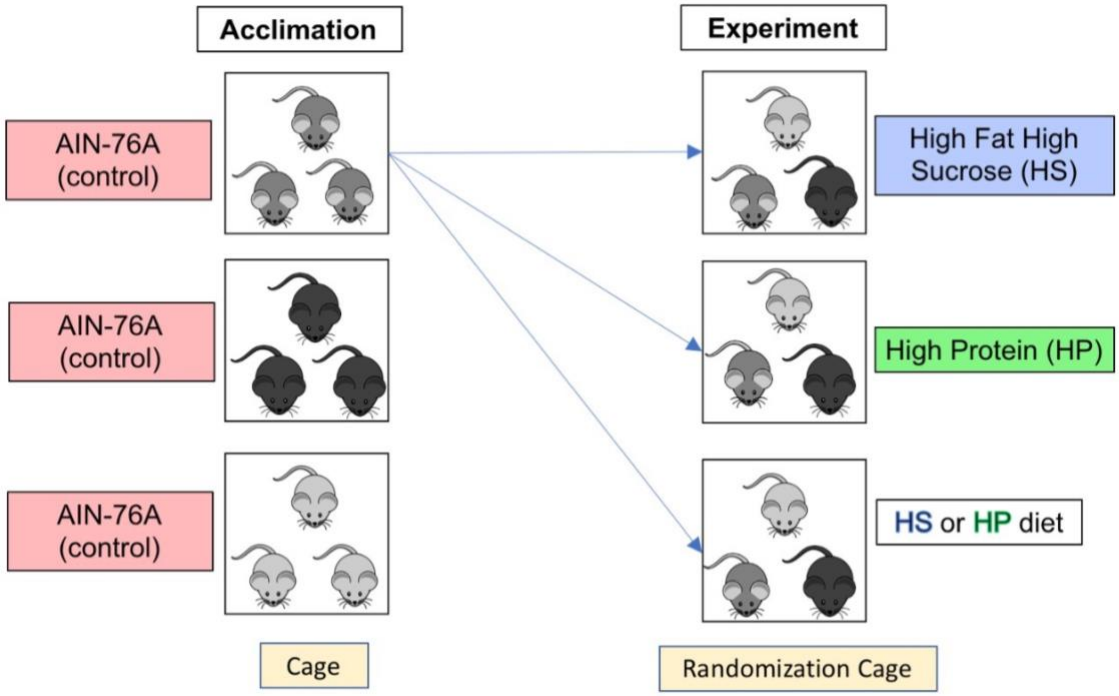


Figure S1-2. PhenoMaster TSE assessment 12-hr light/dark cycles

Indirect calorimetry was assessed over three days using PhenoMaster (TSE Systems) automated home cage phenotyping. Dark cycles began at 6:00 PM and ended at 6:00 AM, and complete light cycles began at 6:00 AM and ended at 6:00 PM. Means of each phenotype measured were calculated for individual light cycles by day (day 1, 2, and 3), for individual dark cycles by day (night 1 and 2), for light cycles for the duration of the experiment (all days combined), for dark cycles for the duration of the experiment (both nights combined), and for individual experiment days (experiment day 1 and 2). Measurements were taken for less than 12 hours during the light cycles at the beginning and end of the experiment (days 1 and 3), while measurements for both dark cycles (nights 1 and 2) and the light cycle between (day 2) for 12 complete hours.

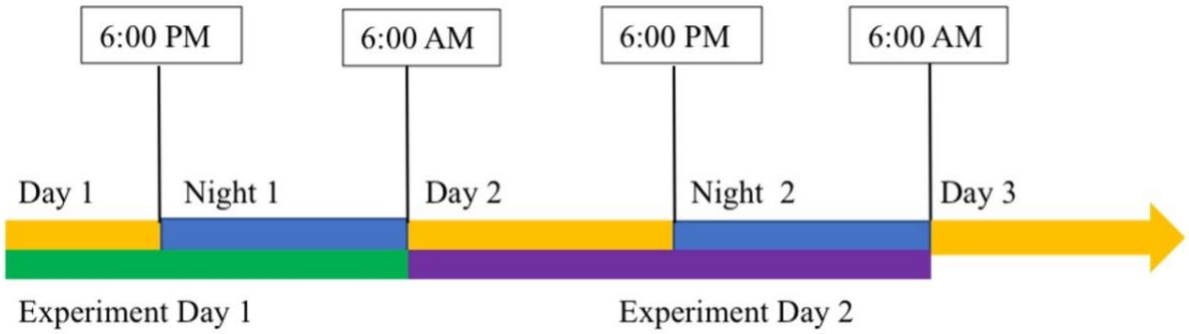


Figure S1-3. Spearman's correlation of baseline body fat % and total weight

Spearman's correlation of baseline body fat % and total weight (g) was performed for each CC strain to estimate whether the relationship between body fat % and weight differed by genetic background. Strains are ordered by significance of correlation from most to least significant.

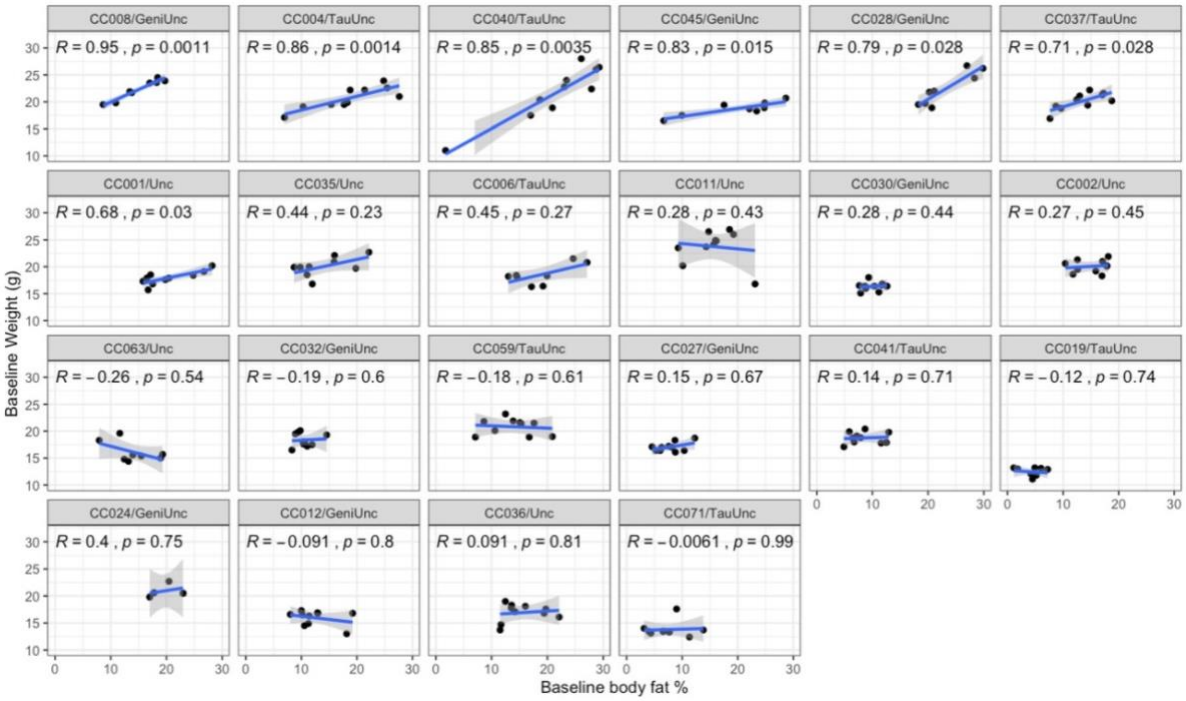


Figure S1-4. Average changes in body fat % for each CC strain on the HP or HS diet

Changes in body fat % for each CC strain on the HP (H-Protein) or HS (H-Sucrose) diet were obtained by subtracting baseline body fat % from post-diet body fat % for each mouse, and then the mean change for each strain on the respective diets was calculated. Data are mean ± SE.

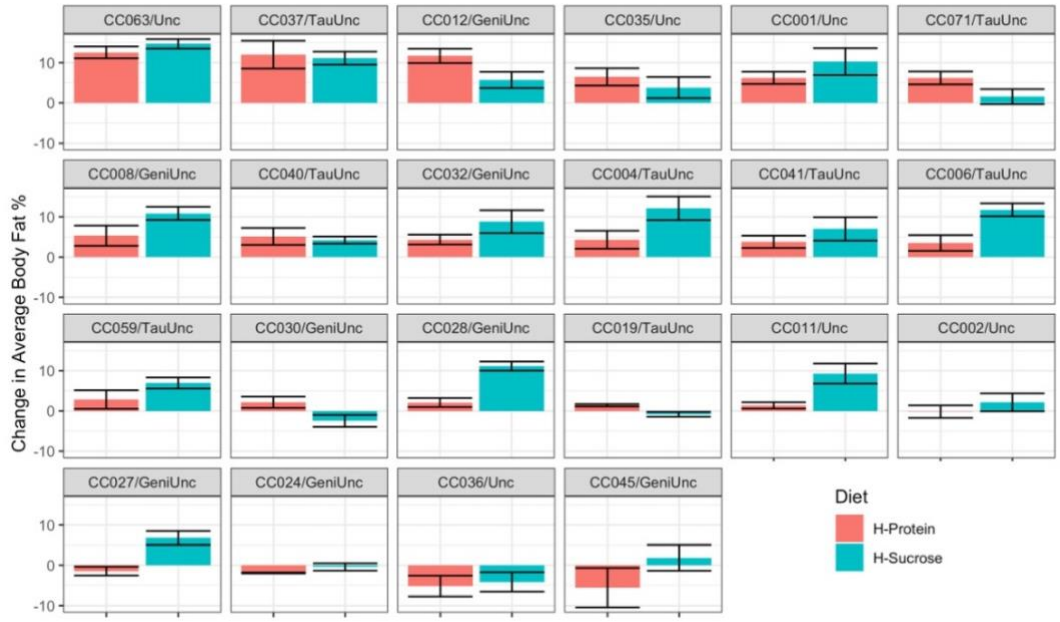


Figure S1-5. Baseline and post-diet measurements of body fat %, total weight, and cholesterol by strain and diet

Baseline and post-diet measurements are shown for (A) body fat %, (B) total weight, and (C) total cholesterol to compare phenotypic differences between strain and diet. Strains are ordered numerically. H-Protein and H-Sucrose represent the HP and HS diets, respectively. Points are measurements obtained for each mouse.

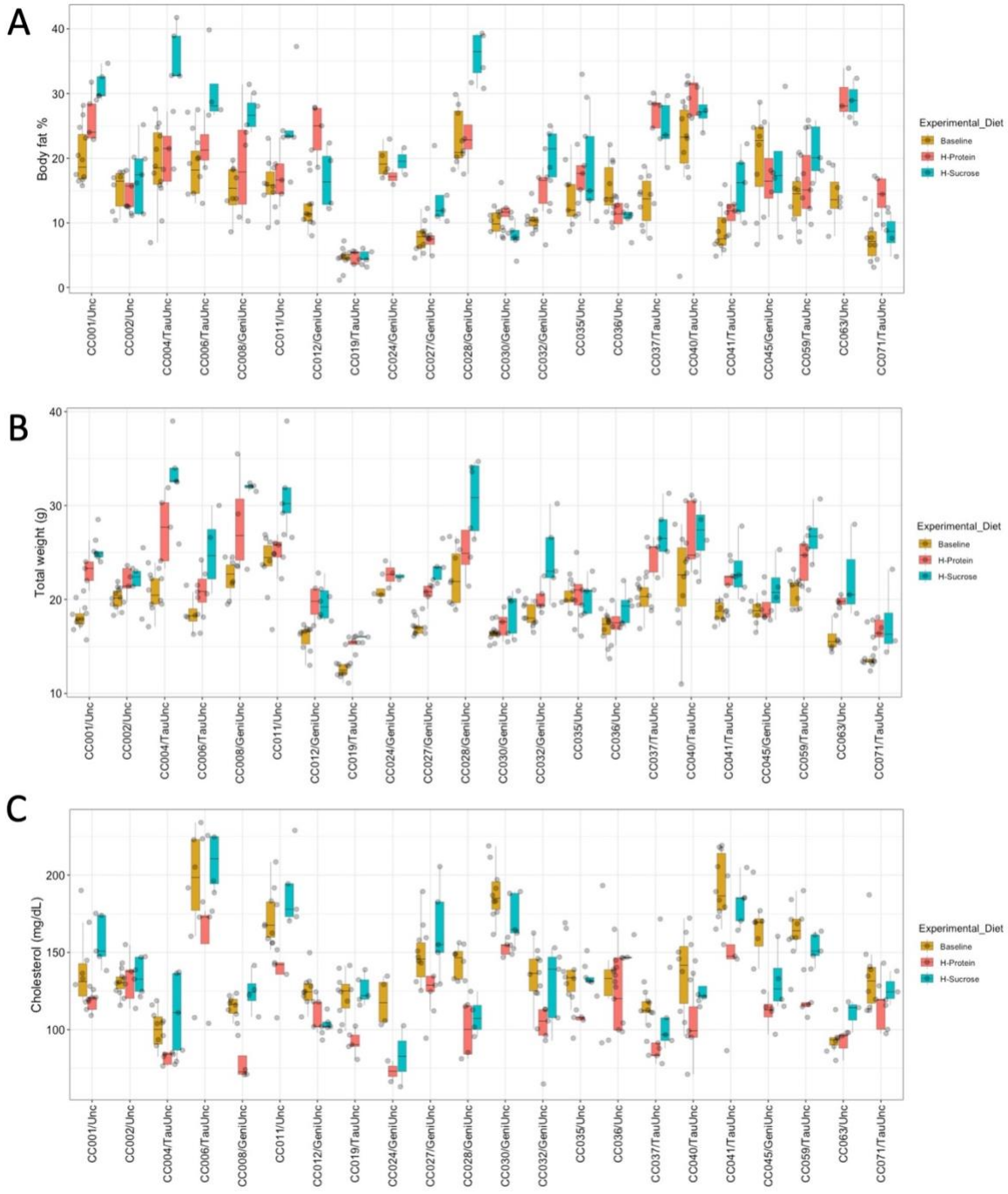


Figure S1-6. Average post-diet heat expenditure adjusted for total body mass (Heat1) and for lean mass only (Heat2)

Post-diet quantification of average (A) heat expenditure adjusted for total body mass (kcal/h/kg) and (B) heat expenditure adjusted for lean mass (kcal/h/kg) for each CC strain on each diet show a wide range of variation across and within strains. Individual points are colored by strain. Data for each strain are ordered by diet within the day cycle followed by diet within the night cycle.

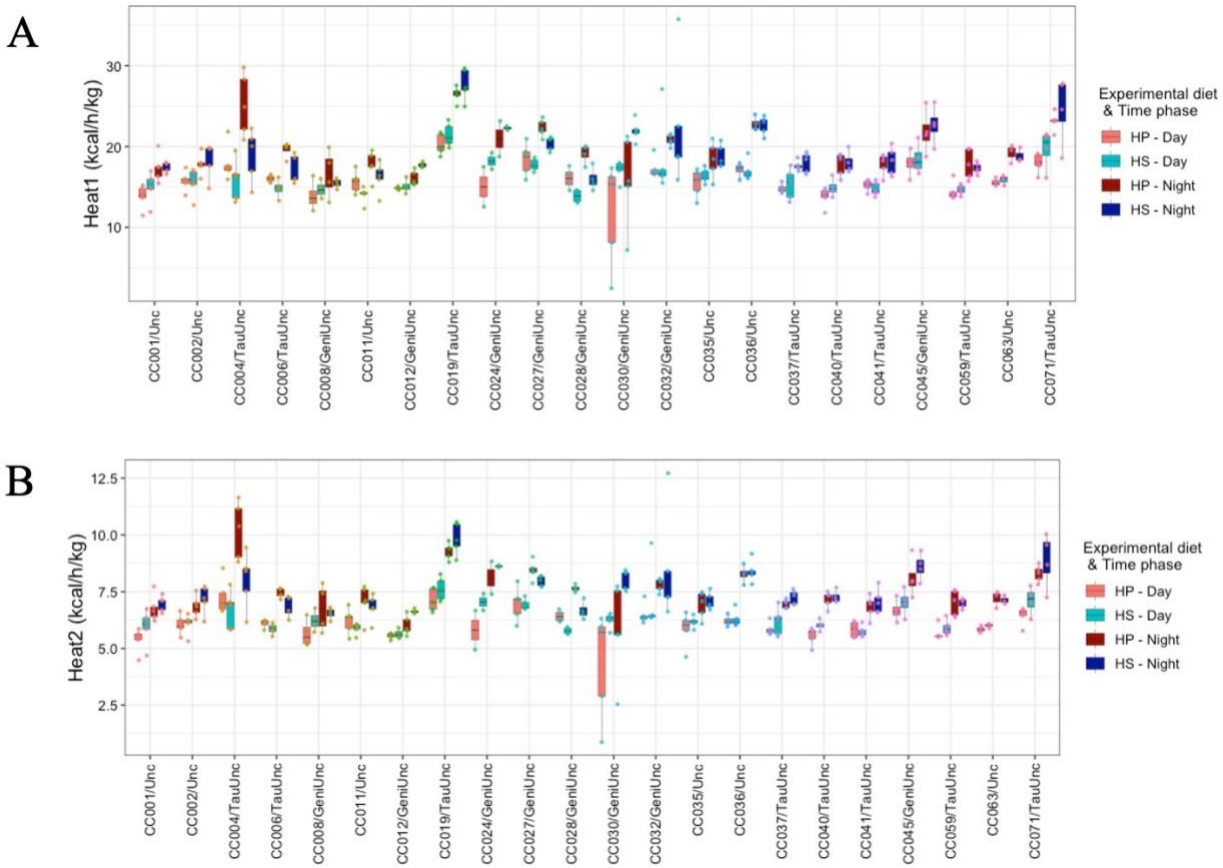


Figure S1-7. Average post-diet RER, energy intake, and total basal activity

Post-diet quantification of average (A) RER, (B) energy intake (kcal/h), and (C) total basal activity (beam breaks/h) for each CC strain on each diet show a wide range of variation across and within strains. Individual points are colored by strain. Data for each strain are ordered by diet within the day cycle followed by diet within the night cycle.

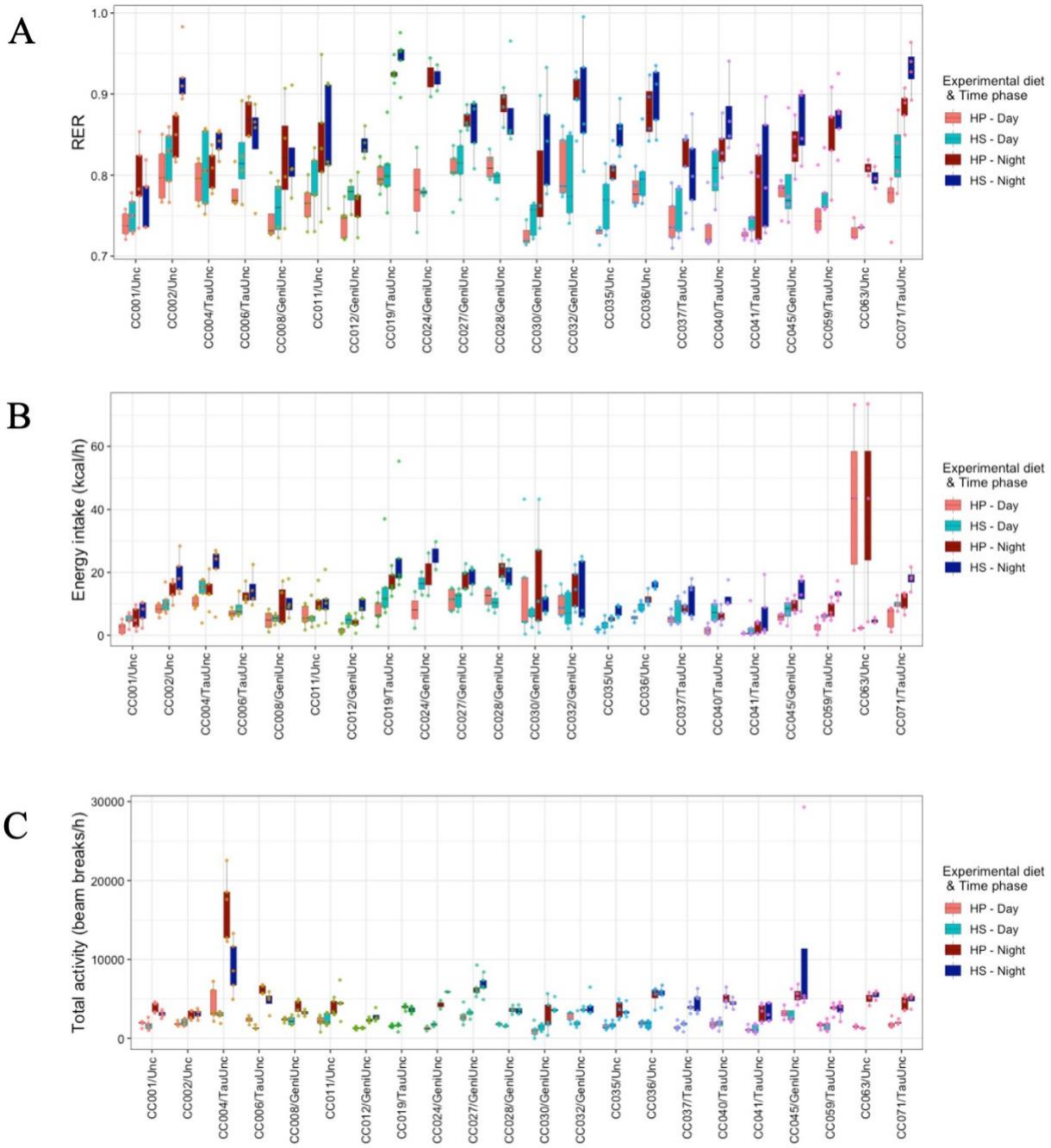
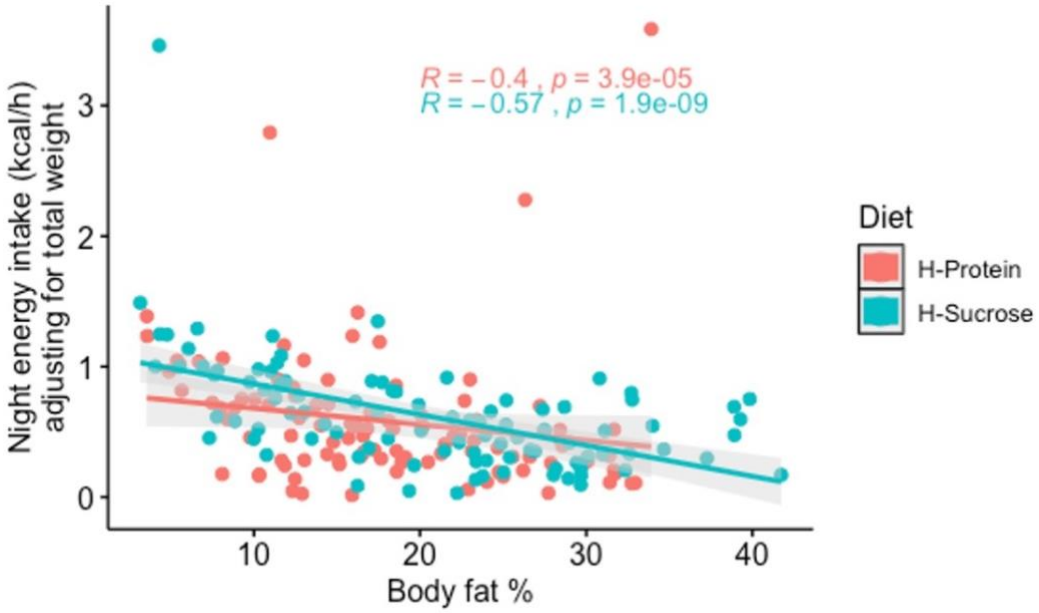


Figure S1-8. Spearman's correlation of post-diet body fat % and night energy intake adjusting for total body weight

Spearman's correlations between post-diet body fat % and nocturnal energy intake adjusting for total weight for the HP (rho=-0.4, p=3.9x10^{-5}) and HS (rho=-0.57, p=1.9x10^{-9}) diets. H-Protein and H-Sucrose represent the HP and HS diets, respectively. R indicate rho values.



Chapter 3:
Genetic background and diet induce hepatic gene expression of different regulatory pathways associated with obesity in the Collaborative Cross

Authors and Affiliations

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Background:

Characterized by excessive accumulation of adiposity resulting in adverse health effects, obesity is a serious disease with a complex etiology. Given the significant role that the liver plays in the biological processes that attenuate adiposity accumulation such as lipogenesis and metabolism of dietary macronutrients, expanding the understanding of how genetics and diet influence hepatic gene expression is crucial to improve strategies of obesity prevention and treatment. To determine how genetics and diet impact obesity development, multiple mice from 22 strains of the genetically diverse recombinant inbred Collaborative Cross (CC) mouse panel were challenged to either a high protein or high fat high sucrose diet, followed by extensive phenotyping and microarray analysis of post-diet hepatic gene expression.

Results:

Genes differentially expressed by diet (1,344) were enriched for biological processes related to metabolic pathways while genes differentially expressed by strain (9,436) were enriched for biological process involved in cell adhesion and signaling. Weighted gene co-expression network analysis identified three biologically relevant gene clusters (modules) whose average expression levels differed by both diet and strain and were significantly correlated with body fat %. Each gene cluster was enriched for distinct types of biological functions.

Conclusions: Genetic background affected hepatic gene expression in the CC overall but differences in diet also altered gene expression for a smaller subset of genes. Generally diet alters hepatic gene expression for metabolic processes sensitive to acute environmental changes, while genetic background more heavily influences overall “stable” cellular functions relative to obesity development.

Introduction

Obesity is characterized by the disproportionate and excessive accumulation of adipose tissue relative to an individual's height, resulting in decreased health and increased risk of developing a myriad of chronic diseases such as atherosclerosis, cardiovascular disease, metabolic syndrome, type 2 diabetes, and certain types of cancer (Williams et al., 2015). Using body mass index (BMI) to classify the extent of obesity, the WHO defines BMI for the status of overweight as 25-29.9 kg/m^2, obese as >30 kg/m^2, and extremely obese as >40kg/m^2 (Després, 2012). In recent decades, the prevalence of overweight and obesity has increased worldwide at an alarming rate, as demonstrated by the increase in the percentage of adults with BMIs >25 kg/m^2 between 1980 and 2013 from 28.8% to 36.9% for men and 29.8% to 38% for women (Ng et al., 2014), necessitating the urgent development of effective methods to prevent and treat obesity on both the individual and population levels.

The prevention and treatment of obesity is challenging, given the complex etiology of obesity. Simplistically, obesity results from the chronic imbalance between energy intake and expenditure, but the mechanisms involved in maintaining energy balance are complex and regulated by numerous factors such as genetic background (C. G. Bell et al., 2005; Loos, 2018; Singh et al., 2017), metabolism (Fernández-Verdejo et al., 2019; Speakman, 2018; Timper & Brüning, 2017), gut microbiome (John & Mullin, 2016; K. B. Martinez et al., 2016; Torres-Fuentes et al., 2017), and environmental factors such as diet in the context of overfeeding (Creasy et al., 2018; Cuthbertson et al., 2017; Danforth, 1985; Schmidt et al., 2012; Sims, 1976), which alter the expression of genes associated with signaling of satiety and hunger.

In addition to the complex interactions between adipose tissue, the central nervous system, nutrients, and hormones that regulate energy balance (C. G. Bell et al., 2005; González-Muniesa et al., 2017), the liver also influences the development of obesity, given its major role in the metabolism of macronutrients including glycogenolysis, production of triglycerides, lipogenesis, and the synthesis of molecules used as building blocks for hormones such as amino acids, cholesterol, and lipoproteins (Rui, 2014; Trefts et al., 2017). Obesity in turn can induce the pathological response of insulin resistance in the liver, which results in an impaired ability of insulin to decrease glucose output from the liver while continuing to stimulate lipogenesis; this disruption of appropriate carbohydrate and lipid metabolism is

thought to contribute to some of the health complications associated with obesity like metabolic syndrome and cardiovascular disease. Adipokines such as adiponectin, adipocyte dysfunction, metabolism, and circulating metabolite levels affect hepatic gene expression (Fischer et al., 2017; Y. Luo et al., 2016), which regulates the mechanisms involved in lipid processing, determination of metabolic rate, and other physiological processes associated with energy imbalance (Langhans, 2003; Trefts et al., 2017). Furthermore, an individual's inherent genetic architecture and factors in an individual's environment such as diet also shape hepatic gene expression and traits that affect hepatic gene expression (Hao et al., 2016; Kozul et al., 2008; Silva & van Booven, 2018). Given that the liver regulates so many biological processes related to obesity development, elucidating the effects of genetic architecture and diet on hepatic gene expression is therefore necessary to understand the full picture of the development of obesity and disease risks associated with obesity for effective prevention and treatment.

Modern molecular biology techniques have revolutionized our ability to detect changes in gene expression (Lis, 2019; Roeder, 2019), which allows one to infer potential candidate genes and pathways underlying metabolic dysfunction (Ellero-Simatos et al., 2014; Houtkooper et al., 2011). Identification of genes and pathways that determine susceptibility to obesity facilitates the understanding of the underlying mechanisms behind the development of obesity, which is instrumental to determining effective methods of prevention and treatment. To find potential candidate genes or functional pathways underlying metabolic dysfunction regulated by diet in a genetically diverse population, we administered a challenge of either high protein (HP) or high fat high sucrose (HS) diet to 22 strains of mice from the Collaborative Cross (CC) mouse panel for eight weeks and performed microarray gene expression analysis of 11,542 genes using high-quality RNA from liver tissue, in addition to extensive phenotyping.

Derived from elaborate intercrosses of eight founder mouse strains (Churchill et al., 2004; Iraqi et al., 2008; Threadgill & Churchill, 2012), the CC is a large recombinant inbred mouse population with tremendous genetic diversity and genetic contribution from the five classically inbred strains A/J, C57BL/6J (B6), 129S1/SvImJ (129), NOD/ShiLtJ (NOD), NZO/HILtJ (NZO), and three wild-derived strains CAST/EiJ (CAST), PWK/PhJ (PWK), and WSB/EiJ (WSB) (Collaborative Cross Consortium, 2012; Philip et al., 2011; Shorter et al., 2019; Srivastava et al., 2017). Utilizing the CC to study the effects of diet and strain on hepatic gene expression enables us to solve some of the challenges caused by confounding

environmental influences in the discernment between the effect of diet compared to the effect of genetics on gene expression in a mouse population with the genetic and phenotypic diversity similar to the human population (Swanzey et al., 2021).

Previously we examined the effects of diet and genetic background on adiposity and other obesity related traits (Yam et al., 2021). In the current follow-up study, our area of focus was to explore the effects of diet and strain (genetic background) on hepatic gene expression and relate the diet- and strain-induced changes in gene expression to phenotypic traits and biological functions. Additionally, we determined which genes' expression in the liver is influenced by diet or strain, potentially identified new obesity-related genes, and confirmed previously found obesity genes in the mouse to narrow down genes for future functional validation studies by identifying genes correlated with adiposity in CC that are relevant in humans found in the GWAS catalog.

Methods

Animals, husbandry, diets and phenotyping: Details on the origin, housing, husbandry, treatment of the CC animals, diet compositions, and phenotyping have been described previously (Yam et al., 2021). Briefly, female mice from 22 CC strains (total n=204) were put on either a high protein (n=102) or high fat high sucrose (n=102) diet for 8 weeks followed by analysis of body composition, metabolic rate, and physical activity. After 8 weeks on experimental diets, mice were euthanized following a 4-hr fast for the collection of blood and liver tissue. Subsequently, cholesterol, triglyceride (TG), glucose, albumin, creatinine, urea, aspartate transaminase (AST), and alanine transaminase (ALT) levels were quantified using the Cobas Integra 400 Plus (Roche Diagnostics, Indianapolis, IN), according to manufacturer's instructions. Circulating insulin was measured using an ultrasensitive mouse insulin ELISA (ALPCO Diagnostics, Salem, NH) per manufacturer's instructions. Trimethylamine N-oxide (TMAO), choline, phosphocholine, glycerophosphocholine (g-phosphocholine), betaine, and carnitine were quantified using liquid chromatography–mass spectrometry (LC-MS) methods as described with modifications (Z. Wang et al., 2014). Health scores were calculated using measurements of several metabolic risk factors (circulating glucose, insulin, glucose/insulin ratio, cholesterol, triglycerides, and body fat %) to approximate overall metabolic health.

Microarray analysis for identification of gene expression levels associated with post-diet traits and differentially expressed genes in liver tissue

Methods of RNA extraction from livers and evaluation of RNA integrity were performed as previously described (Coffey et al., 2017). Randomly selecting 3 mice per stain per diet for microarray analysis, high-quality RNA was available from livers of 127 of the 204 CC mice and hybridized to Affymetrix Mouse Gene 2.1 ST 96-Array Plate using the GeneTitan Affymetrix instrument (Affymetrix, Inc., Santa Clara, CA) according to standard manufacturer's protocol. The robust multiarray average (RMA) method was used to estimate normalized expression levels of transcripts (median polish and sketch-quantile normalization) using the *affy* R package (Gautier et al., 2004). The quality of sample arrays was then assessed using the R package arrayQualityMetrics (Kauffmann et al., 2008) for outlier detection using 3 methods: distance between arrays/principle component analysis, computation of the Kolmogorov-Smirnov statistic K_a between each array's intensity distribution and the intensity distribution of the pooled data to compare individual array intensity to the intensity of all arrays, and computing Hoeffding's statistic D_a to check individual array quality. Sample arrays identified as outliers by all three methods were removed, i.e. a sample array was removed if all three methods indicated that it was an outlier, leaving 123 out of 127 arrays for analysis.

Probes and transcript cluster IDs (TC IDs) were first filtered as described (Que et al., 2020), resulting in the total number of 24,004 unique probes post-filter corresponding to 23,626 genes. Next, TC IDs were kept for analysis if their median expression was above the mean of all TC ID medians or if their median expression was above the mean of all TC ID medians in over 12.5% of samples, based on the assumption that by chance one of the 8 founders may be contributing low/no expression alleles. For TC IDs associated with the same gene, the TC ID with the highest expression was selected to represent that gene so that each gene was represented by a unique TC ID for analysis, resulting in 11,542 TC IDs (genes) used for differential gene expression analysis and correlations between gene expression levels and phenotype data.

After filtering TC IDs and arrays for quality, calculations of multiple biweight midcorrelations (bicor) and their corresponding Student correlation p-values were performed for the unique TC IDs corresponding to 11,542 genes using the bicorAndPvalue function from the weighted gene

co-expression network analysis (WGCNA) R package (Langfelder & Horvath, 2008) to ascertain which genes' expression in the liver were correlated with post-diet traits. Next, differential gene expression analysis was performed using the Linear Models for Microarray Analysis (limma) R package version 3.6.1 (Ritchie et al., 2015) and methods described (Phipson et al., 2016) to find genes that were significantly differentially expressed by diet or CC strain. Genes with a Benjamini-Hochberg (BH) adjusted p value < 0.05 were designated as differentially expressed (DE). Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and gene ontology (GO) enrichment analyses were performed using the *kegga* and *goana* functions in limma for differentially expressed genes with the false discovery rate (FDR) cutoff set to 0.05.

Broad-sense heritability estimates and diet intraclass correlations of hepatic gene expression levels

Broad-sense heritability (H^2) estimates and the intraclass correlations (ICC) for diet were calculated as described previously (Yam et al., 2021) for the 11,542 genes used in limma analysis to assess the degree of influence on gene expression variation from genetics (strain) and diet, respectively. H^2 was estimated by calculating the intraclass correlation (r_I) and the coefficient of genetic determination (g^2) using mean square between (MSB) strains and mean square within (MSW) strains values derived from linear regression analysis (Festing, 1979). The following linear models were fit using the *lm* function and implementing Satterthwaite approximations on the output of *lm* as described (Luke, 2016) to obtain MSB and MSW values for r_I and g^2 calculations: 1) a "full" additive model with strain, diet, and week (mouse "batch") as variables fitted with gene expression data from both experimental diets, 2) a "HP" additive model including strain and week as variables fitted with gene expression data from only mice fed the HP diet, and 3) a "HS" additive model including strain and week as variables fitted with gene expression data from only mice fed the HS diet. H^2 estimates derived from models fitted with data from all mice post-diet compare the contribution of genetics (strain) and diet overall to heritable gene expression level variance, while diet-specific H^2 estimates were calculated to discern differences in heritability affected by differences in macronutrient composition. The diet ICCs were calculated using the mean square between (MSB) diets and mean square within (MSW) diets derived from the "full" additive linear model described above.

Weighted gene co-expression network analysis (WGCNA)

The WGCNA R package was used to identify modules for the 11,542 genes used in microarray analysis of differentially expressed genes since complex traits often result from changes in expression of multiple genes. Expression data from the 123 non-outlier sample arrays were used to detect modules, which are groups of highly correlated genes with similar connection strengths (Ghazalpour et al., 2006; Zhang & Horvath, 2005)**.** The soft threshold was chosen by running the pickSoftThreshold function to determine the best fit to a scale-free topology, and beta was set to 5 because it was the lowest power value where the R^2 value crossed the 0.9 threshold for approximate scale-free topology and connectivity measures implicated the possibility of finding highly connected genes. The *blockwiseModules* function was run to construct the unsigned network in one block, calculate an adjacency matrix with Pearson correlations, calculate the topological overlap matrix (TOM) using the signed method, cluster genes using the default average linkage hierarchical clustering, and establish modules by the Dynamic Hybrid tree cut method (Langfelder & Horvath, 2008). Next, the *mergeCloseModules* function was used to merge closely related and highly correlated modules. Module eigengenes were calculated and Spearman's correlations were performed between module eigengenes and measured phenotypes. KEGG pathway enrichment and gene ontology analyses were performed on genes within each module using Enrichr as described (Que et al., 2020) to see which modules contained genes associated with biological function or diseases.

Human GWAS Catalogue analysis: Entries in the EMBL-EBI Human GWAS catalog v1.0.2 accessed in 2021 were indexed to matching mouse genes (Buniello et al., 2019) to compare the DE genes found in the CC with homologous genes in humans. Human gene symbols from the "MAPPED_GENE" catalog column (described here: https://www.ebi.ac.uk/gwas/docs/methods/curation) were matched against mouse gene symbols after case-normalization, white space removal, and in the case of multiple mapped genes, delimiter separation.

Additional statistical analyses: All statistical analyses were performed in R (v.3.6.1) (R Core Team, 2019). Diet or strain effects on module eigengenes were assessed using the two-group Mann-Whitney U (Wilcoxon rank) test or Kruskal-Wallis statistical test, respectively. Fisher's Exact test was used to determine whether expressed genes differentially expressed in the CC appeared in the GWAS catalog

more frequently than expected by chance; p-values were adjusted using the Benjamini-Hochberg (BH) method where indicated.

Results

Hepatic gene expression levels are correlated with adiposity for 2,552 genes

Microarray gene expression analysis of 11,542 genes was performed using high-quality RNA from livers of 123 CC mice collected after an 8-week challenge of either a high protein (HP) or high fat high sucrose (HS) diet. Correlations of post-diet adiposity with normalized gene expression levels using calculations of multiple biweight midcorrelations (bicor) and their corresponding Student correlation p-values were performed to determine which genes' expression levels were associated with body fat % and obesity-related traits. Post-diet body fat % was significantly correlated with the expression of 2,552 genes out of 11,542 genes with validated annotation, with the top 15 most significant positive and 15 most significant negative correlations shown in **Figure 2-1**; specifically, post-diet body fat % showed significant moderate negative correlation with the gene expression of TBC1 domain family (*Tbc1d30*; bicor = -0.603, p = 1.56 x 10^{-13}), insulin-like growth factor binding protein 2 (*Igfbp2*; bicor = -0.560, p = 1.62 x 10^{-11}), apolipoprotein M (*ApoM*; bicor = -0.530, p = 2.82 x 10^{-10}), inter-alpha globulin inhibitor H5 (*Itih5*; bicor = -0.527, p = 3.76 x 10^{-10}), and flavin containing monooxygenase 3 (*Fmo3*; bicor = -0.483, p = 1.44 x 10^{-8}), as well as moderate positive correlation between post-diet adiposity and gene expression of aldehyde dehydrogenase (*Aldh1a1*; bicor = 0.538, p = 1.29 x 10^{-10}), thyroid hormone receptor interactor 4 (*Trip4*; bicor = 0.493, p = 6.41 x 10^{-9}), plastin 3 (*Pls3*; bicor = 0.469, p = 4.17 x 10^{-8}), lysophospholipase-like 1 (*Lyplal1*; bicor = 0.468, p = 4.81 x 10^{-8}), and adiponectin receptor 2 (*Adipor2*; bicor = 0.425, p = 9.21 x 10^{-7}). Of these highly correlated genes, metabolic health score was also significantly correlated with *Aldh1a1* (bicor = -0.246, p = 0.001), *Trip4* (bicor = -0.246, p = 0.006), and *Igfbp2* (bicor = 0.270, p = 0.002); total weight was also significantly mildly correlated with the expression levels of these top 30 genes (**Figure 2-1**).

The expression levels of many genes that were significantly correlated either negatively or positively with body fat % were also significantly correlated with lean % and heat production "in the opposite direction" (**Figure 2-1**), which confirms that the relationships between hepatic gene expression

levels and manifested phenotypes are biologically consistent. Very few of the expression levels of the top 30 genes showed significant correlations with circulating analytes or metabolites, with the exception of insulin and phenotype estimates derived from insulin measurements (glucose/insulin ratio, HOMA-IR, and HOMA-B).

Differential gene expression analysis identified 1,344 genes differentially expressed by diet.

Both genetics and environmental factors such as diet are critical determinants of obesity. Although genetics have a stronger effect on susceptibility to developing obesity than diet alone (Corrêa et al., 2020; Hainer et al., 2008), the role of diet as an environmental factor that influences gene expression is still important, since changes in dietary patterns can help mitigate the degree of obesity that develops by altering gene expression levels. To assess which genes' expression levels are affected by diet, differential gene expression analysis was performed using the R package limma (Linear Models for MicroArray) on liver gene expression data. Limma analysis comparing the HS diet to the HP diets revealed 1,344 genes that were differentially expressed by diet ($p\ adj < 0.05$, **Figure 2-2A**), with the top 20 most significant hits showing patterns of expression clustering by diet (**Figure 2-2B**), with 16 genes showing increased expression and 4 genes showing decreased expression in mice fed the HP diet relative to the HS diet, though expression patterns exhibited some degree of inter-strain variation depending on the gene and strain. The opposite patterns of expression for these genes were shown in mice fed the HS diet, i.e. genes that showed increased expression in mice fed the HP diet had decreased levels of expression in mice fed the HS diet (**Figure 2-2B**). The expression levels of 389 differentially expressed genes (DEGs) by diet were significantly correlated with body fat % ($p < 0.05$).

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway and gene ontology (GO) enrichment analyses identified 20 significantly over-represented KEGG pathways and 187 significantly over-represented GO terms for DEGs by diet (**Figure 2-3**), with varying degrees of gene richness defined by the number of up- or downregulated DEGs found belonging to each KEGG pathway or GO term out of the total number of genes that comprise each KEGG pathway or GO term. The most significantly over-represented KEGG pathways identified were metabolic pathways, oxidative phosphorylation, and biosynthesis of amino acids ($p\ adj < 5.051 \times 10^{-8}$). In terms of each GO term category, 105 GO biological processes, 45 GO cellular components, and 37 GO molecular functions were significantly over-

represented (p adj < 0.05), with the top 10 most significantly overrepresented GO terms in DEGs by diet are shown in **Figures 2-3B – 2-3D**.

The effect of CC strain on liver gene expression far surpasses the effect of diet for differential gene expression.

Genetics is clearly an important factor affecting susceptibility to metabolic dysfunction. We tested the role of genetics in regulatory gene expression by performing limma differential gene expression analysis by CC strain. Differential gene expression analysis revealed 9,436 DEGs by CC strain (p adj < 0.05, **Figure 2-4**), with the top 20 most significant hits showing patterns of expression clustering by CC strain instead of diet (**Figure 2-4B**). Unlike the inter-strain variation of expression patterns for diet DEGs, expression patterns were consistent across diets for strain DEGs. DEGs by CC strain showed similar levels of expression within each CC strain regardless of the diet fed. 2,367 of DEGs by CC strain were correlated with body fat % (nominal p < 0.05), and 1,131 DEGs by CC strain were also differentially expressed by diet.

KEGG pathway and GO enrichment analyses identified fewer over-represented KEGG pathways and GO terms for genes differentially expressed by CC strain than diet. For DEGs by CC strain, 13 significantly over-represented KEGG pathways and 163 significantly over-represented GO terms were identified (p adj < 0.05, **Figure 2-5**), with varying degrees of gene richness. The most significantly over-represented KEGG pathways identified were cell adhesion molecules (CAMs), ECM-receptor interaction, and focal adhesion (p adj < 0.002), which are pathways important to cell signaling and structural binding between cells. For each GO term category, 95 GO biological processes, 24 GO cellular components, and 37 GO molecular functions were significantly over-represented in DEGs by CC strain (p adj < 0.05), with the top 10 most significantly overrepresented GO terms in DEGs by strain shown in **Figures 2-5B – 2-5D**.

Clinically relevant genes in humans are differentially expressed in the CC

We sought to determine whether DEGs in the CC were relevant to human health and obesity in humans. To examine the DEGs by diet, we dichotomized our 11,542 unique genes by inclusion in the list of genes differentially expressed by diet. To examine the DEGs by strain, we dichotomized our 11,542 unique genes by inclusion in the list of genes differentially expressed by strain. We then generated a list of

unique human GWAS genes from the “Mapped Gene(s)” column of the 2021 EMBL-EBI Human GWAS catalog v1.0.2, which we used to dichotomize our mouse genes based on inclusion in the human GWAS catalogue (in GWAS: 8,017; not in GWAS: 3,525). To focus on obesity traits in humans, we dichotomized our mouse genes based on whether they contained human homologs found in the GWAS catalog specifically associated with obesity traits (GWAS obesity trait gene: 1,819; not GWAS obesity trait gene: 9,723). Using Fisher’s Exact Test, we demonstrated that DEGs in the CC appear in the human GWAS catalogue more frequently than expected by chance for both differential expression by diet (OR: 1.35, 95%CI: 1.19-1.55, $p < 4.13x10^{-6}$) and strain (OR: 1.23, 95%CI: 1.12-1.37, $p < 4.44x10^{-5}$). To determine whether DEGs in the CC had human homologs that were related to obesity more frequently than expected by chance, we performed Fisher’s Exact Test on our mouse genes dichotomized by whether they contained human homologs found in the GWAS catalog specifically associated with obesity traits. Although DEGs in the CC did not appear in the human GWAS catalogue more frequently than expected by chance for obesity traits for diet DEGs (OR: 1.01, 95%CI: 0.86-1.19, p = 0.873) nor strain DEGs (OR: 1.14, 95%CI: 1.00-1.31, p = 0.06), mouse genetic reference panels can still prove useful for making inferences about genes associated with human disease in general.

DEGs in the CC have homologs implicated to cause complex disease traits in human GWAS studies.

We were next interested in identifying genes that are suspected of causing underlying complex traits in humans since 1,819 of the 11,542 annotated mouse genes included in our analysis have human homologs found in the GWAS catalog associated with obesity traits (Buniello et al., 2019). First, we determined the number of DEGs with gene expression levels significantly correlated with body fat % for diet DEGs (39), strain DEGs (2017), and genes differentially expressed by both diet and strain (350) (**Figure 2-6A**). All of the top 30 genes with expression levels most significantly correlated with body fat % were strain DEGs (**Figure 2-1**), and serine incorporator 2 (*Serinc2*), alanyl-tRNA synthetase (*Aars*), and F-box protein 21 (*Fbxo21*) were also differentially expressed by diet.

To identify DEGs suspected of influencing the manifestation of obesity traits in humans, we intersected our list of differentially expressed genes in mouse with homologous human genes from the GWAS catalog and established the number of DEGs with human homologs found to be associated with obesity traits for diet DEGs (28), strain DEGs (1330), and genes differentially expressed by both diet and

strain (186) (**Figure 2-6B**). Of the top 20 diet DEGs,14 genes were also differentially expressed by strain and four genes differentially expressed by both diet and strain were found to be associated with at least one obesity trait in the GWAS catalog (**Figure 2-2B**). Two of the top 20 diet DEGs with mild but significant correlations between expression levels and body fat % also had human homologs in the GWAS catalog associated with at least one obesity trait: pyruvate kinase liver and red blood cell (*Pklr*; bicor = 0.286, p = 0.0012) and carbamoyl-phosphate synthetase 1 (*Cps1*; bicor = -0.178, p = 0.048). Two of the top 20 strain DEGs also had human homologs associated with at least one obesity trait in the GWAS catalog but did not have expression levels significantly correlated with body fat % (**Figure 2-4B**), namely glycerophosphodiester phosphodiesterase domain containing 3 (*Gdpd3*) and neurexophilin and PC-esterase domain family, member 2 (*Nxpe2*). In humans, PKLR is associated with appendicular lean mass and body fat distribution; CPS1 and GDPD3 are associated with body mass index (BMI); and NXPE2 is associated with BMI and waist circumference adjusted for BMI. Though not in either of the top 20 DEGs lists, FBXO21 is also associated with BMI in humans.

Of the 1,344 genes differentially expressed by diet, 214 genes had human homologs that were found to be associated with obesity traits in the GWAS database; 65 of these 214 genes were also significantly correlated with body fat % (**Figure 2-6C**). Out of 9,436 genes differentially expressed by CC strain, 1,516 genes had human homologs that were found to be associated with obesity traits in the GWAS database; 431 of these 1,516 genes were also significantly correlated with body fat % (**Figure 2-6D**). By intersecting our lists of genes across multiple analyses, we found 434 differentially expressed genes with expression levels correlated with body fat % in the CC and human homologs associated with obesity traits in humans (**Figure 2-6E**), with three genes exclusively differentially expressed by diet, 369 genes exclusively differentially expressed by strain, and 62 genes differentially expressed by both diet and strain.

WGCNA identified 13 unique gene modules with different compositions of DEGs

Because polygenic obesity is a complex physiological trait, we used a gene co-expression network approach to characterize the effects of strain and diet on expression of groups of related genes in addition to assessment of genes individually. Weighted gene co-expression network analysis (WGCNA) identified 13 gene modules each assigned an arbitrary color, where the number of genes contained in

each module ranged from 42 to 3,119 (**Figures 2-7A, 2-7B, Table 2-1**). Upon further examination of the gene composition of each module, the percentage of genes significantly correlated with body fat % (15.1-69.0%) and the percentage of DEGs by diet (0-49.5%) showed a wide range of variation across modules, but the percentage of DEGs by CC strain remained consistently high (>69%) for all modules (**Table 2-1, Figure 2-7C**), demonstrating a stronger effect of CC strain than diet. Of the DEGs with expression levels correlated with body fat % and human homologs associated with obesity-related traits in the GWAS catalog, the three diet DEGs were each assigned to different modules (black, blue, and pink); the range of strain DEGs per module was 1 – 106, with the turquoise module containing the highest number of strain DEGs (**Table 2-2**). The range of DEGs differentially expressed by both diet and strain with expression levels correlated with body fat % and human homologs associated with obesity-related traits in the GWAS catalog per module was 0 – 19, where most modules contained at least one DEG and yellow contained the most DEGs (**Table 2-2**).

After establishing the modules, module eigengenes (MEs) were calculated to estimate the average expression of profiles of each module and Spearman's correlations were performed between MEs and phenotype data from all mice to determine the relationships between the modules and measured phenotypic traits, revealing significant correlations between the pink, yellow, salmon, tan, red, and magenta modules and body fat % (**Figure 2-7D**). Concurrent with ME x phenotype data correlations, modules that were significantly correlated with body fat % had relatively higher percentages of individual genes whose expression levels were significantly correlated with body fat %.

Enrichment analysis revealed multiple biologically relevant modules

Because multiple module eigengenes were significantly correlated with measured phenotypes in the CC (**Figure 2-7D**), we performed enrichment analysis to determine which modules may be biologically relevant. Modules varied widely in terms of the number of enrichments for each category (**Table 2-3**), from no enrichments at all (tan) to 419 total enrichments (brown). **Figure 2-8** shows the top enrichments for each module if present. Of the modules that were significantly correlated with body fat % in the CC, the tan module showed no enrichments, the pink module showed enrichment for the RNA binding GO molecular function (GO:0003723) (p adj = 0.042), the salmon module showed enrichment for the

regulation of angiogenesis (GO:0045765) (p adj = 0.009) and cGMP metabolic process GO biological processes (GO:0046068) (p adj = 0.046), and the magenta, red, and yellow modules showed multiple enrichments for GO Biological Processes, GO molecular functions, KEGG pathways, and Jensen diseases (**Figures S2-1 – S2-4**). Genes in the magenta module were significantly enriched for GO terms and KEGG pathways related to endoplasmic reticulum function (**Figure S2-1**), genes assigned to the red module were significantly enriched for GO terms and KEGG pathways involved in steroid, cholesterol, and fatty acid biosynthesis/metabolism (**Figure S2-2**), and genes found in the yellow module were significantly enriched for a variety of functions in terms of GO terms and KEGG pathways, such as photoperiodism, transcription regulation, insulin signaling, and more (**Figure S2-3**). Although the brown module was only correlated with day basal activity in this study (rho=-0.2, p = 0.03), the highest number of significant enrichment terms was found for the brown module compared to all other modules, where almost all enrichment terms were related to immune response (**Figure S2-4**).

Biologically relevant modules associated with body fat % in the CC contain genes associated with obesity-related traits in humans in the GWAS catalog.

WGCNA identified three gene modules with MEs significantly correlated with body fat % in the CC, namely the magenta, red, and yellow modules, that were enriched for biological pathways related to: endoplasmic reticulum function; steroid, cholesterol, and fatty acid biosynthesis/metabolism; and photoperiodism, transcription regulation, and insulin signaling, respectively. To determine whether these modules contained DEGs in the CC associated with obesity in humans, the lists of genes assigned to each module were intersected with the list of mouse genes that found to have human homologs in the GWAS catalog associated with obesity traits, with examples for each module shown in **Table 2-4**. For the magenta module, there were 0 diet DEGs, 16 strain DEGs, and 5 DEGs by diet and strain identified that had homologs in the GWAS catalog associated with obesity traits in humans, many of which were not significantly correlated with body fat % in the CC. Similarly, there were 0 diet DEGs, 47 strain DEGs, and 12 DEGs by diet and strain in the red module identified to have many homologs in the GWAS catalog associated with obesity traits in humans that were not significantly correlated with body fat % in the CC. In contrast, the yellow module had 3 diet DEGs, 87 strain DEGs, and 30 DEGs by diet and strain with homologs associated with at least one obesity trait in humans, where many of the DEGs were

significantly correlated with body fat % in the CC. By intersecting our results across different analyses, DEGs important to obesity in humans were found in biologically relevant modules associated with body fat % in the CC, where the DEG distribution across modules highlighted the larger contribution of differential expression by strain over diet.

Most modules correlated with body fat % exhibited significant differences in average expression by diet and CC strain

After finding modules that were correlated with body fat %, we ascertained whether the average gene expression profile of these modules defined by their ME first principal components (PC1) differed by diet and/or strain. Wilcoxon ranked sum test of the PC1 between mice fed the HP and HS diets for each module (**Figure 2-9**) revealed significant differences by diet for the pink, yellow, tan, red, and magenta modules ($p < 0.001$), but not the salmon module ($p > 0.1$). Interestingly, when the Kruskal-Wallis test was performed to determine whether PC1 differed by strain for each module (**Figure 2-10**), PC1 significantly differed by strain for the yellow ($p = 6.0 \times 10^{-4}$), red ($p = 2.8 \times 10^{-9}$), magenta ($p = 8.1 \times 10^{-4}$), and salmon ($p = 1.3 \times 10^{-8}$) modules, but not the pink nor tan modules ($p > 0.07$). Of the modules with MEs significantly correlated with body fat %, the yellow, red, and magenta modules exhibited differences by diet and CC strain.

The magnitude and direction of associations between MEs and body fat % change depending on genetic background and diet for certain biologically relevant gene modules

Relating module MEs and body fat %, Spearman's correlations performed between MEs and body fat % for the yellow, red, and magenta modules using data from all samples revealed a significant negative correlation between body fat % and the yellow module ($rho = -0.28$, $p = 0.0016$) and significant positive correlations between body fat % and the magenta ($rho = 0.19$, $p = 0.037$) and red ($rho = 0.27$, $p = 0.0027$) modules (**Figure 2-11**). Given the many enrichments in biological pathways found and significant differences in MEs by diet and CC strain for these three modules, Spearman's correlations were performed between MEs and body fat % by individual CC strain and diet for each module (**Figures S2-5 – S2-6**) to determine whether the relationship between MEs and body fat % stayed consistent across different CC strains and diets. Intriguingly, the overall negative correlation between the yellow ME and

body fat % changed depending on the strain (**Figure S2-5**), from suggestive strong positive associations (CC036/Unc, CC045/GeniUnc) to strong negative associates (CC041/TauUnc, CC063/Unc). For certain CC strains (CC037/TauUnc, CC040/TauUnc), the ME correlations demonstrated a lack of relationship between expression of the yellow module and body fat %. The correlation between expression of the yellow module and body fat % was significant and negative for the HS diet only (**Figure S2-6**). Like the overall negative correlation between the yellow ME and body fat %, the overall positive correlation between the magenta ME and body fat % changed depending on the strain (**Figure S2-5**), from suggestive strong positive associations (CC041/TauUnc, CC063/Unc) to strong negative associates (CC036/Unc, CC071/TauUnc). For certain CC strains (CC030/GeniUnc, CC032/GeniUnc), the correlations demonstrated a lack of relationship between the magenta ME and body fat %. The correlation between expression of the magenta module and body fat % was significant and positive for the HS diet only (**Figure S2-6**). In contrast, the overall positive correlation between the red ME and body fat % became weakened for many strains (**Figure S2-5**), except for several moderate positive associations (CC032/GeniUnc, CC071/TauUnc) and one strong negative association (CC027/GeniUnc). Unlike the yellow and magenta modules where the correlations between MEs and body fat % were only significant for the HS diet, the correlation between the red ME and body fat % remained significant and consistently positive for both diets (**Figure S2-6**). In summary, Spearman's correlations performed between MEs and body fat % by individual CC strain and diet for biologically relevant modules illustrated alterations in the direction and magnitude of associations between module MEs and body fat % depending on CC strain and diet for the yellow and magenta modules, in contrast to the red module where the direction and magnitude of associations between module MEs and body fat % for the red module were weakened for many strains, but reflected the overall significant positive association regardless of diet.

Differences in diet macronutrient composition do not strongly affect broad sense heritability (H^2) estimates for gene expression levels

To quantify the degree to which genetic variation influences variation in gene expression levels, we calculated broad sense heritability (H^2) for the 11,542 genes used for differential gene expression analysis. Using the between- and within-strain mean square values (MSB and MSW, respectively) derived

from linear models, H^2 was estimated by calculating the intraclass correlation (r_I) and coefficient of genetic determination (g^2), which determine the proportion of variation in gene expression levels attributed to differences between strain (genetic variation). Estimates of H^2 based on g^2 calculated using MSB and MSW derived from the "full" additive linear models for the 11,542 genes used for differential gene expression analysis ranged from -0.056 – 0.983 with a median g^2 of 0.173. To assess whether differences in macronutrient composition ("diet environment") influenced H^2 by DEG status, r_I and g^2 summary statistics were calculated for all genes used in limma analysis, diet DEGs, and strain DEGs (**Table 2-5**). Similar g^2 estimates for all limma genes, g^2 for diet DEGs ranged from -0.044 – 0.735 with a median of 0.195, while g^2 for strain DEGs ranged from 0.045 – 0.983 with a median of 0.211. For diet-specific g^2, the minimum g^2 values were slightly less than 0, implying that the variation in expression levels for these genes was greater within strains than between strains, but maximum g^2 and median g^2 values were similar both across diets and DEG status, demonstrating that the proportion of variation in gene expression levels attributed to genetic variation stays relatively constant despite differences in macronutrient composition.

To quantify the proportion of the total gene expression variation that is accounted for by differences between diet, we next calculated the diet intraclass correlation (ICC) using the diet MSB and MSW values derived from the "full" additive linear models and then calculated summary statistics by DEG status group i.e., all limma genes, diet DEGs, and strain DEGs (**Table 2-5**). Diet ICC for all limma genes ranged from -0.017 – 0.799 with a median diet ICC of 0.015. Similarly, diet ICC for strain DEGs ranged from -0.017 – 0.787 with a median of 0.019. Though the maximum diet ICC for diet DEGs was like the other diet ICC maximum values (diet ICC = 0.799), the minimum (diet ICC = 0.099) and median (diet ICC = 0.235) estimates were slightly higher, confirming that the proportion of gene expression variation explained by diet differences was mildly increased for diet DEGs.

To investigate the degree to which gene x environmental (diet) effects mediates variation in gene expression relative to genetics and environment, additional linear mixed model analyses with strain, diet, and strain x diet interactions as all random effects were performed for each gene to estimate the relative heritable variation that can be attributed to strain, diet, and strain x diet effects. From the results of these models, we calculated the variance for each of these terms and found that the proportion of heritable

variation for gene expression attributed to strain x diet interactions on average was small (2.6%) and remained the same regardless of DEG status (**Table 2-6**). For all genes used in differential expression analysis, the largest proportion of heritable variation for gene expression can be attributed to genetic background (strain) on average (30.3%), while the proportion of heritable variation for gene expression attributed to diet (3.9%) and strain x diet interactions (2.6%) were much smaller. As expected, the proportion of heritable variation for gene expression attributed to diet was increased in diet DEGs (18.7%), and the proportion of heritable variation for gene expression attributed to strain was increased in strain DEGs (36.0%).

Modules showed a wide range of variation for overall H^2 and diet ICC both within and between modules

To estimate the H^2 of genes according to WGCNA module assignment, summary statistics were calculated for H^2 estimates and diet ICC of individual modules. Across modules, the blue module had the overall lowest median H^2 estimates (g^2 full = 0.117; g^2 HP = 0.103; g^2 HS = 0.116), while the purple module had the overall highest median H^2 estimates (g^2 full = 0.421; g^2 HP = 0.472; g^2 HS = 0.404). Within modules, the modules with the widest ranges in H^2 were the yellow, brown, and red modules, while the modules with the narrowest ranges in H^2 were the salmon and pink modules. The variation in diet ICC across modules was less than the variation in H^2, as shown by the salmon module which had the lowest diet ICC (diet ICC = -0.007) and the pink module which had the highest diet ICC (diet ICC = 0.121). The pink and magenta modules had the widest ranges in diet ICC (pink diet ICC = -0.017 – 0.772; magenta diet ICC = -0.015 – 0.705), while the salmon and purple modules had the narrowest ranges in diet ICC (salmon diet ICC = -0.017 – 0.072; purple diet ICC = -0.017 – 0.453).

The association between H^2 and intramodular gene connectivity and the association between diet ICC and intramodular gene connectivity vary by individual modules

The connectivity of a gene within a single module relative to genes within the same module, also known as intramodular connectivity, can help identify genes that are important to individual modules. Intramodular connectivity (kWithin) was calculated for all genes used in WGCNA, where higher kWithin values for genes indicated higher degrees of connectivity. To determine whether intramodular connectivity and gene expression H^2 were related in general, Spearman's correlations were performed for the log10 of kWithin for all genes used in WGCNA and H^2 estimates (**Figure S2-7**), revealing consistent

but slight significant negative relationships between intramodular connectivity with H^2 (g^2 full rho= -0.22, $p < 2.2 \times 10^{-16}$; g^2 HP rho= -0.24, $p < 2.2 \times 10^{-16}$; and g^2 HS rho= -0.25, $p < 2.2 \times 10^{-16}$). Although differences in diet macronutrient composition did not greatly alter the association between intramodular connectivity and H^2 overall, the relationship between intramodular connectivity and H^2 changed depending on individual modules and differences in diet macronutrient composition (**Figures S2-8**).

Spearman's correlations were performed for the log10 of kWithin for all genes used in WGCNA and diet ICC (**Figure S2-7D**), revealing a weaker association between intramodular connectivity and diet ICC than H^2 (rho= -0.11, $p < 2.2 \times 10^{-16}$). The relationship between intramodular connectivity and diet ICC varied depending on individual modules (**Figure S2-8**), but the association between intramodular connectivity and diet ICC was significant in fewer modules than the association between intramodular connectivity and H^2.

Discussion

Obesity is a complex and heterogeneous disease whose development is caused by numerous biological factors, particularly genetics, diet, and gene expression. Though long established that obesity results from a chronic imbalance between energy intake and expenditure at a fundamental level, our understanding of exactly how diet and genetics interact to influence gene expression and how gene expression regulates the development of obesity remain to be fully elucidated. Because the liver regulates metabolism of macronutrients, cholesterol, and triglycerides, we measured hepatic gene expression in the CC to gain insight of how diet and genetic background impact obesity and related obesity-related traits. Correlations performed between hepatic gene expression levels and post-diet phenotype data revealed 2,552 genes whose expression levels were significantly correlated with body fat % in the CC, some which were negatively correlated such as *ApoM* and *Fmo3*, but also positively correlated such as *Aldh1a1* and *Adipor2*. *ApoM* encodes a membrane-bound apolipoprotein associated with high density lipoproteins, low density lipoproteins, and triglyceride-rich lipoproteins; secreted through the plasma membrane, alipoprotein M is involved in lipid transport (N. Xu & Dahlbäck, 1999). In the mouse, leptin the "satiety" hormone and leptin receptor are essential for expression of *ApoM*, but excess concentrations of leptin inhibited *ApoM* mRNA expression in a dose-dependent manner in the human hepatoma cell line HepG2,

suggesting that leptin may mediate *ApoM* expression (G. Luo et al., 2005). Although FMO3 is more well-known for its role in preventing trimethylaminuria (fishy odor syndrome) in humans when present at sufficient levels and functioning properly by converting trimethylamine (TMA) to odorless trimethylamine-N-oxide (TMAO) through oxidation (Treacy, 1998), FMO3 also functions as a drug-metabolizing enzyme to catalyze the NADPH-dependent oxygenation of various molecules including therapeutic drugs and dietary compounds (Phillips & Shephard, 2020). Intriguingly, studies in the mouse have suggested additional roles for FMO3 in health and disease, such as modulating cholesterol metabolism (Warrier et al., 2015), glucose and lipid homeostasis (Shih et al., 2015), and as a target for down-regulation by insulin (Miao et al., 2015). Since adipocyte secretion of leptin and insulin occurs in proportion with the volume of adipose tissue under "normal" circumstances, this may partially explain the negative correlations between body fat % and expression of *ApoM* and *Fmo3*.

In the current study, the hepatic gene expression levels of *Aldh1a1* and *Adipor2* were positively correlated with body fat %. *Aldh1a1* encodes the protein aldehyde dehydrogenase 1 family, member A1 (ALDH1A1), also known as retinaldehyde dehydrogenase 1 (RALDH1), which is a prominent enzyme in the oxidative pathway of alcohol metabolism; in humans, mutations in this gene have been linked to alcoholism (Liu et al., 2011; Sherva et al., 2009). However, various studies in mice have shown that ALDH1A1 also modulates hepatic gluconeogenesis and lipid metabolism through its role in retinoid metabolism (Kiefer et al., 2012) and up-regulation of ALDH1A1 is associated with reduced adiponectin expression in adipose tissue after high-fat diet feeding (Landrier et al., 2017). Furthermore, mice without ALDH1A1 are resistant to diet-induced obesity and inhibition of ALDH1A1 in mice suppresses weight gain (Haenisch et al., 2018, 2021), which is consistent with our finding and illustrates the potential for ALDH1A1 as a drug target for obesity prevention or treatment. Adipor2 encodes adiponectin receptor 2 which interacts with adiponectin to mediate fatty acid oxidation and glucose uptake (Yamauchi et al., 2003). An agonist of adiponectin receptor 2, the adipokine adiponectin is inversely correlated with body fat mass and visceral adiposity in humans, though the mechanisms of how adiponectin's interactions with its receptors to elicit anti-diabetic, anti-atherogenic, and anti-inflammatory effects are not fully understood (Parida et al., 2019).

After confirming the relationship between expression of genes related to obesity and body fat % in the CC, we investigated the effects of genetic background (strain) and diet on hepatic gene expression levels. Similar to adiposity and the obesity-related traits examined in our previous study (Yam et al., 2021), genetic background had a far stronger effect on hepatic gene expression than diet, as shown by the overwhelmingly larger number of significant DEGs by strain (9,436) compared to the number of DEGs by diet (1,344). Interestingly, gene expression of 28.9% of DEGs by diet were significantly correlated with adiposity (389/1,344) compared to 25% of DEGs by strain (2,367/9,436). Of the top 20 most significant diet DEGs identified in the CC, carbamoyl-phosphate synthase 1 (*Cps1)*, isovaleryl-CoA dehydrogenase (*Ivd)*, neuropilin 1 (*Nrp1)*, and pyruvate kinase L/R (*Pklr)* have human homologs associated with obesity traits (Kichaev et al., 2019; Locke et al., 2015; Pulit et al., 2019; Rask-Andersen et al., 2019; Zhu et al., 2020), but only one of the top 20 most significant strain DEGs also had a human homolog associated with at least one obesity traits, namely glycerophosphodiester phosphodiesterase domain containing 3 (*Gdpd3)* (Zhu et al., 2020).

Gene enrichment analysis of DEGs revealed different trends between DEGs by diet compared to strain. DEGs by diet showed enrichment for KEGG pathways and Gene Ontology (GO) biological processes related to numerous types of metabolism, amino acid synthesis, and non-alcoholic fatty liver disease, whereas DEGs by strain showed enrichment for cell function pathways, type I diabetes, and fatty acid metabolism. Like KEGG pathway enrichment, GO term enrichment for cellular components and molecular functions also showed distinct differences between DEGs by diet compared to strain; DEGs by diet showed enrichment for multiple cellular components related to the mitochondrion, endoplasmic reticulum, and cell membrane, while DEGs by strain showed enrichment for cellular components related to the cell membrane, extracellular components, and cell surface. In terms of molecular functions, DEGs by diet showed enrichment for metabolism and binding for nutrients and small molecules such as cofactor binding, vitamin B6 binding, catalytic activity, and electron transfer activity, while DEGs by strain showed enrichment for binding related to general cell and tissue functions, such as extracellular matrix, collagen, signaling receptor, and fibronectin binding. The culmination of our results suggests that generally diet alters gene expression for “acute” metabolic processes sensitive to environmental changes, but genetic background more heavily influences overall “stable” cellular function.

Having identified genes with expression strongly influenced by diet or strain, we performed further analysis using the GWAS catalog to determine which DEGs may be most relevant to human health in general and specifically to obesity-related traits in humans, revealing 300 DEGs by diet and 1,704 DEGs by strain with expression significantly correlated with body fat % in the CC and a corresponding human homolog in the GWAS catalog associated with at least one disease or trait. Of these 300 DEGs by diet and 1,704 DEGs by strain found in the GWAS catalog, 65 DEGs by diet and 431 DEGs by strain were specifically associated with obesity-related traits such as body fat distribution, BMI, waist-hip ratio, weight, and fat body mass. In our list of genes whose gene expression levels were significantly correlated with body fat % that have previously been associated with obesity-related traits in humans, some diet DEGs not differentially expressed by strain found in our current study include increased sodium tolerance 1 homolog (*Ist1*) (Hoffmann et al., 2018), chromodomain protein, Y chromosome-like (*Cdyl*) (Tachmazidou et al., 2017), and NIPBL cohesin loading factor (*Nipbl*) (Tachmazidou et al., 2017), while strain DEGs not differentially expressed by diet were lysophospholipase-like 1 (*Lyplal1*) (Fox et al., 2012; Kichaev et al., 2019; Lindgren et al., 2009; Pulit et al., 2019; Tachmazidou et al., 2017; Wang et al., 2019), leucine rich repeat (in FLII) interacting protein 1 (*Lrrfip1*) (Plourde et al., 2013), and neurotrophic tyrosine kinase, receptor, type 2 (*Ntrk2*) (Akiyama et al., 2017; Kichaev et al., 2019; Pulit et al., 2019; Zhu et al., 2020), and lastly genes differentially expressed by both strain and diet include F-box protein 21(*Fbxo21*) (Kichaev et al., 2019; Pulit et al., 2019; Zhu et al., 2020), alanyl-tRNA synthetase (*Aars*) (Kichaev et al., 2019; Zhu et al., 2020), and BRCA1 associated protein (*Brap*) (Hoffmann et al., 2018; The LifeLines Cohort Study et al., 2015; Winkler et al., 2015). Our findings highlight which candidate genes previously described in the literature have the highest potential for successful future validation studies.

Since obesity is a complex trait regulated by multiple genes, we used a gene co-expression network approach to find groups of genes that are similarly regulated by diet or strain and identified 13 gene modules comprised of a wide number of genes from 42 to 3,319. Consistent with our DEG analyses, all modules were comprised largely of genes that were strain DEGs (>69%) while the proportion of diet DEGs (0-49.5%) and genes with expression significantly correlated with body fat % (15.1-69.0%) varied much more widely, illustrating the variable effect of diet on gene expression compared to genetic background. Spearman's correlation of the MEs for identified modules with

phenotypic data revealed six modules related to body fat %: tan, pink, salmon, magenta, red, and yellow. The MEs for all of these modules differed significantly by diet, except for the salmon module, suggesting that differences in diet macronutrient composition induce changes in gene expression for entire groups of genes. Similar to diet, the MEs for most of the modules also differed significantly by strain, except for the pink and tan modules. However, it is important to note that the ME variation within each strain appeared much higher for these two modules than the magenta, red, and salmon modules, an observation shown through the ability of utilizing genetic "replicates" with high genotypic and phenotypic diversity that is inherent to the CC; in fact, increasing the number of "replicates" would enhance the ability to find significant strain-by-diet differences. Thus, we show that both diet and strain may strongly affect hepatic gene expression.

Enrichment analysis performed using the lists of genes assigned to each module allowed us to assess which modules identified in the CC may be most biologically relevant to obesity and human health. Of the six modules whose MEs were significantly correlated with body fat %, the number of enrichment terms were few to none for the salmon, pink, and tan modules, but the magenta, red, and yellow modules were significantly enriched for numerous functional pathways, biological processes, and/or diseases. The magenta module was enriched for pathways related to endoplasmic reticulum (ER) function and contained 163 genes total, with 16 strain DEGs and five DEGs by both diet and strain with human homologs associated with at least one obesity trait. Two DEGs with homologs associated with obesity in humans from the magenta module that merit further study are serpin family A member 6 (*Serp1*) and UDP-glucose glycoprotein glucosyltransferase 1(*Uggt1*). Differentially expressed only by strain in the CC, *Serp1* participates in the metabolism of proteins in the ER by protecting target proteins against degradation and interacting with target proteins during their translocation (Yamaguchi et al., 1999). Similarly, *Uggt1* encodes the enzyme UDP-glucose:glycoprotein glucosyltransferase (UGT), which is also located in the lumen of the ER and provides quality control for protein transport by selectively reglucosylates unfolded glycoproteins (Dejgaard et al., 2004), but *Uggt1* was differentially expressed by both diet and strain in the CC. Studies have demonstrated that hepatic ER stress induced by obesity can lead to the development of hepatic insulin resistance and gluconeogenesis, likely through the activation of the JNK pathway (Kim et al., 2015; Ozcan, 2004; Yilmaz, 2017). Our findings reaffirm the association

between obesity and alterations in hepatic gene expression related to ER function and suggest potential candidate genes for future study in relation to patient screening for diabetes risk, and provide a link between diet, five hepatic ER genes, obesity, and insulin resistance.

The red module was enriched for pathways clearly related to obesity which involve steroid, cholesterol, and fatty acid biosynthesis/metabolism. Containing 378 genes total with 47 strain DEGs and 12 DEGs by both diet and strain with human homologs associated with at least one obesity trait, potential candidate genes from the red module belong to biological pathways clearly related to substrate metabolism. For example, strain GWAS DEG *Fasn* encodes the multifunction protein fatty acid synthase whose primary function is to catalyze the synthesis of palmitate from acetyl-CoA and malonyl-CoA into long-chain saturated fatty acids in the presence of NADPH (Alberts et al., 1975), processes important to maintaining lipid homeostasis. Like *Fasn*, the *Acac* gene is also a strain GWAS DEG that encodes a protein crucial to fatty acid synthesis - acetyl-CoA carboxylase (ACC), which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA (the rate-limiting step in fatty acid synthesis) (Tong, 2005). Of the DEGs by both diet and strain, *Mipep* also shows promise as a potential therapeutic target. In humans, *MIPEP* encodes mitochondrial intermediate peptidase, a critical component of mitochondrial protein import machinery involved in the maturing process of nuclear-coded mitochondrial proteins (Chew et al., 1997). In mice, calorie restriction upregulates MIPEP in white adipocytes, while MIPEP subsequently upregulates sirtuin-3, a protein that regulates mitochondrial quality (Kobayashi et al., 2017). In addition to dyslipidemia, further investigation of candidate DEGs found in the red module could offer insights on how diet or specific genetic variants influence cardiometabolic disease risk through impaired cholesterol metabolism.

The yellow module was enriched for a variety of pathways, such as photoperiodism, transcription regulation, insulin signaling, and more, containing 665 genes total, with strain DEGs and DEGs by both diet and strain with human homologs associated with at least one obesity trait. Unlike the magenta and red modules, the yellow module also contained 3 genes associated with obesity in humans only differentially expressed by diet. Three DEGs with homologs associated with obesity in humans from the yellow module that warrant further exploration are *Fars2*, *Pnpla7*, and *Mgrn1*. The nuclear *Fars2* gene is

differentially expressed only by diet in the CC and encodes phenylalanyl-tRNA synthetase, mitochondrial (FARS2), which localizes to the mitochondrion to assist in mitochondrial protein translation (Bullard et al., 1999); in humans, mutations in the FARS2 gene typically result in early-onset epilepsy, neurological deficits, or complex IV deficiency (Almalki et al., 2014). Differentially expressed by strain, *Pnpla7* encodes patatin-like phospholipase domain-containing protein 7 (PNPLA7), a lipid-metabolizing transmembrane protein in the ER linked to insulin signaling and energy metabolism that promotes hydrolysis of lysophosphatidylcholine in mammalian cells (Heier et al., 2017). Differentially expressed by both diet and strain *Mgrn1* encodes the E3 ubiquitin ligase mahogunin ring finger-1 (MGRN1), an accessory protein for melanocortin receptor (MCR) signaling in the mouse; MGRN1 has been found to inhibit MCR signaling, which modulates body weight by influencing appetite signaling (Pérez-Oliva et al., 2009). Our findings provide context on how disruptions in specific biological pathways may contribute to obesity development, as well as identify potential candidate genes within these pathways that explain the relationship between obesity and other traits such as chronotype.

Interestingly, our gene network approach allowed us to identify other potentially relevant gene modules outside of the focus of the current study, specifically the brown module. The brown module had the highest number of enrichment terms (419) where almost all enrichment terms were related to immune response, such as neutrophil immune responses, cytokine-mediated and chemokine signaling, T cell receptor binding, and others. Since obesity is usually accompanied by mild, chronic, systemic inflammation, the identification of a module enriched for immune response pathways may provide novel insights about the interactions between liver function, immunity, and obesity in future studies if additional phenotypic data were included, such as measurements of circulating cytokine levels.

Using the between- and within-strain mean square values derived from linear models, we calculated H^2 estimates to quantify the degree to which genetic variation affects hepatic gene expression level variation. For the 11,542 genes included in our analysis, the range of coefficient of genetic determination (g^2) was broad (g^2 = -0.056 – 0.983) as expected, but the median was lower than anticipated (g^2 = 0.173) given the strong effect of strain on the expression of most genes. Median H^2 estimates by DEG status increased slightly but not drastically (diet DEG g^2 = 0.195, strain DEG g^2 = 0.211), while H^2 estimates remained similar, suggesting that differences in macronutrient composition did

not have a large impact on hepatic gene expression in this study. Upon examination of the relative heritable variation that can be attributed to strain, diet, and strain x diet effects for all genes, the largest proportion of heritable variation for gene expression can be attributed to genetic background (strain) on average (30.3%), while the proportion of heritable variation for gene expression attributed to diet (3.9%) and strain x diet interactions (2.6%) were much smaller, which reaffirms the strong effect of strain (genetic background) on gene expression relative to diet and strain x diet effects. However, one caveat of these approximations is that increasing the sample size would provide a better estimation of the relative heritable variation since the number of mice per strain per diet is relatively low, so the estimation of strain x diet effect may not be precise.

One limitation of the current study is the number of strain-by-diet replicates. With an increased sample size, significant effects of strain-by-diet interactions would be more detectable and more detailed conclusions could be drawn regarding the effects of strain-by-diet interactions on both individual genes and in the identified gene modules. For future studies, investigating the transcriptome and epigenome of both adipose tissue and hepatic tissue together would further clarify the genetic and dietary mechanisms that drive the crosstalk between tissue types to modulate energy balance in the context of obesity development. If possible, integrating microbiome data would provide yet another “piece of the puzzle” for the elucidation of how genetic and environmental factors interact in the development of obesity. Nonetheless, our findings show that both variation in genetic background and diet can strongly influence hepatic gene expression of both individual genes and groups of related genes.

References

Akiyama, M., Okada, Y., Kanai, M., Takahashi, A., Momozawa, Y., Ikeda, M., Iwata, N., Ikegawa, S., Hirata, M., Matsuda, K., Iwasaki, M., Yamaji, T., Sawada, N., Hachiya, T., Tanno, K., Shimizu, A., Hozawa, A., Minegishi, N., Tsugane, S., … Kamatani, Y. (2017). Genome-wide association study identifies 112 new loci for body mass index in the Japanese population. *Nature Genetics*, *49*(10), 1458–1467. https://doi.org/10.1038/ng.3951

Alberts, A. W., Strauss, A. W., Hennessy, S., & Vagelos, P. R. (1975). Regulation of synthesis of hepatic fatty acid synthetase: Binding of fatty acid synthetase antibodies to polysomes. *Proceedings of the National Academy of Sciences of the United States of America*, *72*(10), 3956–3960. https://doi.org/10.1073/pnas.72.10.3956

Almalki, A., Alston, C. L., Parker, A., Simonic, I., Mehta, S. G., He, L., Reza, M., Oliveira, J. M. A., Lightowlers, R. N., McFarland, R., Taylor, R. W., & Chrzanowska-Lightowlers, Z. M. A. (2014). Mutation of the human mitochondrial phenylalanine-tRNA synthetase causes infantile-onset epilepsy and cytochrome c oxidase deficiency. *Biochimica Et Biophysica Acta*, *1842*(1), 56–64. https://doi.org/10.1016/j.bbadis.2013.10.008

Bell, C. G., Walley, A. J., & Froguel, P. (2005). The genetics of human obesity. *Nature Reviews Genetics*, *6*(3). https://doi.org/10.1038/nrg1556

Bullard, J. M., Cai, Y. C., Demeler, B., & Spremulli, L. L. (1999). Expression and characterization of a human mitochondrial phenylalanyl-tRNA synthetase. *Journal of Molecular Biology*, *288*(4), 567–577. https://doi.org/10.1006/jmbi.1999.2708

Chew, A., Buck, E. A., Peretz, S., Sirugo, G., Rinaldo, P., & Isaya, G. (1997). Cloning, expression, and chromosomal assignment of the human mitochondrial intermediate peptidase gene (MIPEP). *Genomics*, *40*(3), 493–496. https://doi.org/10.1006/geno.1996.4586

Churchill, G. A., Airey, D. C., Allayee, H., Angel, J. M., Attie, A. D., Beatty, J., Beavis, W. D., Belknap, J. K., Bennett, B., Berrettini, W., Bleich, A., Bogue, M., Broman, K. W., Buck, K. J., Buckler, E., Burmeister, M., Chesler, E. J., Cheverud, J. M., Clapcote, S., … Complex Trait Consortium. (2004). The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nature Genetics*, *36*(11). https://doi.org/10.1038/ng1104-1133

Coffey, A. R., Smallwood, T. L., Albright, J., Hua, K., Kanke, M., Pomp, D., Bennett, B. J., & Sethupathy, P. (2017). Systems genetics identifies a co-regulated module of liver microRNAs associated with plasma LDL cholesterol in murine diet-induced dyslipidemia. *Physiological Genomics*, *49*(11). https://doi.org/10.1152/physiolgenomics.00050.2017

Collaborative Cross Consortium. (2012). The Genome Architecture of the Collaborative Cross Mouse Genetic Reference Population. *Genetics*, *190*(2). https://doi.org/10.1534/genetics.111.132639

Corrêa, T. A. F., Quintanilha, B. J., Norde, M. M., Pinhel, M. A. de S., Nonino, C. B., & Rogero, M. M. (2020). Nutritional genomics, inflammation and obesity. *Archives of Endocrinology and Metabolism*, *64*(3), 205–222. https://doi.org/10.20945/2359-3997000000255

Creasy, S. A., Rynders, C. A., Bergouignan, A., Kealey, E. H., & Bessesen, D. H. (2018). Free-Living Responses in Energy Balance to Short-Term Overfeeding in Adults Differing in Propensity for Obesity. *Obesity*, *26*(4). https://doi.org/10.1002/oby.22121

Cuthbertson, D. J., Steele, T., Wilding, J. P., Halford, J. C., Harrold, J. A., Hamer, M., & Karpe, F. (2017). What have human experimental overfeeding studies taught us about adipose tissue expansion and susceptibility to obesity and metabolic complications? *International Journal of Obesity*, *41*(6). https://doi.org/10.1038/ijo.2017.4

Danforth, E. (1985). Diet and obesity. *The American Journal of Clinical Nutrition*, *41*(5). https://doi.org/10.1093/ajcn/41.5.1132

Dejgaard, S., Nicolay, J., Taheri, M., Thomas, D. Y., & Bergeron, J. J. M. (2004). The ER glycoprotein quality control system. *Current Issues in Molecular Biology*, *6*(1), 29–42.

Després, J.-P. (2012). Body Fat Distribution and Risk of Cardiovascular Disease: An Update. *Circulation*, *126*(10), 1301–1313. https://doi.org/10.1161/CIRCULATIONAHA.111.067264

Ellero-Simatos, S., Fleuren, W. W., Bauerschmidt, S., Dokter, W. H., & Toonen, E. J. (2014). Identification of gene signatures for prednisolone-induced metabolic dysfunction in collagen-induced arthritic mice. *Pharmacogenomics*, *15*(5). https://doi.org/10.2217/pgs.14.3

Fernández-Verdejo, R., Marlatt, K. L., Ravussin, E., & Galgani, J. E. (2019). Contribution of brown adipose tissue to human energy metabolism. *Molecular Aspects of Medicine*, *68*. https://doi.org/10.1016/j.mam.2019.07.003

Festing, M. F. W. (1979). *Inbred Strains in Biomedical Research*. Macmillan Education UK. https://doi.org/10.1007/978-1-349-03816-9

Fischer, I. P., Irmler, M., Meyer, C. W., Sachs, S. J., Neff, F., Hrabě de Angelis, M., Beckers, J., Tschöp, M. H., Hofmann, S. M., & Ussar, S. (2017). A history of obesity leaves an inflammatory fingerprint in liver and adipose tissue. *International Journal of Obesity*, *42*(3). https://doi.org/10.1038/ijo.2017.224

Fox, C. S., Liu, Y., White, C. C., Feitosa, M., Smith, A. V., Heard-Costa, N., Lohman, K., GIANT Consortium, MAGIC Consortium, GLGC Consortium, Johnson, A. D., Foster, M. C., Greenawalt, D. M., Griffin, P., Ding, J., Newman, A. B., Tylavsky, F., Miljkovic, I., Kritchevsky, S. B., … Borecki, I. B. (2012). Genome-Wide Association for Abdominal Subcutaneous and Visceral Adipose Reveals a Novel Locus for Visceral Fat in Women. *PLoS Genetics*, *8*(5), e1002695. https://doi.org/10.1371/journal.pgen.1002695

Gautier, L., Cope, L., Bolstad, B. M., & Irizarry, R. A. (2004). affy—Analysis of Affymetrix GeneChip data at the probe level. *Bioinformatics*, *20*(3). https://doi.org/10.1093/bioinformatics/btg405

Ghazalpour, A., Doss, S., Zhang, B., Wang, S., Plaisier, C., Castellanos, R., Brozell, A., Schadt, E. E., Drake, T. A., Lusis, A. J., & Horvath, S. (2006). Integrating Genetic and Network Analysis to Characterize Genes Related to Mouse Weight. *PLoS Genetics*, *2*(8), e130. https://doi.org/10.1371/journal.pgen.0020130

González-Muniesa, P., Mártinez-González, M.-A., Hu, F. B., Després, J.-P., Matsuzawa, Y., Loos, R. J. F., Moreno, L. A., Bray, G. A., & Martinez, J. A. (2017). Obesity. *Nature Reviews Disease Primers*, *3*. https://doi.org/10.1038/nrdp.2017.34

Haenisch, M., Nguyen, T., Fihn, C. A., Goldstein, A. S., Amory, J. K., Treuting, P., Brabb, T., & Paik, J. (2021). Investigation of an ALDH1A1-specific inhibitor for suppression of weight gain in a diet-induced mouse model of obesity. *International Journal of Obesity (2005)*, *45*(7), 1542–1552. https://doi.org/10.1038/s41366-021-00818-1

Haenisch, M., Treuting, P. M., Brabb, T., Goldstein, A. S., Berkseth, K., Amory, J. K., & Paik, J. (2018). Pharmacological inhibition of ALDH1A enzymes suppresses weight gain in a mouse model of diet-induced obesity. *Obesity Research & Clinical Practice*, *12*(1), 93–101. https://doi.org/10.1016/j.orcp.2017.08.003

Hainer, V., Zamrazilová, H., Spálová, J., Hainerová, I., Kunesová, M., Aldhoon, B., & Bendlová, B. (2008). Role of hereditary factors in weight loss and its maintenance. *Physiological Research*, *57 Suppl 1*, S1-15.

Hao, L., Huang, K.-H., Ito, K., Sae-tan, S., Lambert, J. D., & Ross, A. C. (2016). Fibroblast Growth Factor 21 (Fgf21) Gene Expression Is Elevated in the Liver of Mice Fed a High-Carbohydrate Liquid Diet and Attenuated by a Lipid Emulsion but Is Not Upregulated in the Liver of Mice Fed a High-Fat Obesogenic Diet. *The Journal of Nutrition*, *146*(2). https://doi.org/10.3945/jn.115.216572

Heier, C., Kien, B., Huang, F., Eichmann, T. O., Xie, H., Zechner, R., & Chang, P.-A. (2017). The phospholipase PNPLA7 functions as a lysophosphatidylcholine hydrolase and interacts with lipid droplets through its catalytic domain. *The Journal of Biological Chemistry*, *292*(46), 19087–19098. https://doi.org/10.1074/jbc.M117.792978

Hoffmann, T. J., Choquet, H., Yin, J., Banda, Y., Kvale, M. N., Glymour, M., Schaefer, C., Risch, N., & Jorgenson, E. (2018). A Large Multiethnic Genome-Wide Association Study of Adult Body Mass Index Identifies Novel Loci. *Genetics*, *210*(2), 499–515. https://doi.org/10.1534/genetics.118.301479

Houtkooper, R. H., Argmann, C., Houten, S. M., Cantó, C., Jeninga, E. H., Andreux, P. A., Thomas, C., Doenlen, R., Schoonjans, K., & Auwerx, J. (2011). The metabolic footprint of aging in mice. *Scientific Reports*, *1*(1). https://doi.org/10.1038/srep00134

Iraqi, F. A., Churchill, G., & Mott, R. (2008). The Collaborative Cross, developing a resource for mammalian systems genetics: A status report of the Wellcome Trust cohort. *Mammalian Genome*, *19*(6). https://doi.org/10.1007/s00335-008-9113-1

John, G. K., & Mullin, G. E. (2016). The Gut Microbiome and Obesity. *Current Oncology Reports*, *18*(7). https://doi.org/10.1007/s11912-016-0528-7

Kauffmann, A., Gentleman, R., & Huber, W. (2008). arrayQualityMetrics—A bioconductor package for quality assessment of microarray data. *Bioinformatics*, *25*(3). https://doi.org/10.1093/bioinformatics/btn647

Kichaev, G., Bhatia, G., Loh, P.-R., Gazal, S., Burch, K., Freund, M. K., Schoech, A., Pasaniuc, B., & Price, A. L. (2019). Leveraging Polygenic Functional Enrichment to Improve GWAS Power. *The American Journal of Human Genetics*, *104*(1), 65–75. https://doi.org/10.1016/j.ajhg.2018.11.008

Kiefer, F. W., Orasanu, G., Nallamshetty, S., Brown, J. D., Wang, H., Luger, P., Qi, N. R., Burant, C. F., Duester, G., & Plutzky, J. (2012). Retinaldehyde dehydrogenase 1 coordinates hepatic gluconeogenesis and lipid metabolism. *Endocrinology*, *153*(7), 3089–3099. https://doi.org/10.1210/en.2011-2104

Kim, H. J., Oh, S. Y., Choi, D.-W., & Park, E.-C. (2019). The Association between Eating-Out Rate and BMI in Korea. *International Journal of Environmental Research and Public Health*, *16*(17), 3186. https://doi.org/10.3390/ijerph16173186

Kim, O.-K., Jun, W., & Lee, J. (2015). Mechanism of ER Stress and Inflammation for Hepatic Insulin Resistance in Obesity. *Annals of Nutrition and Metabolism*, *67*(4), 218–227. https://doi.org/10.1159/000440905

Kobayashi, M., Takeda, K., Narita, T., Nagai, K., Okita, N., Sudo, Y., Miura, Y., Tsumoto, H., Nakagawa, Y., Shimano, H., & Higami, Y. (2017). Mitochondrial intermediate peptidase is a novel regulator of sirtuin-3 activation by caloric restriction. *FEBS Letters*, *591*(24), 4067–4073. https://doi.org/10.1002/1873-3468.12914

Kozul, C. D., Nomikos, A. P., Hampton, T. H., Warnke, L. A., Gosse, J. A., Davey, J. C., Thorpe, J. E., Jackson, B. P., Ihnat, M. A., & Hamilton, J. W. (2008). Laboratory diet profoundly alters gene expression and confounds genomic analysis in mouse liver and lung. *Chemico-Biological Interactions*, *173*(2). https://doi.org/10.1016/j.cbi.2008.02.008

Landrier, J.-F., Kasiri, E., Karkeni, E., Mihály, J., Béke, G., Weiss, K., Lucas, R., Aydemir, G., Salles, J., Walrand, S., de Lera, A. R., & Rühl, R. (2017). Reduced adiponectin expression after high-fat diet is associated with selective up-regulation of ALDH1A1 and further retinoic acid receptor signaling in adipose tissue. *FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology*, *31*(1), 203–211. https://doi.org/10.1096/fj.201600263RR

Langfelder, P., & Horvath, S. (2008). WGCNA: an R package for weighted correlation network analysis. *BMC Bioinformatics*, *9*(1). https://doi.org/10.1186/1471-2105-9-559

Langhans, W. (2003). Role of the liver in the control of glucose-lipid utilization and body weight. *Current Opinion in Clinical Nutrition and Metabolic Care*, *6*(4). https://doi.org/10.1097/01.mco.0000078993.96795.16

Lindgren, C. M., Heid, I. M., Randall, J. C., Lamina, C., Steinthorsdottir, V., Qi, L., Speliotes, E. K., Thorleifsson, G., Willer, C. J., Herrera, B. M., Jackson, A. U., Lim, N., Scheet, P., Soranzo, N., Amin, N., Aulchenko, Y. S., Chambers, J. C., Drong, A., Luan, J., … McCarthy for the GIANT consortium, M. I. (2009). Genome-Wide Association Scan Meta-Analysis Identifies Three Loci Influencing Adiposity and Fat Distribution. *PLoS Genetics*, *5*(6), e1000508. https://doi.org/10.1371/journal.pgen.1000508

Lis, J. T. (2019). A 50 year history of technologies that drove discovery in eukaryotic transcription regulation. *Nature Structural & Molecular Biology*, *26*(9). https://doi.org/10.1038/s41594-019-0288-9

Liu, J., Zhou, Z., Hodgkinson, C. A., Yuan, Q., Shen, P.-H., Mulligan, C. J., Wang, A., Gray, R. R., Roy, A., Virkkunen, M., Goldman, D., & Enoch, M.-A. (2011). Haplotype-based study of the association of alcohol-metabolizing genes with alcohol dependence in four independent populations. *Alcoholism, Clinical and Experimental Research*, *35*(2), 304–316. https://doi.org/10.1111/j.1530-0277.2010.01346.x

Locke, A. E., Kahali, B., Berndt, S. I., Justice, A. E., Pers, T. H., Day, F. R., Powell, C., Vedantam, S., Buchkovich, M. L., Yang, J., Croteau-Chonka, D. C., Esko, T., Fall, T., Ferreira, T., Gustafsson, S., Kutalik, Z., Luan, J., Mägi, R., Randall, J. C., … Speliotes, E. K. (2015). Genetic studies of body mass index yield new insights for obesity biology. *Nature*, *518*(7538), 197–206. https://doi.org/10.1038/nature14177

Loos, R. J. (2018). The genetics of adiposity. *Current Opinion in Genetics & Development*, *50*. https://doi.org/10.1016/j.gde.2018.02.009

Luke, S. G. (2016). Evaluating significance in linear mixed-effects models in R. *Behavior Research Methods*, *49*(4). https://doi.org/10.3758/s13428-016-0809-y

Luo, G., Hurtig, M., Zhang, X., Nilsson-Ehle, P., & Xu, N. (2005). Leptin inhibits apolipoprotein M transcription and secretion in human hepatoma cell line, HepG2 cells. *Biochimica Et Biophysica Acta*, *1734*(2), 198–202. https://doi.org/10.1016/j.bbalip.2005.02.005

Luo, Y., Burrington, C. M., Graff, E. C., Zhang, J., Judd, R. L., Suksaranjit, P., Kaewpoowat, Q., Davenport, S. K., O'Neill, A. M., & Greene, M. W. (2016). Metabolic phenotype and adipose and liver features in a high-fat Western diet-induced mouse model of obesity-linked NAFLD. *American Journal of Physiology-Endocrinology and Metabolism*, *310*(6). https://doi.org/10.1152/ajpendo.00319.2015

Martinez, K. B., Pierre, J. F., & Chang, E. B. (2016). The Gut Microbiota. *Gastroenterology Clinics of North America*, *45*(4). https://doi.org/10.1016/j.gtc.2016.07.001

Miao, J., Ling, A. V., Manthena, P. V., Gearing, M. E., Graham, M. J., Crooke, R. M., Croce, K. J., Esquejo, R. M., Clish, C. B., Morbid Obesity Study Group, Vicent, D., & Biddinger, S. B. (2015). Flavin-containing monooxygenase 3 as a potential player in diabetes-associated atherosclerosis. *Nature Communications*, *6*, 6498. https://doi.org/10.1038/ncomms7498

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E. C., Biryukov, S., Abbafati, C., Abera, S. F., Abraham, J. P., Abu-Rmeileh, N. M. E., Achoki, T., AlBuhairan, F. S., Alemu, Z. A., Alfonso, R., Ali, M. K., Ali, R., Guzman, N. A., … Gakidou, E. (2014). Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: A systematic analysis for the Global Burden of Disease Study 2013. *The Lancet*, *384*(9945), 766–781. https://doi.org/10.1016/S0140-6736(14)60460-8

Ozcan, U. (2004). Endoplasmic Reticulum Stress Links Obesity, Insulin Action, and Type 2 Diabetes. *Science*, *306*(5695), 457–461. https://doi.org/10.1126/science.1103160

Parida, S., Siddharth, S., & Sharma, D. (2019). Adiponectin, Obesity, and Cancer: Clash of the Bigwigs in Health and Disease. *International Journal of Molecular Sciences*, *20*(10), 2519. https://doi.org/10.3390/ijms20102519

Pérez-Oliva, A. B., Olivares, C., Jiménez-Cervantes, C., & García-Borrón, J. C. (2009). Mahogunin ring finger-1 (MGRN1) E3 ubiquitin ligase inhibits signaling from melanocortin receptor by competition with Galphas. *The Journal of Biological Chemistry*, *284*(46), 31714–31725. https://doi.org/10.1074/jbc.M109.028100

Philip, V. M., Sokoloff, G., Ackert-Bicknell, C. L., Striz, M., Branstetter, L., Beckmann, M. A., Spence, J. S., Jackson, B. L., Galloway, L. D., Barker, P., Wymore, A. M., Hunsicker, P. R., Durtschi, D. C., Shaw, G. S., Shinpock, S., Manly, K. F., Miller, D. R., Donohue, K. D., Culiat, C. T., … Chesler, E. J. (2011). Genetic analysis in the Collaborative Cross breeding population. *Genome Research*, *21*(8). https://doi.org/10.1101/gr.113886.110

Phillips, I. R., & Shephard, E. A. (2020). Flavin-containing monooxygenase 3 (FMO3): Genetic variants and their consequences for drug metabolism and disease. *Xenobiotica*, *50*(1), 19–33. https://doi.org/10.1080/00498254.2019.1643515

Phipson, B., Lee, S., Majewski, I. J., Alexander, W. S., & Smyth, G. K. (2016). Robust hyperparameter estimation protects against hypervariable genes and improves power to detect differential expression. *The Annals of Applied Statistics*, *10*(2). https://doi.org/10.1214/16-aoas920

Plourde, M., Vohl, M.-C., Bellis, C., Carless, M., Dyer, T., Dolley, G., Marette, A., Després, J.-P., Bouchard, C., Blangero, J., & Pérusse, L. (2013). A variant in the *LRRFIP1* gene is associated with adiposity and inflammation: A Variant in the *LRRFIP1* Gene. *Obesity*, *21*(1), 185–192. https://doi.org/10.1002/oby.20242

Pulit, S. L., Stoneman, C., Morris, A. P., Wood, A. R., Glastonbury, C. A., Tyrrell, J., Yengo, L., Ferreira, T., Marouli, E., Ji, Y., Yang, J., Jones, S., Beaumont, R., Croteau-Chonka, D. C., Winkler, T. W., GIANT Consortium, Hattersley, A. T., Loos, R. J. F., Hirschhorn, J. N., … Lindgren, C. M. (2019). Meta-analysis of genome-wide association studies for body fat distribution in 694 649 individuals of European ancestry. *Human Molecular Genetics*, *28*(1), 166–174. https://doi.org/10.1093/hmg/ddy327

Que, E., James, K. L., Coffey, A. R., Smallwood, T. L., Albright, J., Huda, M. N., Pomp, D., Sethupathy, P., & Bennett, B. J. (2020). Genetic Architecture Modulates Diet-Induced Hepatic mRNA and miRNA Expression Profiles in Diversity Outbred Mice. *Genetics*, *216*(1). https://doi.org/10.1534/genetics.120.303481

R Core Team. (2019). *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. https://www.R-project.org/

Rask-Andersen, M., Karlsson, T., Ek, W. E., & Johansson, Å. (2019). Genome-wide association study of body fat distribution identifies adiposity loci and sex-specific genetic effects. *Nature Communications*, *10*(1), 339. https://doi.org/10.1038/s41467-018-08000-4

Ritchie, M. E., Phipson, B., Wu, D., Hu, Y., Law, C. W., Shi, W., & Smyth, G. K. (2015). Limma powers differential expression analyses for RNA-sequencing and microarray studies. *Nucleic Acids Research*, *43*(7). https://doi.org/10.1093/nar/gkv007

Roeder, R. G. (2019). 50+ years of eukaryotic transcription: An expanding universe of factors and mechanisms. *Nature Structural & Molecular Biology*, *26*(9). https://doi.org/10.1038/s41594-019-0287-x

Rui, L. (2014). Energy Metabolism in the Liver. In R. Terjung (Ed.), *Comprehensive Physiology* (pp. 177–197). John Wiley & Sons, Inc. https://doi.org/10.1002/cphy.c130024

Schmidt, S. L., Harmon, K. A., Sharp, T. A., Kealey, E. H., & Bessesen, D. H. (2012). The Effects of Overfeeding on Spontaneous Physical Activity in Obesity Prone and Obesity Resistant Humans. *Obesity*, *20*(11). https://doi.org/10.1038/oby.2012.103

Sherva, R., Rice, J. P., Neuman, R. J., Rochberg, N., Saccone, N. L., & Bierut, L. J. (2009). Associations and interactions between SNPs in the alcohol metabolizing genes and alcoholism phenotypes in European Americans. *Alcoholism, Clinical and Experimental Research*, *33*(5), 848–857. https://doi.org/10.1111/j.1530-0277.2009.00904.x

Shih, D. M., Wang, Z., Lee, R., Meng, Y., Che, N., Charugundla, S., Qi, H., Wu, J., Pan, C., Brown, J. M., Vallim, T., Bennett, B. J., Graham, M., Hazen, S. L., & Lusis, A. J. (2015). Flavin containing monooxygenase 3 exerts broad effects on glucose and lipid metabolism and atherosclerosis. *Journal of Lipid Research*, *56*(1), 22–37. https://doi.org/10.1194/jlr.M051680

Shorter, J. R., Najarian, M. L., Bell, T. A., Blanchard, M., Ferris, M. T., Hock, P., Kashfeen, A., Kirchoff, K. E., Linnertz, C. L., Sigmon, J. S., Miller, D. R., McMillan, L., & Pardo-Manuel de Villena, F. (2019). Whole Genome Sequencing and Progress Toward Full Inbreeding of the Mouse Collaborative Cross Population. *G3, Genes, Genomes, Genetics*, *9*(5). https://doi.org/10.1534/g3.119.400039

Silva, J. P., & van Booven, D. (2018). Analysis of diet-induced differential methylation, expression, and interactions of lncRNA and protein-coding genes in mouse liver. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-29993-4

Sims, E. A. H. (1976). Experimental obesity, dietary-induced thermogenesis, and their clinical implications. *Clinics in Endocrinology and Metabolism*, *5*(2). https://doi.org/10.1016/s0300-595x(76)80027-8

Singh, R. K., Kumar, P., & Mahalingam, K. (2017). Molecular genetics of human obesity: A comprehensive review. *Comptes Rendus Biologies*, *340*(2). https://doi.org/10.1016/j.crvi.2016.11.007

Speakman, J. R. (2018). Obesity and thermoregulation. *Handbook of Clinical Neurology*, *Jun*(1;10(6):679-689). https://doi.org/10.1016/b978-0-444-63912-7.00026-6

Srivastava, A., Morgan, A. P., Najarian, M. L., Sarsani, V. K., Sigmon, J. S., Shorter, J. R., Kashfeen, A., McMullan, R. C., Williams, L. H., Giusti-Rodríguez, P., Ferris, M. T., Sullivan, P., Hock, P., Miller, D. R., Bell, T. A., McMillan, L., Churchill, G. A., & de Villena, F. P.-M. (2017). Genomes of the Mouse Collaborative Cross. *Genetics*, *206*(2). https://doi.org/10.1534/genetics.116.198838

Swanzey, E., O'Connor, C., & Reinholdt, L. G. (2021). Mouse Genetic Reference Populations: Cellular Platforms for Integrative Systems Genetics. *Trends in Genetics*, *37*(3), 251–265. https://doi.org/10.1016/j.tig.2020.09.007

Tachmazidou, I., Süveges, D., Min, J. L., Ritchie, G. R. S., Steinberg, J., Walter, K., Iotchkova, V., Schwartzentruber, J., Huang, J., Memari, Y., McCarthy, S., Crawford, A. A., Bombieri, C., Cocca, M., Farmaki, A.-E., Gaunt, T. R., Jousilahti, P., Kooijman, M. N., Lehne, B., … Zeggini, E. (2017). Whole-Genome Sequencing Coupled to Imputation Discovers Genetic Signals for Anthropometric Traits. *The American Journal of Human Genetics*, *100*(6), 865–884. https://doi.org/10.1016/j.ajhg.2017.04.014

The LifeLines Cohort Study, The ADIPOGen Consortium, The AGEN-BMI Working Group, The CARDIOGRAMplusC4D Consortium, The CKDGen Consortium, The GLGC, The ICBP, The MAGIC Investigators, The MuTHER Consortium, The MIGen Consortium, The PAGE Consortium, The ReproGen Consortium, The GENIE Consortium, The International Endogene Consortium, Locke, A. E., Kahali, B., Berndt, S. I., Justice, A. E., Pers, T. H., … Speliotes, E. K.

(2015). Genetic studies of body mass index yield new insights for obesity biology. *Nature*, *518*(7538), 197–206. https://doi.org/10.1038/nature14177
Threadgill, D. W., & Churchill, G. A. (2012). Ten Years of the Collaborative Cross. *Genetics*, *190*(2). https://doi.org/10.1534/genetics.111.138032
Timper, K., & Brüning, J. C. (2017). Hypothalamic circuits regulating appetite and energy homeostasis: Pathways to obesity. *Disease Models & Mechanisms*, *10*(6). https://doi.org/10.1242/dmm.026609
Tong, L. (2005). Acetyl-coenzyme A carboxylase: Crucial metabolic enzyme and attractive target for drug discovery. *Cellular and Molecular Life Sciences: CMLS*, *62*(16), 1784–1803. https://doi.org/10.1007/s00018-005-5121-4
Torres-Fuentes, C., Schellekens, H., Dinan, T. G., & Cryan, J. F. (2017). The microbiota–gut–brain axis in obesity. *The Lancet Gastroenterology & Hepatology*, *2*(10). https://doi.org/10.1016/s2468-1253(17)30147-4
Treacy, E. (1998). Mutations of the flavin-containing monooxygenase gene (FMO3) cause trimethylaminuria, a defect in detoxication. *Human Molecular Genetics*, *7*(5), 839–845. https://doi.org/10.1093/hmg/7.5.839
Trefts, E., Gannon, M., & Wasserman, D. H. (2017). The liver. *Current Biology*, *27*(21). https://doi.org/10.1016/j.cub.2017.09.019
Wang, H., Zhang, F., Zeng, J., Wu, Y., Kemper, K. E., Xue, A., Zhang, M., Powell, J. E., Goddard, M. E., Wray, N. R., Visscher, P. M., McRae, A. F., & Yang, J. (2019). Genotype-by-environment interactions inferred from genetic effects on phenotypic variability in the UK Biobank. *Science Advances*, *5*(8), eaaw3538. https://doi.org/10.1126/sciadv.aaw3538
Wang, Z., Levison, B. S., Hazen, J. E., Donahue, L., Li, X.-M., & Hazen, S. L. (2014). Measurement of trimethylamine-N-oxide by stable isotope dilution liquid chromatography tandem mass spectrometry. *Analytical Biochemistry*, *455*. https://doi.org/10.1016/j.ab.2014.03.016
Warrier, M., Shih, D. M., Burrows, A. C., Ferguson, D., Gromovsky, A. D., Brown, A. L., Marshall, S., McDaniel, A., Schugar, R. C., Wang, Z., Sacks, J., Rong, X., Vallim, T. de A., Chou, J., Ivanova, P. T., Myers, D. S., Brown, H. A., Lee, R. G., Crooke, R. M., … Brown, J. M. (2015). The TMAO-Generating Enzyme Flavin Monooxygenase 3 Is a Central Regulator of Cholesterol Balance. *Cell Reports*, *10*(3), 326–338. https://doi.org/10.1016/j.celrep.2014.12.036
Williams, E. P., Mesidor, M., Winters, K., Dubbert, P. M., & Wyatt, S. B. (2015). Overweight and Obesity: Prevalence, Consequences, and Causes of a Growing Public Health Problem. *Current Obesity Reports*, *4*(3). https://doi.org/10.1007/s13679-015-0169-4
Winkler, T. W., Justice, A. E., Graff, M., Barata, L., Feitosa, M. F., Chu, S., Czajkowski, J., Esko, T., Fall, T., Kilpeläinen, T. O., Lu, Y., Mägi, R., Mihailov, E., Pers, T. H., Rüeger, S., Teumer, A., Ehret, G. B., Ferreira, T., Heard-Costa, N. L., … Loos, R. J. F. (2015). The Influence of Age and Sex on Genetic Associations with Adult Body Size and Shape: A Large-Scale Genome-Wide Interaction Study. *PLOS Genetics*, *11*(10), e1005378. https://doi.org/10.1371/journal.pgen.1005378
Xu, N., & Dahlbäck, B. (1999). A Novel Human Apolipoprotein (apoM). *Journal of Biological Chemistry*, *274*(44), 31286–31290. https://doi.org/10.1074/jbc.274.44.31286
Yam, P., Albright, J., VerHague, M., Gertz, E. R., Pardo-Manuel de Villena, F., & Bennett, B. J. (2021). Genetic Background Shapes Phenotypic Response to Diet for Adiposity in the Collaborative Cross. *Frontiers in Genetics*, *11*, 1772. https://doi.org/10.3389/fgene.2020.615012
Yamaguchi, A., Hori, O., Stern, D. M., Hartmann, E., Ogawa, S., & Tohyama, M. (1999). Stress-Associated Endoplasmic Reticulum Protein 1 (Serp1)/Ribosome-Associated Membrane Protein 4 (Ramp4) Stabilizes Membrane Proteins during Stress and Facilitates Subsequent Glycosylation. *Journal of Cell Biology*, *147*(6), 1195–1204. https://doi.org/10.1083/jcb.147.6.1195
Yamauchi, T., Kamon, J., Ito, Y., Tsuchida, A., Yokomizo, T., Kita, S., Sugiyama, T., Miyagishi, M., Hara, K., Tsunoda, M., Murakami, K., Ohteki, T., Uchida, S., Takekawa, S., Waki, H., Tsuno, N. H., Shibata, Y., Terauchi, Y., Froguel, P., … Kadowaki, T. (2003). Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. *Nature*, *423*(6941), 762–769. https://doi.org/10.1038/nature01705
Yilmaz, E. (2017). Endoplasmic Reticulum Stress and Obesity. In A. B. Engin & A. Engin (Eds.), *Obesity and Lipotoxicity* (Vol. 960, pp. 261–276). Springer International Publishing. https://doi.org/10.1007/978-3-319-48382-5_11

Zhang, B., & Horvath, S. (2005). A General Framework for Weighted Gene Co-Expression Network Analysis. *Statistical Applications in Genetics and Molecular Biology*, *4*(1). https://doi.org/10.2202/1544-6115.1128

Zhu, Z., Guo, Y., Shi, H., Liu, C.-L., Panganiban, R. A., Chung, W., O'Connor, L. J., Himes, B. E., Gazal, S., Hasegawa, K., Camargo, C. A., Qi, L., Moffatt, M. F., Hu, F. B., Lu, Q., Cookson, W. O. C., & Liang, L. (2020). Shared genetic and experimental links between obesity-related traits and asthma subtypes in UK Biobank. *Journal of Allergy and Clinical Immunology*, *145*(2), 537–549. https://doi.org/10.1016/j.jaci.2019.09.035

Figure 2-1. Top 30 genes most significantly correlated with body fat % and their correlation with other phenotypes

Multiple biweight midcorrelations (bicor) and their corresponding Student correlation p-values were calculated between phenotypic and microarray liver gene expression data to properly take into account the actual number of observations when determining which genes' expression levels were correlated with post-diet phenotypes of interest. The top 15 genes whose expression are most significantly positively correlated with body fat % (bicor > 0.410, $p < 2.53 \times 10^{-6}$) and top 15 genes whose expression are most significantly negatively correlated with body fat % (bicor < -0.466, $p < 5.42 \times 10^{-8}$) are shown. With the exception of insulin and insulin-derived measures, most of the top 30 genes' expression most significantly correlated with body fat % were not significantly correlated with circulating analytes, but were significantly correlated with metabolic (energy regulation) traits. Genes are ordered on the y axis in descending order of bicor with the strongest positive correlation at the top and the strongest negative correlation at the bottom. Scale indicates bicor value with color darkness as indicator of correlation strength. † indicates genes that are also differentially expressed by diet; all 30 genes were found to be differentially expressed by strain. * indicate genes with human homologs found in the GWAS catalog to be associated with at least one obesity-related trait.

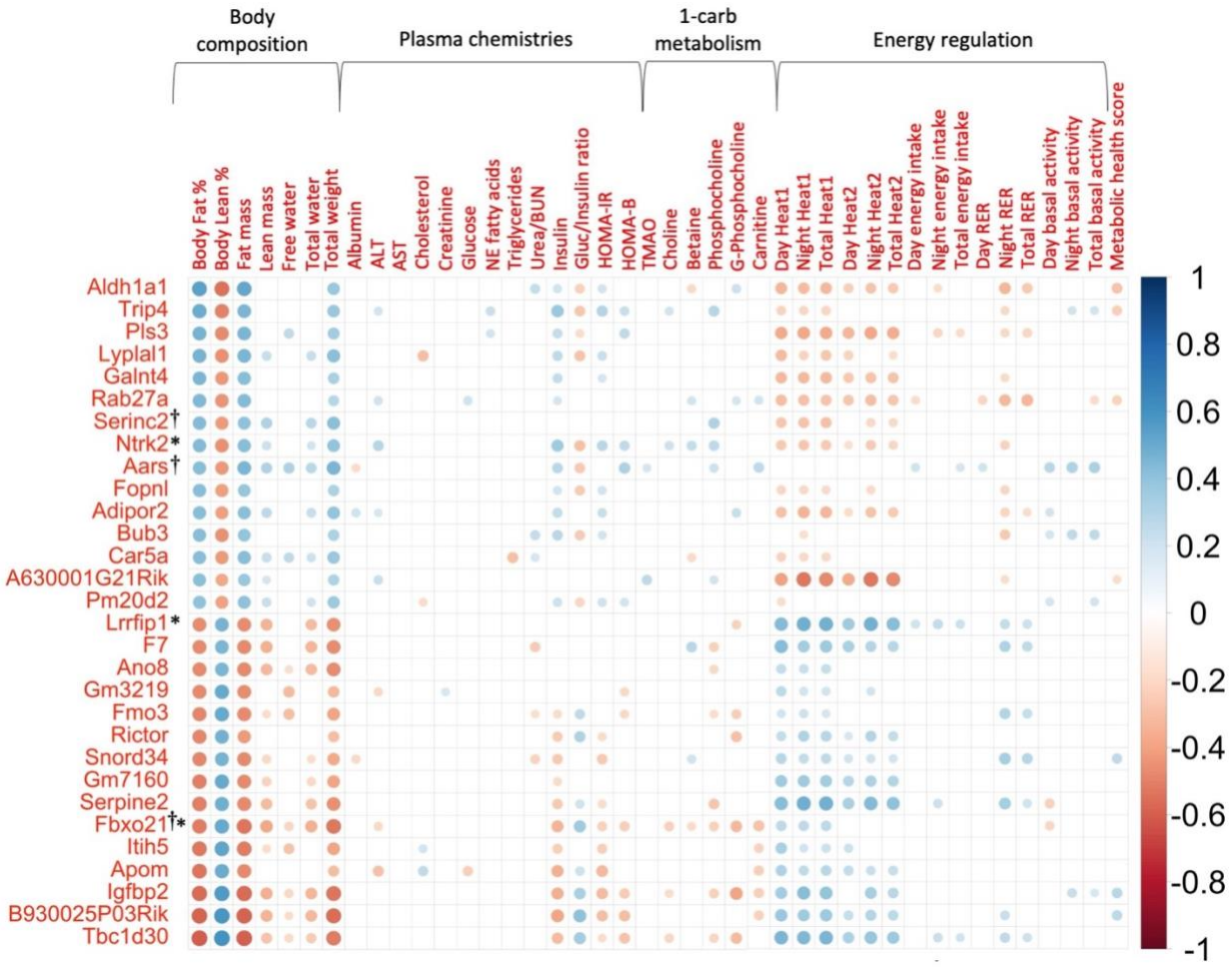


Figure 2-2. Top differentially expressed genes by diet show expression patterns more similar by diet than CC strain

(A) Linear Models for Microarray Data (limma) analysis of microarray data revealed 1344 genes differentially expressed (DE) by diet in the liver shown in the volcano plot, with a positive log fold change value indicating increased expression in mice fed the HP diet relative to mice fed the HS diet. Extremely significant genes or significant genes with > |1| log fold change of expression by diet are highlighted. (B) The top 20 most significant (BH-adjusted $p < 2.37 \times 10^{-8}$) diet DE genes' average Z scores of median Robust Multi-array Average normalized (RMA) gene expression for each CC strain on either the high protein (HP) or high fat high sucrose (HS) diet shown ordered from top to bottom by level of gene expression on the HP diet (highest to lowest). The genes' average Z scores for each CC strain and diet are clustered by Euclidean distance on the x axis. ‡ denotes genes also differentially expressed by strain. * indicate genes with human homologs found in the GWAS catalog to be associated with at least one obesity-related trait.

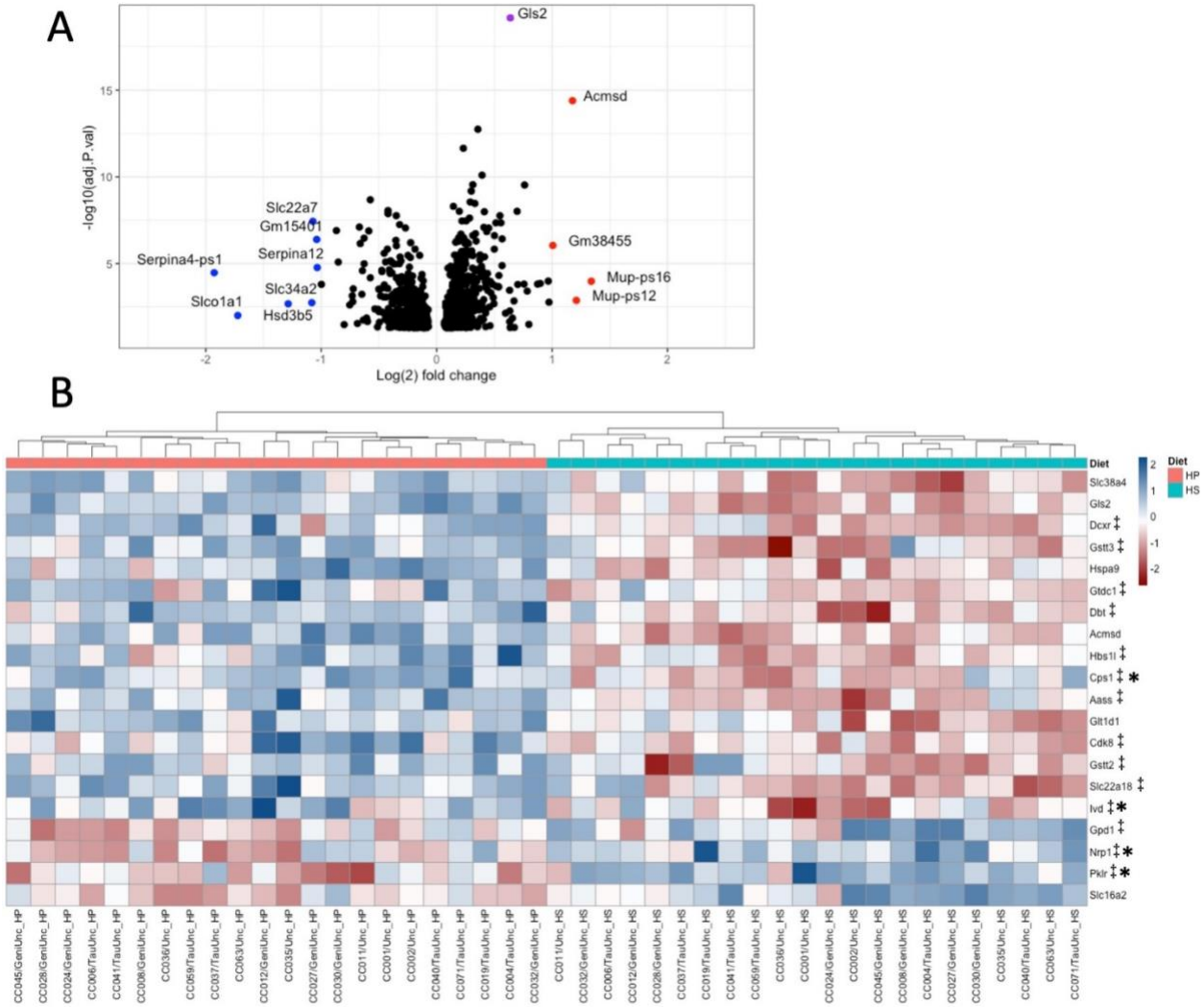


Figure 2-3. Top 10 enriched KEGG and gene ontology (GO) pathways by diet are related to metabolism and energy regulation

Limma analysis of microarray data revealed genes differentially expressed by diet showing significant enrichment (p adj < 0.05) for (A) KEGG (20 total), (B) GO biological pathways (105 total), (C) GO cellular components for upregulated genes (45 total), and (D) GO molecular functions (37 total). Pathways are ordered from top to bottom by significance (highest to lowest) and colored by gene richness. The top 10 enrichments for each ontology category were all upregulated on the HP diet, except for the GO Cellular Component “integral component of membrane”, which was downregulated.

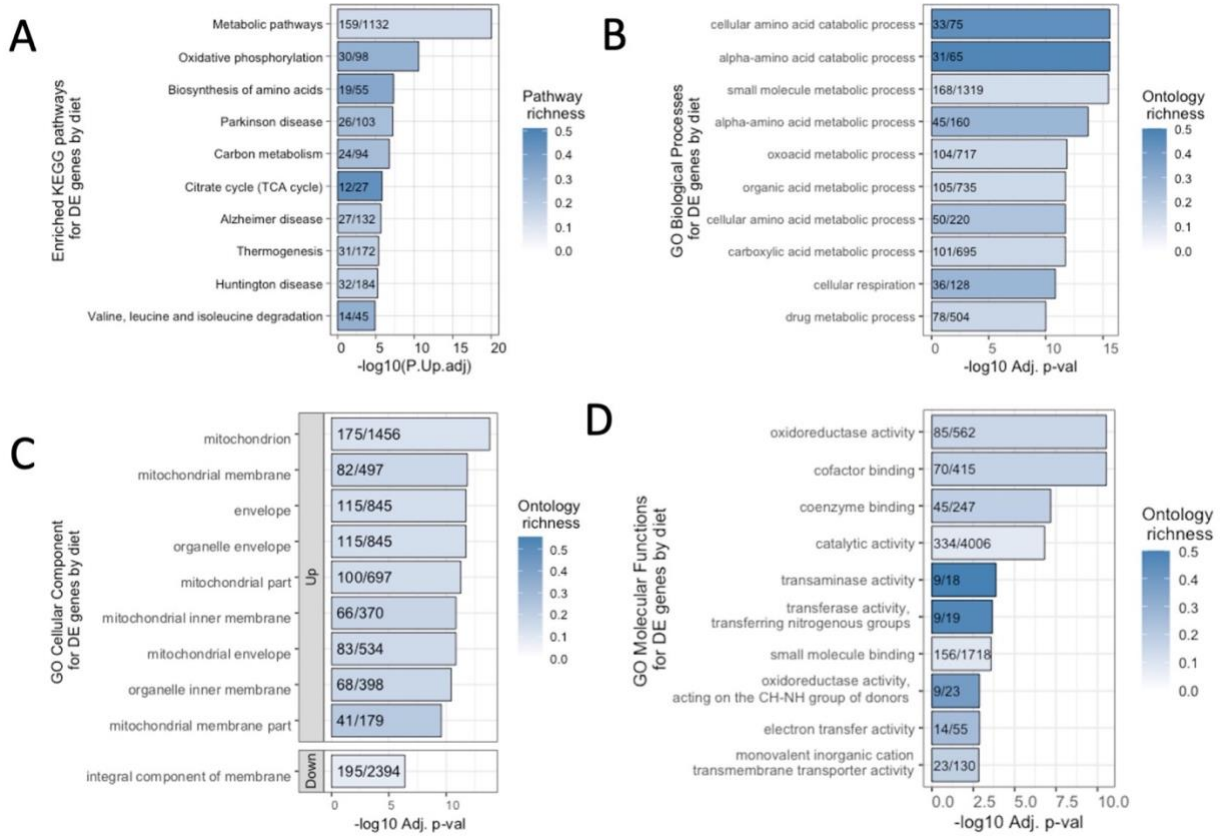


Figure 2-4. Top 20 differentially expressed genes by CC strain clearly demonstrate expression patterns preferentially according to CC Strain than diet
(A) Linear Models for Microarray Data (limma) analysis of microarray data revealed 9436 genes differentially expressed by CC strain in the liver whose Robust Multi-array Average normalized (RMA) expression Z scores are shown, where a higher proportion of significant genes have higher expression. Genes with > |1| standard deviation of expression levels are highlighted. (B) The top 20 most significant (BH-adjusted $p < 9.42 \times 10^{-55}$) strain DE genes' average Z scores of median Robust Multi-array Average normalized (RMA) gene expression for each CC strain on either the high protein (HP) or high fat high sucrose (HS) diet shown. Gene average RMA z scores for each CC strain and diet are clustered according to Euclidean distance by CC strain and diet on the x axis and by gene on the y axis. * indicates genes that have homologous genes in humans found in GWAS catalog to be associated with trait or disease.

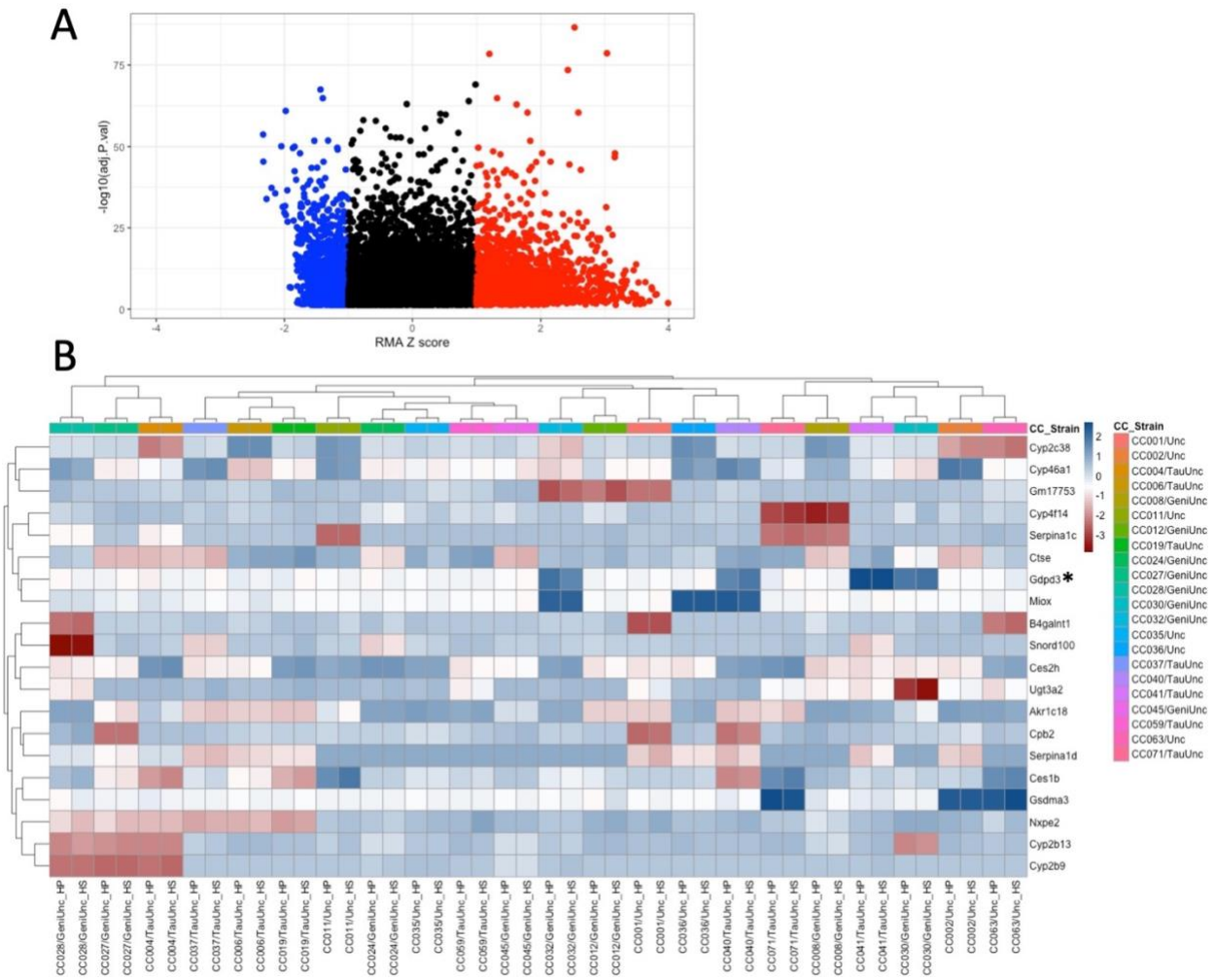


Figure 2-5. Top 10 enriched KEGG and gene ontology (GO) pathways by CC strain are related to general cellular functions

Limma analysis of microarray data revealed genes differentially expressed by strain showing significant enrichment (p adj < 0.05) for (A) KEGG (13 total), (B) GO biological pathways (95 total), (C) GO cellular components for upregulated genes (44 total), and (D) GO molecular functions (24 total). Pathways are ordered from top to bottom by significance (highest to lowest) and colored by gene richness. The top 10 enrichments for each ontology category were all upregulated on the HP diet, except for the linoleic acid metabolism KEGG pathway, GO Molecular Functions “monooxygenase activity” and “oxidoreductase activity, acting on paired donors…”, which were downregulated.

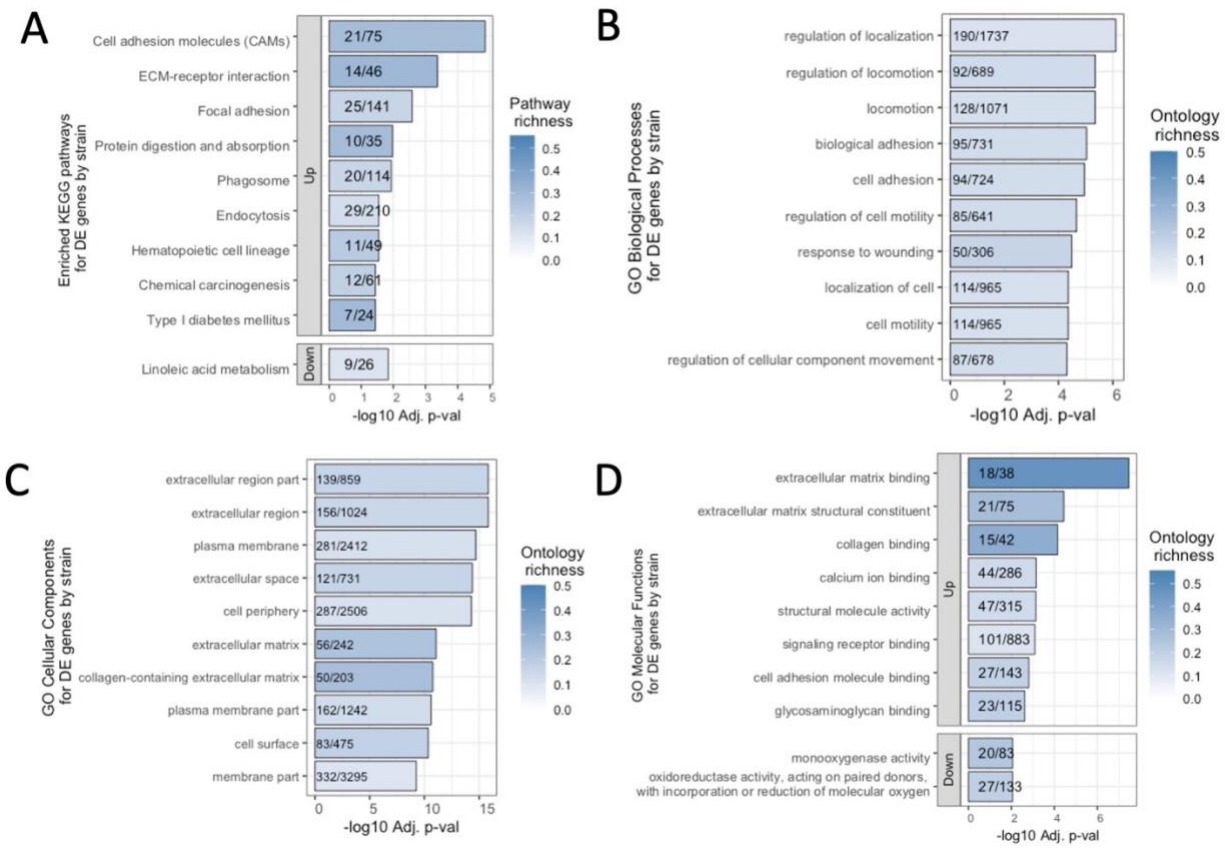


Figure 2-6. Differentially expressed genes in the CC have human homologs identified to be associated with obesity-related traits in the GWAS catalog
Comparisons of differentially expressed genes, genes with expression levels significantly correlated with body fat % (BF%), and annotated mouse genes with human homologs associated with obesity-related traits in the GWAS catalog revealed (A) the number of genes differentially expressed by both diet and strain whose expression levels were significantly correlated with body fat % (350), (B) the number of genes differentially expressed by both diet and strain that had human homologs associated with obesity traits (186), (C) the number of genes differentially expressed by diet that also had expression levels significantly correlated with body fat % and human homologs associated with obesity traits (65), (D) the number of genes differentially expressed by CC strain that also had expression levels significantly correlated with body fat % and human homologs associated with obesity traits (431), and (E) the number of genes that fall under all four categories (62).

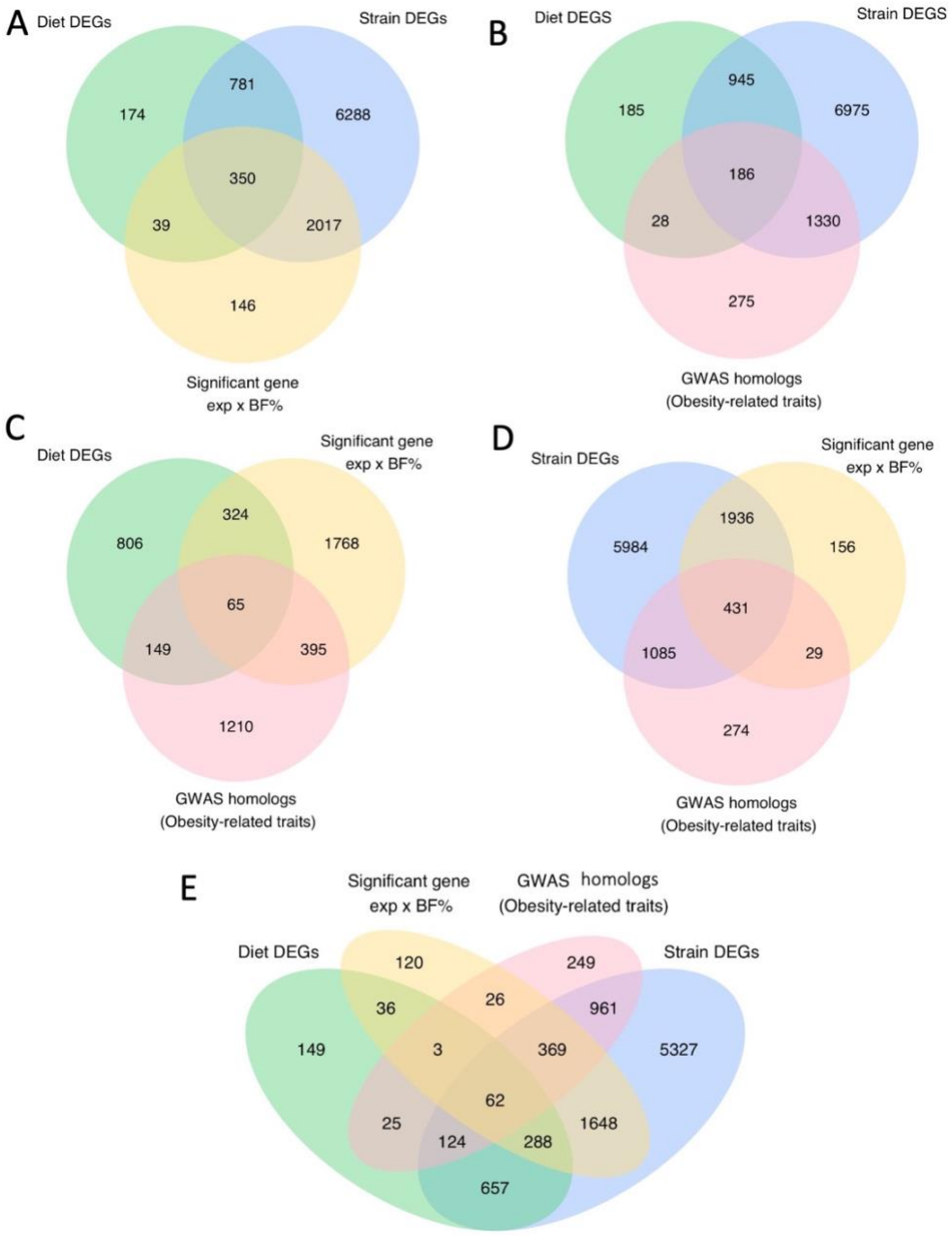
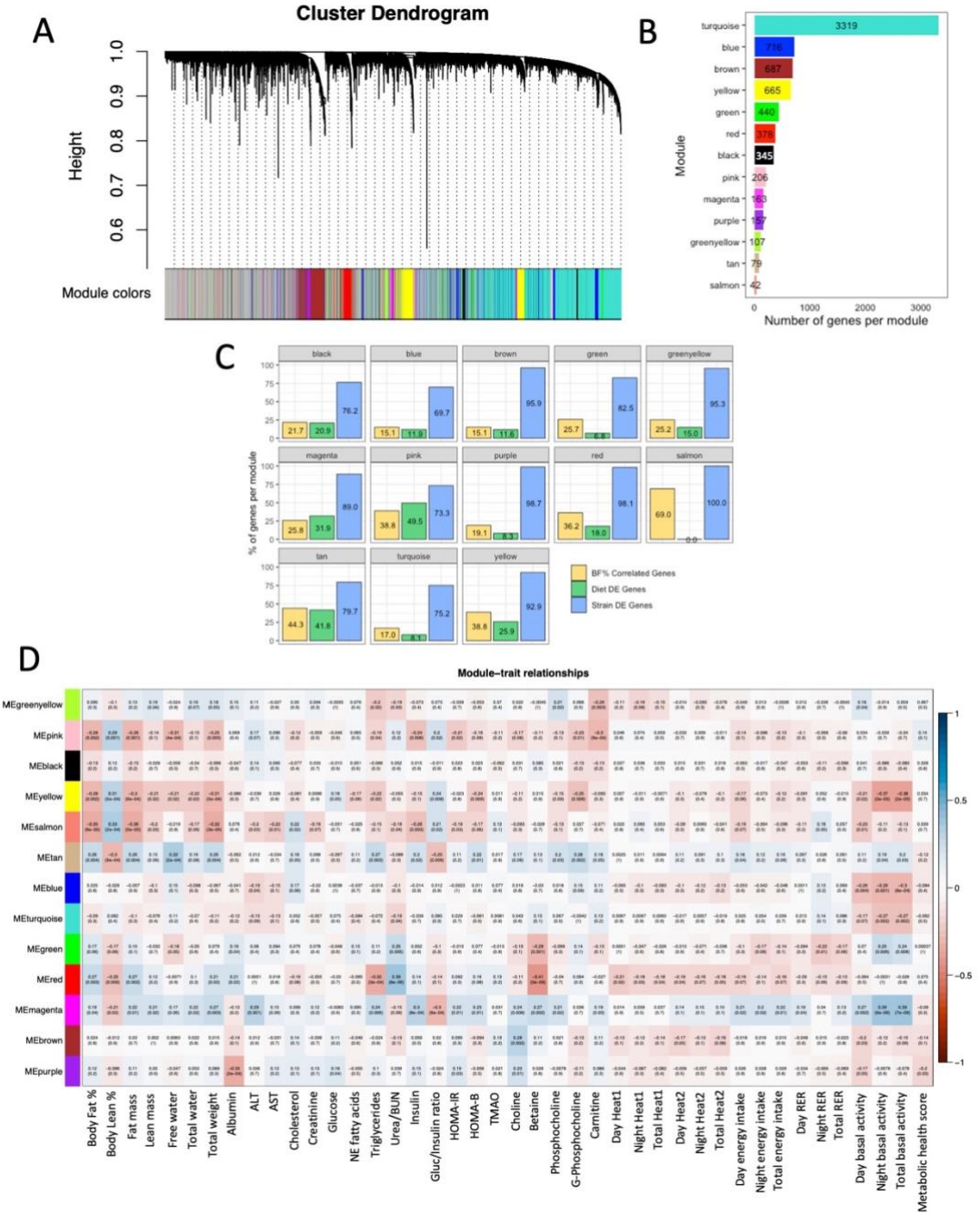


Figure 2-7. WGCNA identifies gene co-regulated modules correlated with phenotypic traits
Using the cleaned and filtered hepatic gene expression data from mice fed the HP diet and mice fed the HS diet, (A) WGCNA identified 13 modules with arbitrarily assigned colors. (B) The 11,542 genes from the limma analysis were used to form the modules, which varied widely in terms of the number genes within each module. (C) Modules demonstrated a wide compositional range in terms of genes with expression levels significantly with body fat % (BF%) (15.1 – 69.0%) and differential expression by diet (0 - 49.5%), but consistently contained a high proportion of genes differentially expressed by CC strain (69.7 – 100%). (D) The heatmap of Spearman's correlations between module eigengenes and phenotypic traits measured in the CC mice revealed significant correlations between the pink, yellow, salmon, tan, red, and magenta modules and BF%.



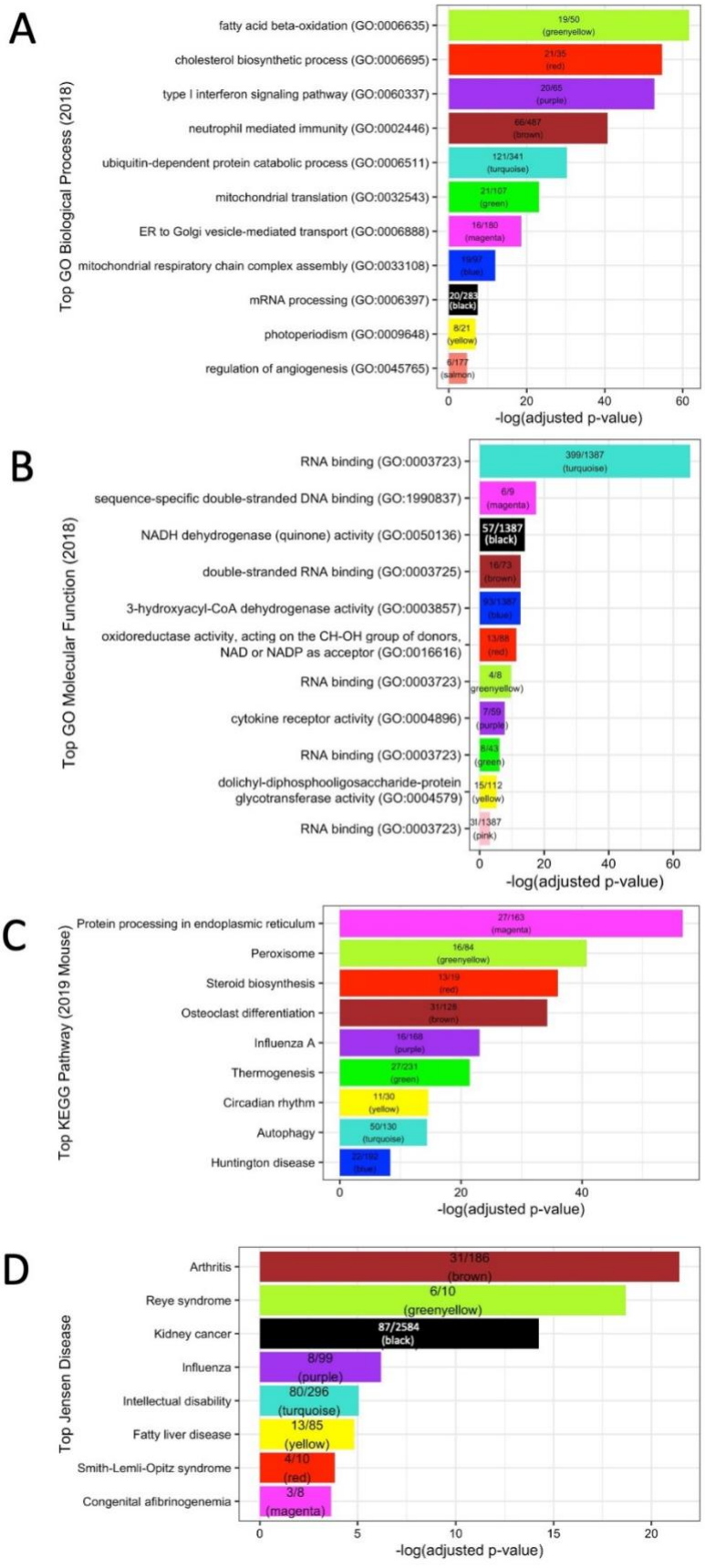


Figure 2-8. Modules showed significant enrichment for different types of functions and pathways both related and unrelated to energy regulation EnrichR analysis performed using the most recent versions of respective databases identified the top significant enrichment for each module, if available; genes belonging to the tan modules did not show any significant enrichment. (A) All modules showed significant enrichment for at least one GO Biological Process, except for the tan and pink modules. (B) Similarly, all modules showed significant enrichment for at least one GO Molecular Function, except for the tan and salmon modules. Fewer modules were enriched for (C) KEGG Pathways and (D) Jensen's Diseases.

Figure 2-9. Module eigengenes (PC1) for most modules significantly correlated with body fat % differ by diet

Most module eigengene (ME) average gene expression profiles significantly correlated with body fat % also significantly differed by diet to different degrees, as ascertained with Wilcoxon ranked sum tests. The MEs that significantly differed by diet were (A) pink ($p < 0.001$), (B) yellow ($p < 0.001$), (C) tan ($p < 0.001$), (D) red ($p < 0.01$), and (E) magenta ($p < 0.001$), but not (F) salmon ($p > 0.1$). Points indicate individual calculated ME expression for each mouse.

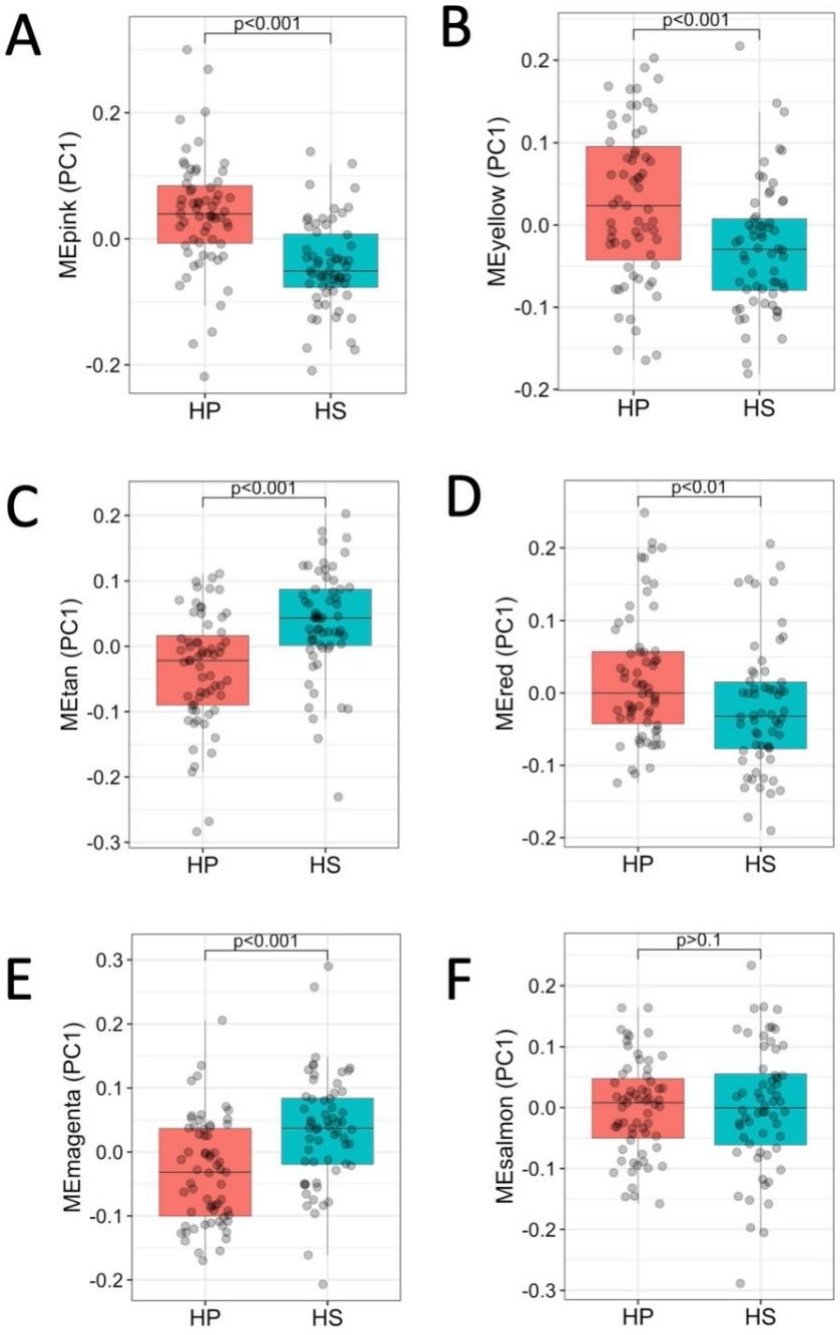


Figure 2-10. Module eigengenes (PC1) for most modules significantly correlated with body fat % differ by CC strain

Most module eigengene (ME) average gene expression profiles significantly correlated with body fat % also significantly differed by CC strain to different degrees, as ascertained with Kruskal-Wallis tests. The (A) pink and (C) tan MEs did not differ significantly by CC strain ($p > 0.07$), but the MEs for the (B) yellow ($p = 6.0 \times 10^{-4}$), (D) red ($p = 2.8 \times 10^{-9}$), (E) magenta ($p = 8.1 \times 10^{-3}$), and (F) salmon ($p = 1.3 \times 10^{-8}$) modules differed significantly by CC strain. Points indicate individual calculated ME expression for each mouse, and CC strains are ordered numerically.

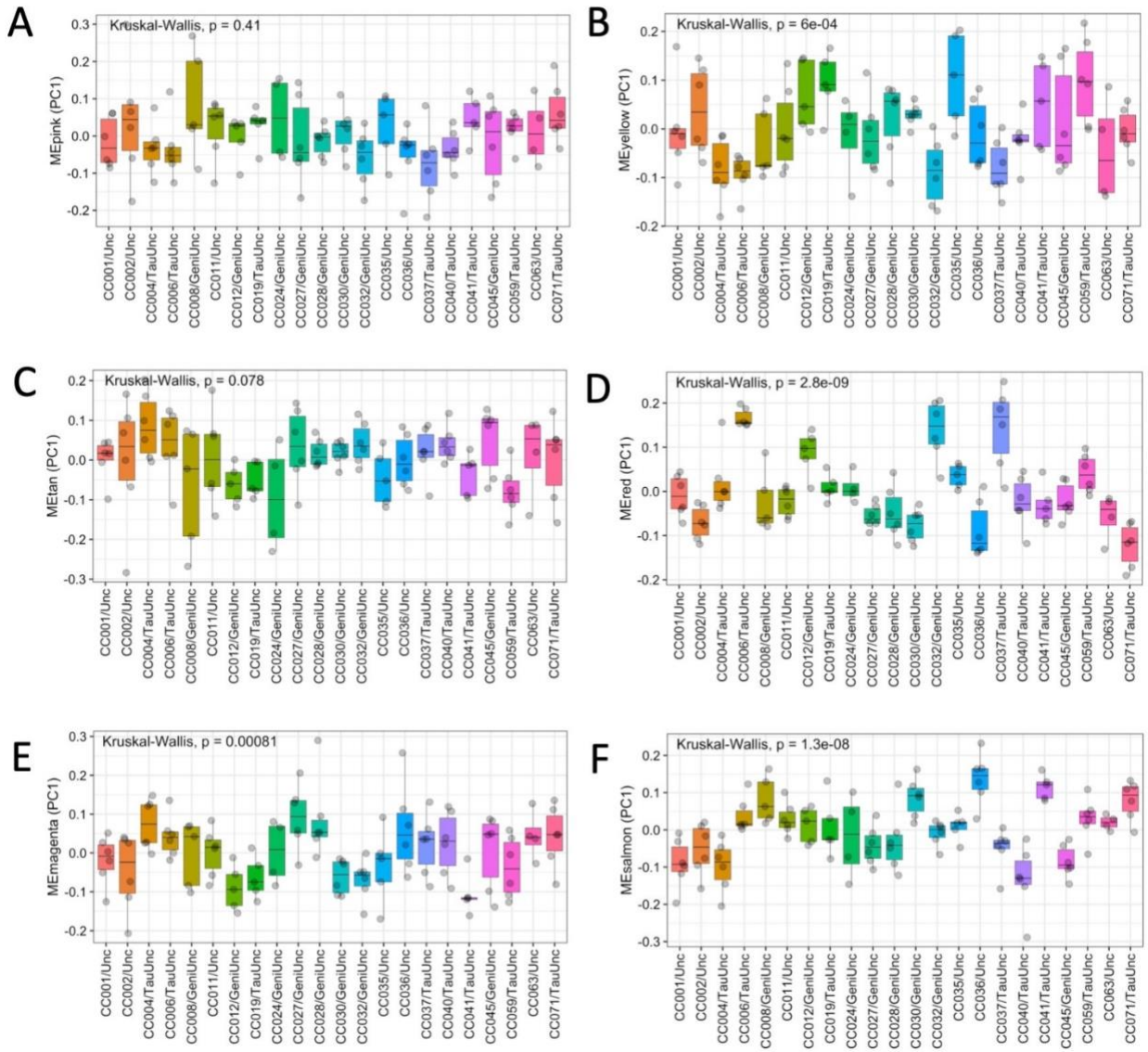


Figure 2-11. Significant correlations between MEs of biologically relevant modules and body fat %

Spearman's correlations between baseline body fat % and (A) yellow ME (PC1) (rho = -0.28, p = 0.0016), (B) magenta ME (PC1) (rho = 0.19, p = 0.037), and (C) red ME (PC1) (rho = 0.27, p = 0.0027) show significant overall associations between average expression profiles of modules identified by WGCNA and body fat %.

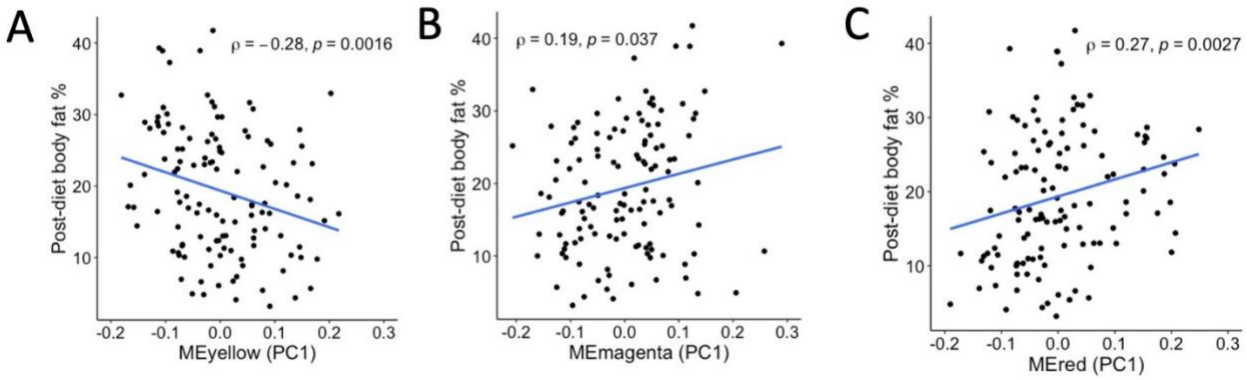


Table 2-1. Module gene composition

WCGNA identified 13 gene modules each assigned an arbitrary color with the number of genes contained in each module ranging from 42 (salmon) to 3,119 (turquoise). Each gene module showed variation in terms of the number of genes with expression significantly correlated with post-diet body fat (BF%), genes differentially expressed by diet, genes differentially expressed by strain, genes with human homologs in the GWAS catalog associated with a disease/trait, and genes with human homologs in the GWAS catalog associated specifically with obesity traits.

Module colors	BF% correlated genes	Diet DE genes	Strain DE genes	GWAS genes	GWAS obesity traits genes	Total genes
turquoise	564	268	2497	2476	719	3319
blue	108	85	499	493	104	716
brown	104	80	659	531	142	687
yellow	258	172	618	520	123	665
green	113	30	363	287	71	440
red	137	68	371	254	74	378
black	75	72	263	272	75	345
pink	80	102	151	156	43	206
magenta	42	52	145	121	27	163
purple	30	13	155	98	23	157
greenyellow	27	16	102	77	10	107
tan	35	33	63	61	20	79
salmon	29	0	42	40	11	42

Table 2-2. DEGs with expression levels correlated with body fat % in the CC associated with obesity in humans

By intersecting lists of genes across multiple analyses, 434 DEGs in the CC were found to have gene expression levels significantly correlated with body fat % and human homologs associated with obesity traits in the GWAS catalog, with 3 diet DEGs, 369 strain DEGs, and 62 genes differentially expressed by both diet and strain. The number of genes belonging to each category and assigned to the respective modules are shown above, with 148 genes not assigned to any module.

Module colors	Diet DEGs	Gene DE by Diet and Strain	Strain DEGs
turquoise	0	9	106
yellow	0	19	35
red	0	0	22
brown	0	1	15
green	0	1	15
black	1	2	13
blue	1	2	8
pink	1	6	7
purple	0	0	6
salmon	0	0	5
tan	0	3	5
greenyellow	0	1	1
magenta	0	0	1

Table 2-3. Distribution of significant enrichment terms across modules

In EnrichR analysis, genes assigned to each module were used to determine whether modules were significantly enriched for functional terms, pathways, or diseases (enrichment terms). Modules varied widely in terms of the number of enrichments for each category, from no enrichments at all (tan) to 419 total enrichments (brown).

	GO Biological Process 2018	GO Molecular Function 2018	Jensen DISEASES	KEGG 2019 Mouse	Total
brown	296	25	18	80	419
turquoise	289	55	1	49	394
greenyellow	25	11	18	16	70
red	37	2	3	24	66
purple	34	2	2	14	52
yellow	27	11	2	6	46
magenta	35	4	2	2	43
black	18	17	3	0	38
green	24	5	0	8	37
blue	22	3	0	8	33
salmon	2	0	0	0	2
pink	0	1	0	0	1
tan	0	0	0	0	0

Table 2-4. DEGs in the CC assigned to enriched modules with human homologs associated with obesity traits in the GWAS catalog

The number of DEGs for the magenta, red, and yellow modules identified by WGCNA illustrate the larger contribution of differential expression by strain over diet. Examples of genes with human homologs associated with obesity traits in the GWAS catalog are shown for each module, where * denotes genes that are significantly correlated with body fat % in the CC.

	Magenta module		**Red module**		**Yellow module**	
	Number of DEGs	Genes associated with obesity traits in humans	Number of DEGs	Genes associated with obesity traits in humans	Number of DEGs	Genes associated with obesity traits in humans
Diet DEGs	0	NA	0	NA	3	*Fars2, Mdfic, Abhd15*
Strain DEGs	16	*Macrod1*, Vegfb, Serp1*	47	*Fasn*, Acac*, Asrgl1*, Ppil1**	87	*Nicn1*, Pnpla7*, Syne3*, Clock**
DEGs by diet and strain	5	*Uggt1, Itih1, Serpina6*	12	*Spc24, Mipep, Cyb5b, Dlat*	30	*Fbxo21*, Brap*, Mgrn1**

Table 2-5. Summary statistics for broad sense heritability estimates and diet intraclass correlations for all limma genes, diet DEGs, and strain DEGs

Post-diet heritability estimates were calculated from linear models including strain, diet, and week as covariates (r_I or g^2 "full") for gene expression of the 11,542 genes used in limma differential gene expression analysis. Diet-specific estimations of broad sense heritability were also calculated accordingly for gene expression levels represented by intraclass correlations (r_I) and coefficients of genetic determination (g^2) for each trait using the MSB and MSW for strain derived from linear models with strain and week as covariates using only data from each experimental diet per model as indicated to assess how different diet "environments" affect heritability. The intraclass correlation for diet (Diet ICC), which is the proportion of the total phenotypic variation that is accounted for by differences between diet, was calculated to compare the proportion of variation in gene expression attributed to diet in general or genetics. Summary statistics were calculated for each group of genes after heritability estimates and diet ICC were obtained. g^2 accounts for the additive genetic variance that doubles during inbreeding and may be a more appropriate estimate for broad sense heritability in this study. However, both r_I and g^2 values are presented to facilitate comparisons with other findings in the literature.

Heritability estimate or diet ICC	Mean ± SE	Median (Q1, Q3)	Min	Max
rI full - All limma genes	0.327 ± 0.002	0.295 (0.157, 0.471)	-0.12	0.991
rI full - Diet DEGs	0.341 ± 0.005	0.327 (0.202, 0.471)	-0.091	0.848
rI full - Strain DEGs	0.387 ± 0.002	0.348 (0.232, 0.513)	0.087	0.991
rI HP - All limma genes	0.324 ± 0.002	0.305 (0.136, 0.498)	-0.332	0.99
rI HP - Diet DEGs	0.339 ± 0.006	0.34 (0.179, 0.497)	-0.288	0.899
rI HP - Strain DEGs	0.388 ± 0.002	0.367 (0.221, 0.545)	-0.194	0.99
rI HS - All limma genes	0.328 ± 0.002	0.313 (0.146, 0.498)	-0.359	0.993
rI HS - Diet DEGs	0.348 ± 0.006	0.345 (0.203, 0.5)	-0.264	0.887
rI HS - Strain DEGs	0.389 ± 0.002	0.372 (0.228, 0.539)	-0.234	0.993
g2 full - All limma genes	0.218 ± 0.002	0.173 (0.085, 0.308)	-0.056	0.983
g2 full - Diet DEGs	0.221 ± 0.004	0.195 (0.112, 0.308)	-0.044	0.735
g2 full - Strain DEGs	0.26 ± 0.002	0.211 (0.131, 0.345)	0.045	0.983
g2 HP - All limma genes	0.223 ± 0.002	0.18 (0.073, 0.331)	-0.142	0.98
g2 HP - Diet DEGs	0.226 ± 0.005	0.205 (0.098, 0.331)	-0.126	0.816
g2 HP - Strain DEGs	0.267 ± 0.002	0.224 (0.124, 0.374)	-0.089	0.98
g2 HS - All limma genes	0.224 ± 0.002	0.186 (0.079, 0.331)	-0.152	0.985
g2 HS - Diet DEGs	0.232 ± 0.005	0.208 (0.113, 0.333)	-0.116	0.797
g2 HS - Strain DEGs	0.267 ± 0.002	0.229 (0.129, 0.369)	-0.105	0.985
Diet ICC - All limma genes	0.055 ± 0.001	0.015 (-0.009, 0.079)	-0.017	0.799
Diet ICC - Diet DEGs	0.266 ± 0.003	0.235 (0.172, 0.327)	0.099	0.799
Diet ICC - Strain DEGs	0.061 ± 0.001	0.019 (-0.008, 0.089)	-0.017	0.787

Table 2-6. Estimating the average relative heritable gene expression variation attributed to CC strain, experimental diet, and CC strain x diet interaction

To estimate the relative heritable variation that can be attributed to genetics, environment (diet), and gene x environmental effects, linear mixed model analyses with strain, diet, and strain x diet interactions as all random effects were performed to quantify the proportions of variance attributed to each term relative to each other for the 11,542 genes used in limma differential gene expression analysis. The mean approximate values for proportion of variance for strain, diet, and interaction were calculated by dividing the variance for each term by the sum of the variance for all terms in the model and multiplied by 100.

	CC Strain		Diet		CC Strain x Diet	
	Variance	**Proportion of Variance (%)**	**Variance**	**Proportion of Variance (%)**	**Variance**	**Proportion of Variance (%)**
All limma genes	0.069	30.3	0.007	3.9	0.004	2.6
Diet DEGs	0.055	26.2	0.033	18.7	0.004	2.6
Strain DEGs	0.083	36	0.007	3.9	0.004	2.6

Figure S2-1. Significantly enriched pathways and ontologies for the magenta module
Genes in the magenta module were significantly enriched in 35 GO Biological Processes, four GO Molecular Functions, two Jensen Diseases, and two KEGG pathways in mice related to endoplasmic reticulum function. The top 10 most significantly enriched GO Biological Processes are shown. The number of genes present in the magenta module and total number of genes that belong to respective enriched pathways/ontologies are displayed in each bar.

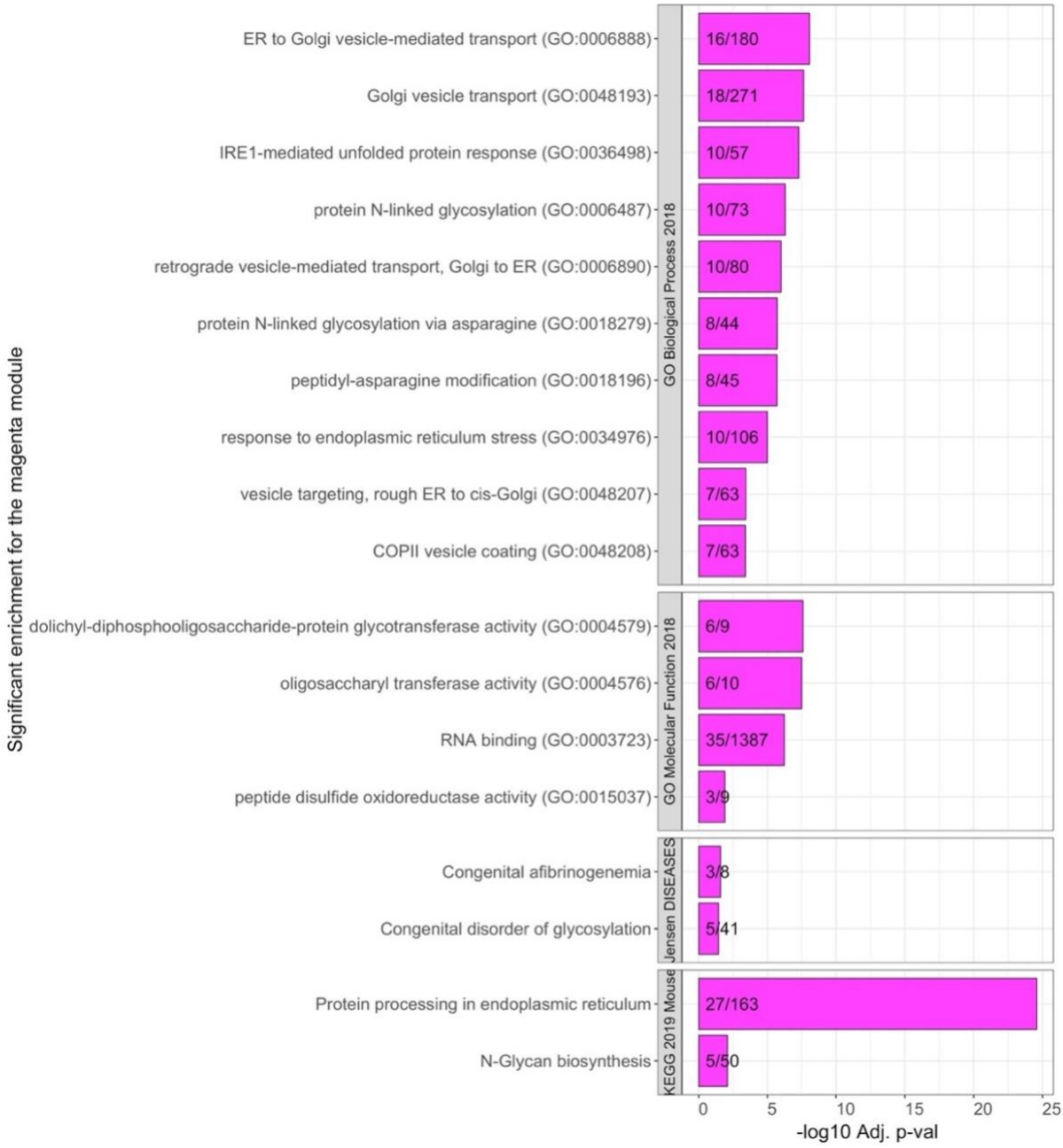


Figure S2-2. Significantly enriched pathways and ontologies for the red module
Genes in the red module were significantly enriched in 37 GO Biological Processes, two GO Molecular Functions (MF), three Jensen Diseases, and 24 KEGG pathways in mice related to steroid, cholesterol, and fatty acid biosynthesis/metabolism. The top 10 most significantly enriched GO Biological Processes and KEGG pathways are shown. The number of genes present in the red module and total number of genes that belong to respective enriched pathways/ontologies are displayed in each bar.

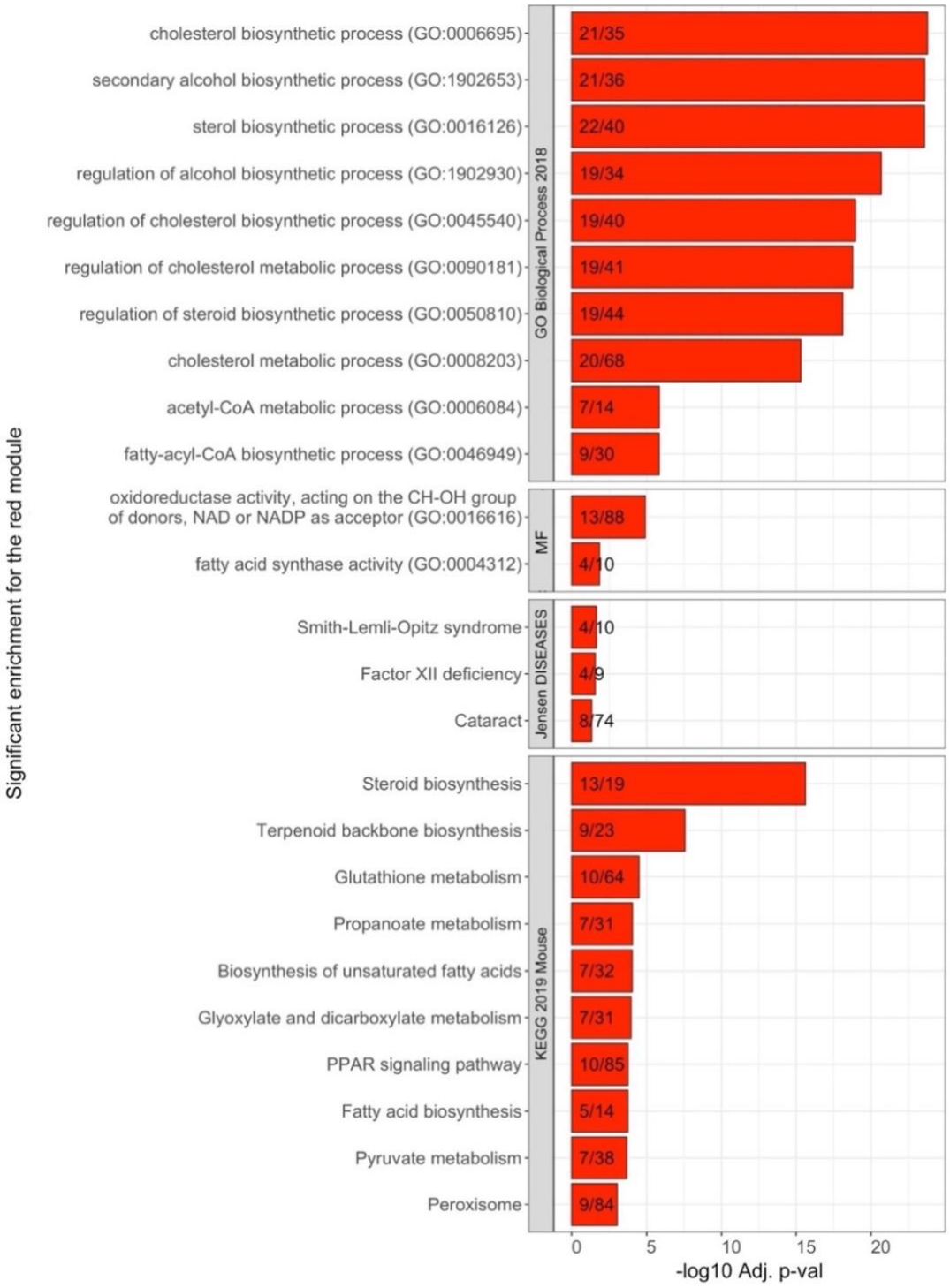


Figure S2-3. Significantly enriched pathways and ontologies for the yellow module
Genes in the yellow module were significantly enriched in 27 GO Biological Processes, 11 GO Molecular Functions, two Jensen Diseases (J's DISEASES), and six KEGG pathways in mice related to a variety of functions such as photoperiodism, transcription regulation, insulin signaling, etc. The top 10 most significantly enriched GO Biological Processes and Molecular Functions are shown. The number of genes present in the yellow module and total number of genes that belong to respective enriched pathways/ontologies are displayed in each bar.

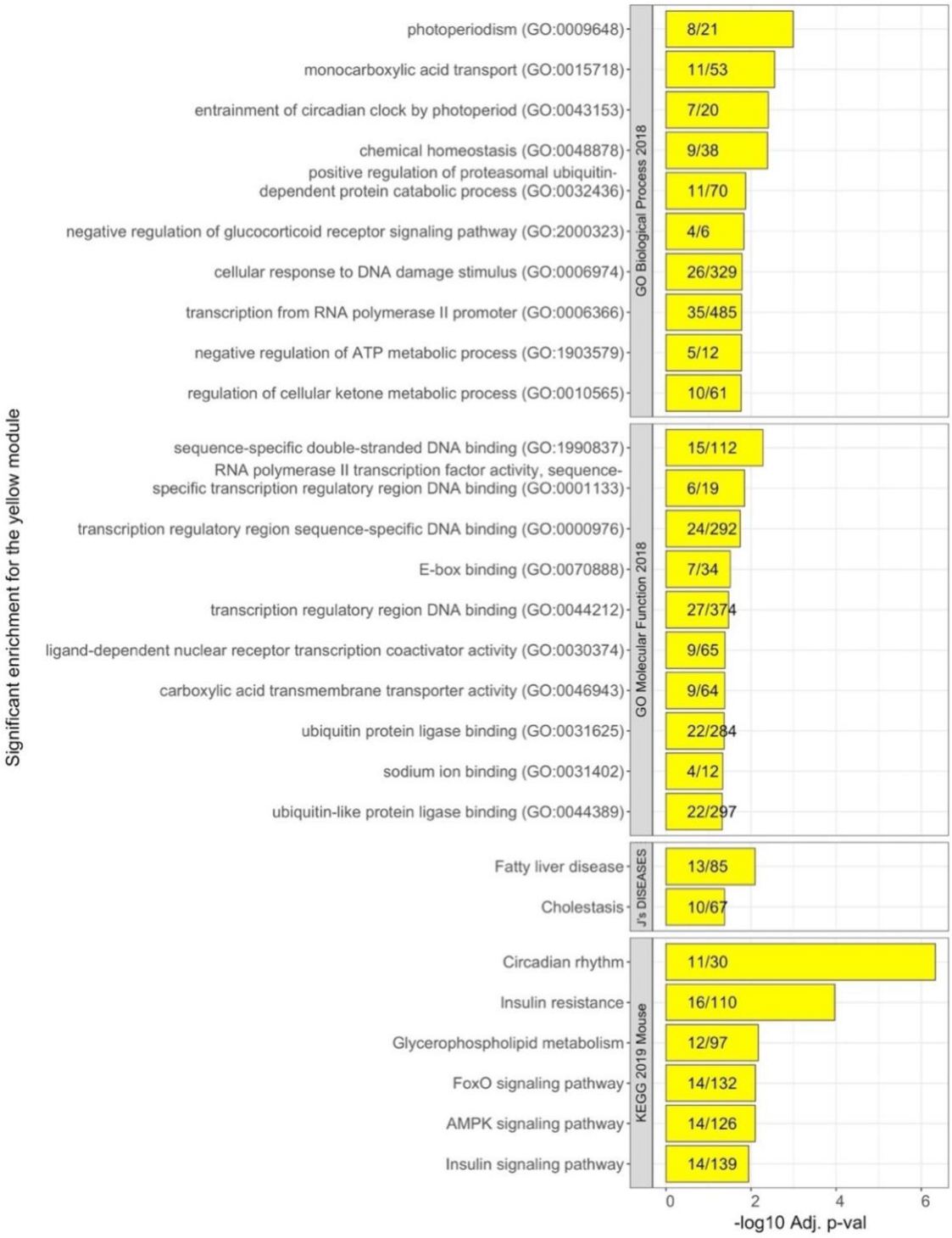


Figure S2-4. Significantly enriched pathways and ontologies for the brown module
Genes in the brown module were significantly enriched in 296 GO Biological Processes, 25 GO Molecular Functions, 18 Jensen Diseases, and 80 KEGG pathways in mice related to immune response; only the top 10 most significantly enriched pathways/ontologies are shown. The number of genes present in the brown module and total number of genes that belong to respective enriched pathways/ontologies are displayed in each bar.

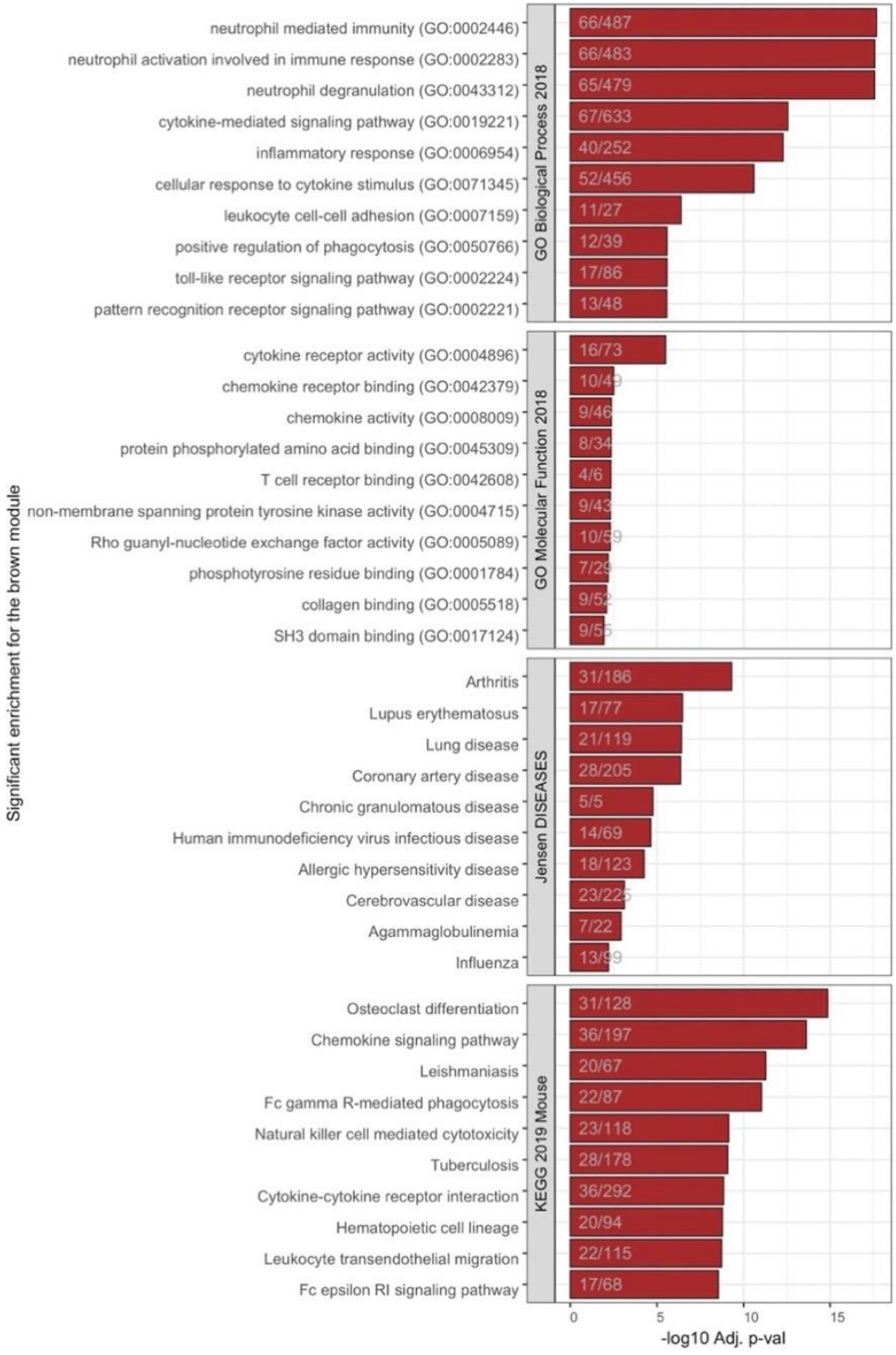


Figure S2-5. Correlations between the magenta, red, and yellow MEs with body fat % by CC strain
Spearman's correlations performed between the magenta, red, and yellow module eigengenes (PC1) and body fat % by CC strain suggest that the magnitude and direction of correlation in gene expression between these modules change depending on genetic background (CC strain).

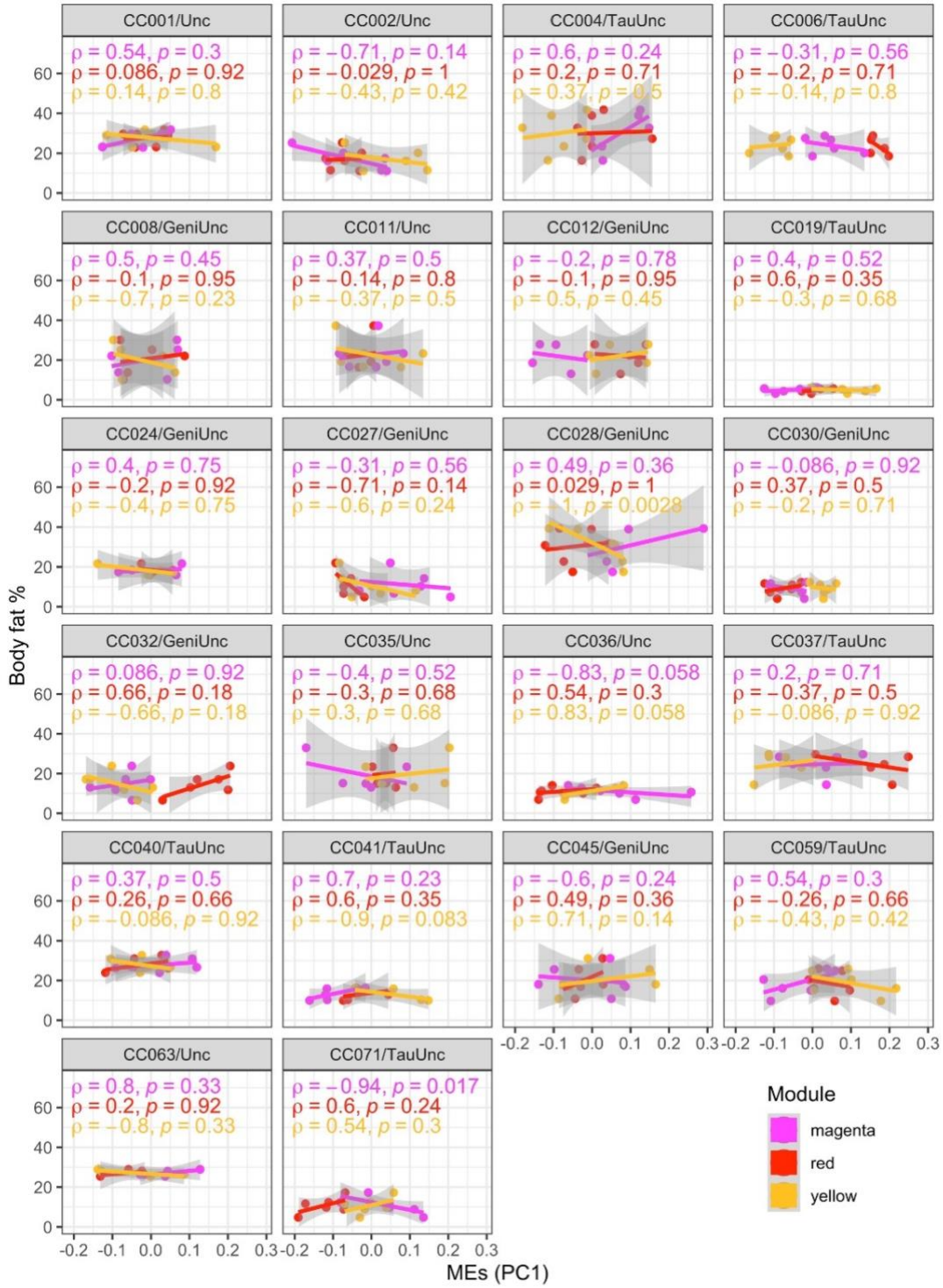


Figure S2-6. Correlations between the magenta, red, and yellow MEs with body fat % by diet
Spearman's correlations performed between the magenta, red, and yellow module eigengenes (PC1) and body fat % by diet suggest that the magnitude and direction of correlation in gene expression between these modules change depending on diet.

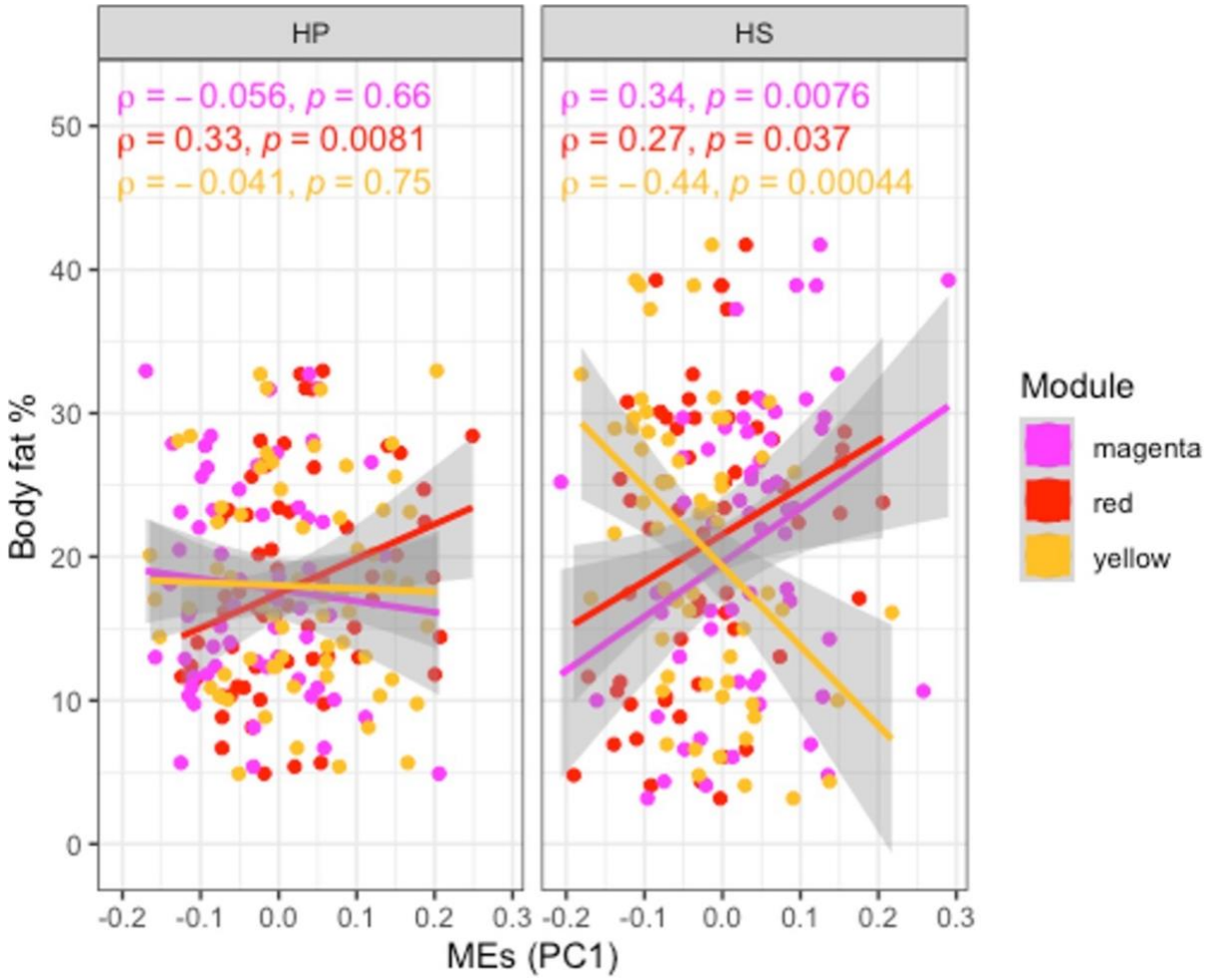


Figure S2-7. Gene module connectivity is negatively associated with gene expression H^2 and diet intraclass correlation

For the 11,542 genes used in WGCNA, Spearman's correlations were performed between log-transformed gene module connectivity and (A) coefficients of genetic determination calculated using MSB and MSW derived from "full" additive linear models (g2 full) (rho = -0.22, $p < 2.2 \times 10^{-16}$), (B) HP-diet coefficients of genetic determination (g2 HP) (rho = -0.24, $p < 2.2 \times 10^{-16}$), (C) HS-diet coefficients of genetic determination (g2 HS) (rho = -0.25, $p < 2.2 \times 10^{-16}$), and (D) diet intraclass correlation estimates (Diet ICC) (rho = -0.11, $p < 2.2 \times 10^{-16}$).

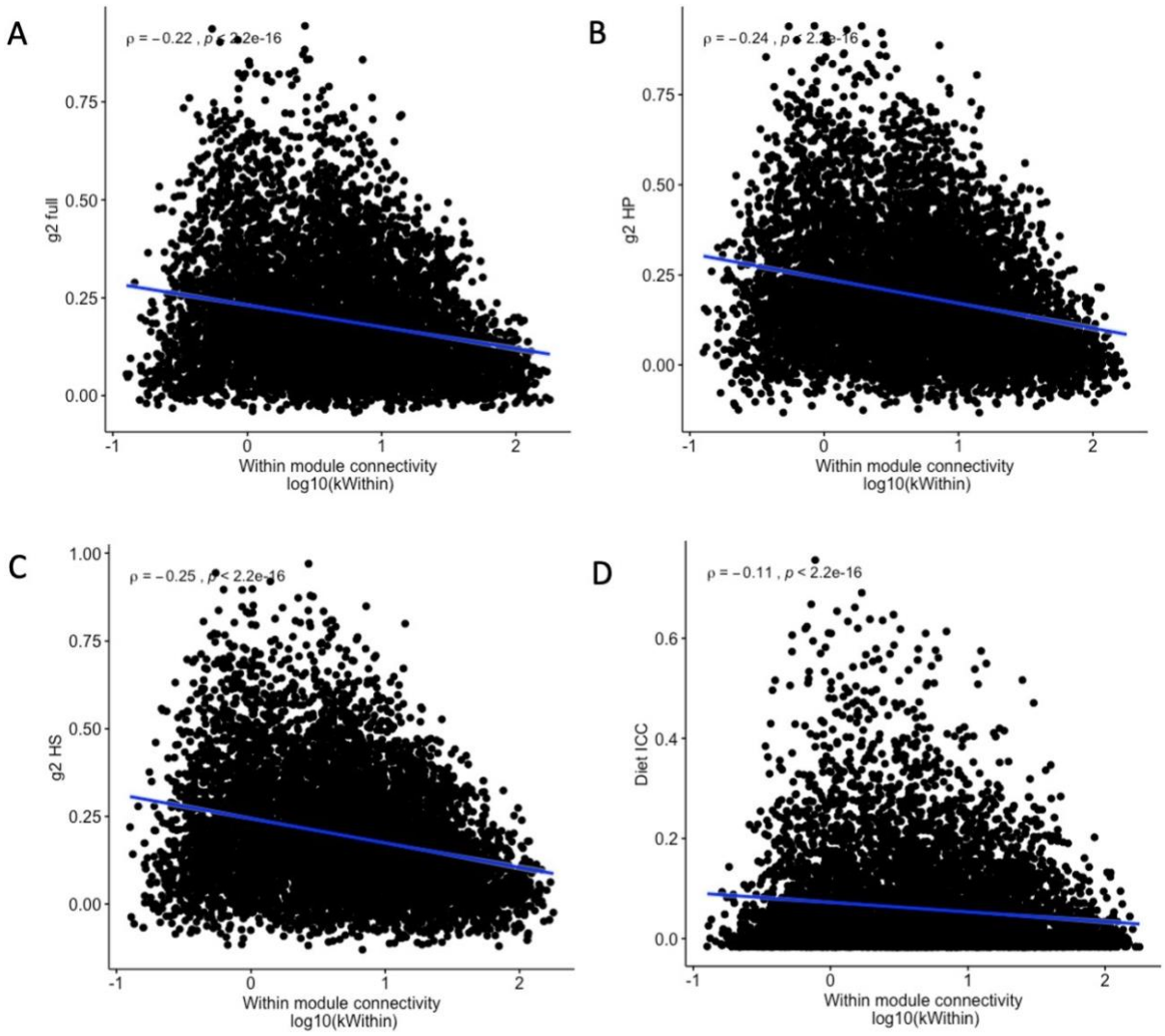
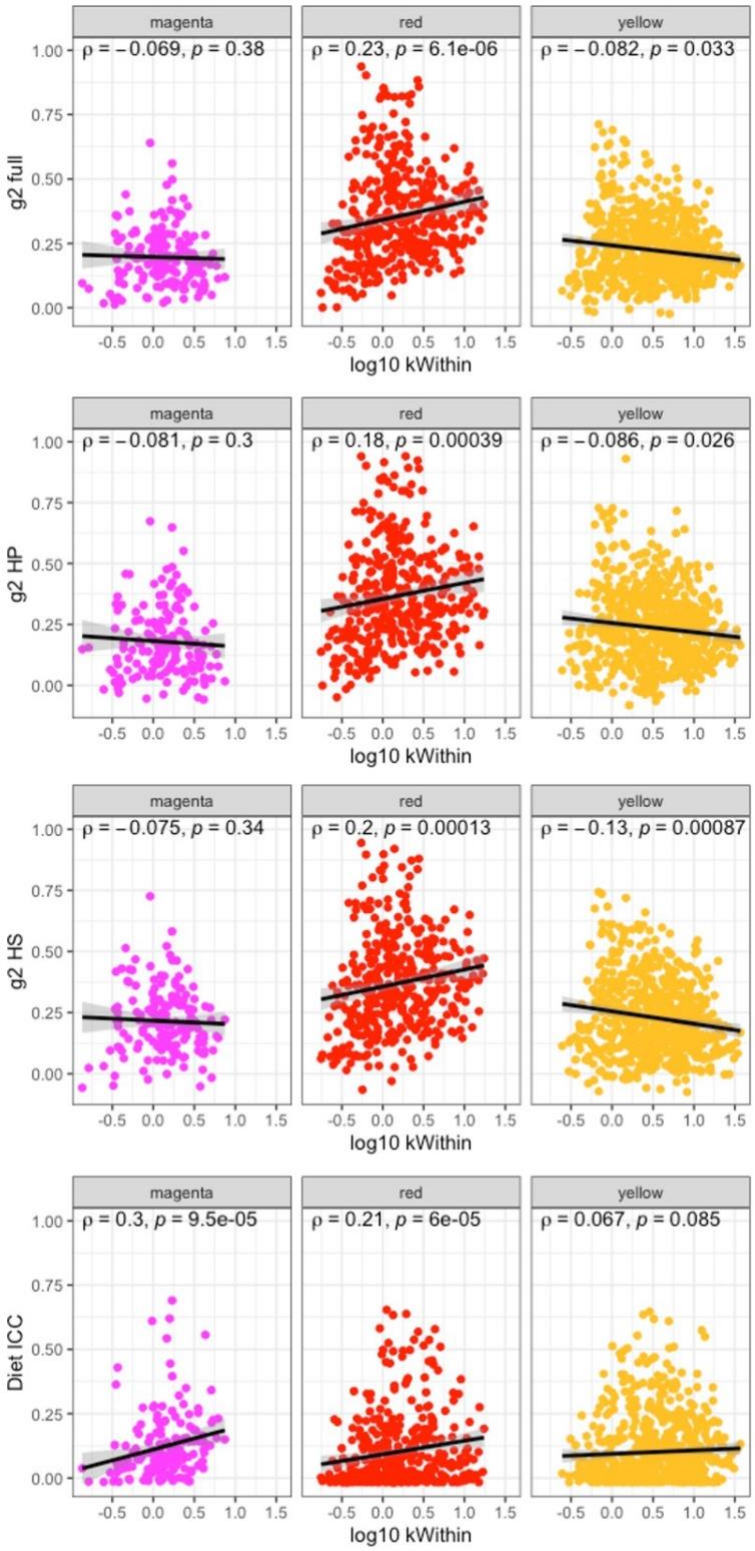


Figure S2-8. Correlations between H^2 and diet ICC with gene module connectivity by module



Spearman's correlations performed between log-transformed gene module connectivity and H2 estimates, as well as correlations performed between log-transformed gene module connectivity and diet intraclass correlations (diet ICC) for the magenta, red, and yellow modules demonstrate that the relationship between gene module connectivity and H2 varies by module; the relationship between gene module connectivity and diet ICC also varies by module. H2 and connectivity were not significantly correlated regardless of dietary composition, H2 and connectivity were consistently significantly correlated across different "diet environments," and H2 and connectivity were inversely correlated across different "diet environments" where the correlation was slightly stronger for the HS diet. Interestingly, diet ICC and connectivity were significantly correlated for the magenta and red modules but not the yellow module.

Chapter 4: Conclusion

In this dissertation the effects of two important determinants of obesity development, genetics and diet, were studied using the Collaborative Cross, a highly genetically diverse mouse panel designed for systems genetics. The aim of chapter 2 of this dissertation was to determine the degree that both genetic background and diet contribute to the development of obesity; some subobjectives were to investigate whether diet alters susceptibility to developing obesity, assess whether differences in diet macronutrient composition result in more beneficial phenotypic outcomes, and relate adiposity and metabolism to clinical traits associated with various diseases such as type 2 diabetes and cardiovascular disease, such as levels of circulating analytes. The results of chapter 2 demonstrate the large effect size of genetics on obesity development relative to diet alone and confirm that genetics largely determines whether an individual will become obese. However, our findings also show that diet can attenuate the severity of obesity depending on the individual and emphasize the importance of accounting for genetics when recommending a weight-loss plan, since certain individuals may require therapeutics in addition to modification of diet. The broad sense heritability estimates and diet effect sizes in chapter 2 highlight which traits are more sensitive to differences in macronutrient composition compared to genetic effects. Furthermore, we identified CC strains that are more responsive to diet for various traits. Given the relationship between obesity and inflammation, measuring circulating cytokine and/or adipokine levels in addition to the traditional markers of metabolic syndrome (e.g. glucose, triglycerides, insulin, and cholesterol) would provide additional insight on the effect of macronutrient composition and phenotypic differences attributed to genetics.

In chapter 3, genetics was found to have a much greater influence on hepatic gene expression compared to diet, as evinced by the over 9,000 genes discovered to be differentially expressed by strain compared to 1,344 gene differentially expressed by diet. By combining differential gene expression analysis and WGCNA into one study, we demonstrated that both strain and diet influence expression of individual genes as well as the expression for groups of related genes. By integrating the phenotype data into the analysis, we found both individual genes and gene modules expressed in the liver that were related to adiposity and other clinical traits. The work in chapter 3 sheds light on one way that genetic background and diet influence adiposity, where the identification of genes expressed in the liver related to

adiposity provides concrete preliminary suggestions of specific “intermediary” mechanisms that bridge genetics and diet with obesity, which may be validated in future studies.

The complexity of obesity leaves many questions regarding its etiology to be elucidated, but this dissertation sets the foundation for more targeted approaches to uncover some of the specific mechanisms that influence the development of obesity. In this dissertation, we identified CC strains that were lean non-responders to diet (CC019/TauUnc), fat non-responders to diet (CC040/TauUnc), responders that gained more weight on the high protein diet relative to the high fat high sucrose diet (CC012/GeniUnc), and responders that gained more weight on the high fat high sucrose diet relative to the high protein diet (CC028/GeniUnc). One possible follow-up study that could be performed to investigate specific mechanisms that contribute to obesity development using a systems biology approach would involve administering a diet challenge to multiple male and female replicates from these four CC strains followed by clinical phenotyping of body composition, indirect calorimetry, and measuring circulating analytes, as well as exploring the epigenome and transcriptome of the liver, subcutaneous fat, gonadal fat, and hypothalamus in one study. Including both male and female mice would facilitate the exploration of sexual dimorphism and effects from interactions between sex, genetics, and diet on obesity relative to health risks such as cardiovascular disease, for which sex differences are well characterized but not in the context of obesity currently (Kamon et al., 2021; Lin et al., 2016). An alternative approach would be to perform genetic crosses between non-responder and responder strains and administering the diet challenge to the F2 generation to explore changes in phenotype and identify genes whose expression levels are influenced by specific diets.

To delve deeper into the effects of specific macronutrients on obesity, two different approaches to refining diet choice could be used. In one approach, one of four diets would be administered to mice from each of the four CC strains comprised of either high protein, high sucrose, high fat, or the control diet. Although fat has been implicated in regulating energy intake and causing obesity in several inbred mouse strains (S. Hu et al., 2018), the effect of dietary fat on obesity still warrants further study using mice with higher genetic diversity such as the CC to better reflect the obesogenic effects of fat consumption in humans. Furthermore, sucrose also contributes to obesity development by altering serotonergic neuroplasticity and dopamine signaling to decrease dietary control, as well as inducing cravings for

sweetened food (Avena et al., 2006, 2008; Beecher et al., 2021; Reichelt, 2016); elucidating the differences in the epigenome and transcriptome between CC strains that respond differently to a high sucrose diet would provide insight on the genetics-by-diet effects on different tissue types in the context of obesity. An alternative approach would be similar to the approach used by Barrington et al., where the diets administered would mimic different diets that humans typically consume, such as the American, Mediterranean, ketogenic, or Japanese diet (Barrington et al., 2017). Utilizing this diet challenge would address questions regarding dietary effects in a more holistic manner; although comparing effects from differences in individual macronutrient content would be difficult, this approach would reveal the overall synergistic effects on obesity from each diet that humans already consume, which may more readily translate to improving dietary recommendations.

Important phenotyping for both baseline measurements prior to the diet challenge and after the diet challenge would include assessment of fasting circulating clinical markers of disease such as glucose, insulin, triglycerides, cholesterol; metabolic phenotyping to estimate food consumption and energy expenditure; body composition measured using MRI; and weekly weight measurements from baseline to the end of the diet challenge. In this experimental design with the control diet fed from the beginning until the end of the experiment, the effect of genetic background, diet, and genetic by diet interactions on obesity could be directly compared while accounting for effects due to age. In addition, assessing food consumption both before and after the diet challenge would clarify whether the effects of diet on obesity were due to differences in dietary composition or the amount of diet consumed.

Chapter 3 of this dissertation demonstrated that both strain and diet influence the expression of genes in the liver related to obesity. To expand on this work and discover the specific mechanisms of *how* strain and diet exert their influence on obesity development, investigating the epigenome and transcriptome of the liver, subcutaneous fat, gonadal fat, and hypothalamus in one study would reveal the strain-specific, diet-specific, and strain-by-diet-specific alterations in the crosstalk between different tissue types leading to different obesity phenotypic outcomes. The epigenome is characterized by heritable changes in gene function that occur without alterations in DNA sequence and includes processes such as histone modifications, DNA methylation, chromatin remodeling, and non-coding RNA interactions (Gao et al., 2021; Pagiatakis et al., 2021). Techniques that measure changes in some of these processes include

ATAC-seq (Assay for Transposase-Accessible Chromatin) (Buenrostro et al., 2013), whole genome bisulfite sequencing (Stevens et al., 2013), Hi-C (Belton et al., 2012), and ChiRP-seq (Chromatin Isolation by RNA purification) (Tian & Hu, 2020), the data of which can then be analyzed in tandem with RNA-seq data as demonstrated by Xu et al. (B. Xu et al., 2021), or used in differential expression analysis (e.g. limma), pathway analysis (e.g. EnRichr), or a network approach as demonstrated by Pomp et al. (Dobrin et al., 2009). Because the epigenome displays variation across cell types and modifications enable cells to respond to environmental stimuli, the epigenome may function as the interface of energy metabolism gene-environment interactions that lead to obesity development (Ling & Rönn, 2019; Loh et al., 2019; van Dijk et al., 2015). For example, several review papers have summarized findings from human studies where genes involved with energy metabolism exhibited altered DNA methylation (Hyun & Jung, 2020; Ling & Rönn, 2019; Loh et al., 2019), such as the positive association between DNA methylation levels at the adiponectin gene locus in subcutaneous adipose tissue with BMI and waist girth (Houde et al., 2015). Therefore, characterizing the epigenome and transcriptome in this follow-up study where environment, diet, and genetic background are defined and controlled would relate the effects of specific diets and epigenetic modifications on genes associated with obesity.

Lastly, numerous studies in both humans and animals demonstrated that the complex bacterial community that resides within the intestine called the gut microbiota plays an incredibly important role in the development of obesity, since this community of microbes induces the expression of genes related to macronutrient metabolism and even mediates energy balance by affecting feeding behavior through activation of vagal afferent neurons and parasympathetic reflexes (Gérard, 2016; Hamilton & Raybould, 2016; John & Mullin, 2016; Raybould & Zumpano, 2021). The complex relationship between the gut microbiota and host suggests myriad possible mechanisms that the microbiota may use to alter energy intake and body weight regulation in relation to host genetics and diet, which currently remain unclear, but advances in sequencing technology have made research of the gut microbiota in studies with large sample sizes much more affordable while still generating informative data sets. Shallow shotgun sequencing is one relatively new method that balances cost with sensitivity and power, producing data sets with the sequencing depths of approximately two million reads per sample (A. J. Johnson et al., 2019). In a future study, the fecal samples collected at baseline and post-diet from the CC cohort studied

in this dissertation will be used for shallow shotgun sequencing to examine how host genetics and differences in diet alter gut microbial composition and the expression of microbial genes relative to obesity. The phenotype and hepatic gene expression data will be integrated with the shallow shotgun sequencing data to identify obesogenic genes in the liver that have expression levels modified by microbial taxa, gene expression, or functional pathways. The results from this study will highlight additional candidate genes for functional validation studies and provide further understanding of the mechanisms associated with the gut microbiota and development of obesity.

References

Avena, N. M., Rada, P., & Hoebel, B. G. (2008). Evidence for sugar addiction: Behavioral and neurochemical effects of intermittent, excessive sugar intake. *Neuroscience & Biobehavioral Reviews*, *32*(1), 20–39. https://doi.org/10.1016/j.neubiorev.2007.04.019

Avena, N. M., Rada, P., Moise, N., & Hoebel, B. G. (2006). Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience*, *139*(3), 813–820. https://doi.org/10.1016/j.neuroscience.2005.12.037

Barrington, W. T., Wulfridge, P., Wells, A. E., Rojas, C. M., Howe, S. Y. F., Perry, A., Hua, K., Pellizzon, M. A., Hansen, K. D., Voy, B. H., Bennett, B. J., Pomp, D., Feinberg, A. P., & Threadgill, D. W. (2017). Improving Metabolic Health Through Precision Dietetics in Mice. *Genetics*, *208*(1). https://doi.org/10.1534/genetics.117.300536

Beecher, K., Wang, J., Jacques, A., Chaaya, N., Chehrehasa, F., Belmer, A., & Bartlett, S. E. (2021). Sucrose Consumption Alters Serotonin/Glutamate Co-localisation Within the Prefrontal Cortex and Hippocampus of Mice. *Frontiers in Molecular Neuroscience*, *14*, 678267. https://doi.org/10.3389/fnmol.2021.678267

Belton, J.-M., McCord, R. P., Gibcus, J. H., Naumova, N., Zhan, Y., & Dekker, J. (2012). Hi-C: A comprehensive technique to capture the conformation of genomes. *Methods (San Diego, Calif.)*, *58*(3), 268–276. https://doi.org/10.1016/j.ymeth.2012.05.001

Buenrostro, J. D., Giresi, P. G., Zaba, L. C., Chang, H. Y., & Greenleaf, W. J. (2013). Transposition of native chromatin for fast and sensitive epigenomic profiling of open chromatin, DNA-binding proteins and nucleosome position. *Nature Methods*, *10*(12), 1213–1218. https://doi.org/10.1038/nmeth.2688

Dobrin, R., Zhu, J., Molony, C., Argman, C., Parrish, M. L., Carlson, S., Allan, M. F., Pomp, D., & Schadt, E. E. (2009). Multi-tissue coexpression networks reveal unexpected subnetworks associated with disease. *Genome Biology*, *10*(5), R55. https://doi.org/10.1186/gb-2009-10-5-r55

Gao, W., Liu, J.-L., Lu, X., & Yang, Q. (2021). Epigenetic regulation of energy metabolism in obesity. *Journal of Molecular Cell Biology*, mjab043. https://doi.org/10.1093/jmcb/mjab043

Gérard, P. (2016). Gut microbiota and obesity. *Cellular and Molecular Life Sciences: CMLS*, *73*(1), 147–162. https://doi.org/10.1007/s00018-015-2061-5

Hamilton, M. K., & Raybould, H. E. (2016). Bugs, guts and brains, and the regulation of food intake and body weight. *International Journal of Obesity Supplements*, *6*(Suppl 1), S8–S14. https://doi.org/10.1038/ijosup.2016.3

Houde, A.-A., Légaré, C., Biron, S., Lescelleur, O., Biertho, L., Marceau, S., Tchernof, A., Vohl, M.-C., Hivert, M.-F., & Bouchard, L. (2015). Leptin and adiponectin DNA methylation levels in adipose tissues and blood cells are associated with BMI, waist girth and LDL-cholesterol levels in severely obese men and women. *BMC Medical Genetics*, *16*, 29. https://doi.org/10.1186/s12881-015-0174-1

Hu, S., Wang, L., Yang, D., Li, L., Togo, J., Wu, Y., Liu, Q., Li, B., Li, M., Wang, G., Zhang, X., Niu, C., Li, J., Xu, Y., Couper, E., Whittington-Davies, A., Mazidi, M., Luo, L., Wang, S., … Speakman, J. R. (2018). Dietary Fat, but Not Protein or Carbohydrate, Regulates Energy Intake and Causes Adiposity in Mice. *Cell Metabolism*, *28*(3). https://doi.org/10.1016/j.cmet.2018.06.010

Hyun, J., & Jung, Y. (2020). DNA Methylation in Nonalcoholic Fatty Liver Disease. *International Journal of Molecular Sciences*, *21*(21), E8138. https://doi.org/10.3390/ijms21218138

John, G. K., & Mullin, G. E. (2016). The Gut Microbiome and Obesity. *Current Oncology Reports*, *18*(7). https://doi.org/10.1007/s11912-016-0528-7

Johnson, A. J., Vangay, P., Al-Ghalith, G. A., Hillmann, B. M., Ward, T. L., Shields-Cutler, R. R., Kim, A. D., Shmagel, A. K., Syed, A. N., Walter, J., Menon, R., Koecher, K., & Knights, D. (2019). Daily Sampling Reveals Personalized Diet-Microbiome Associations in Humans. *Cell Host & Microbe*, *25*(6), 789-802.e5. https://doi.org/10.1016/j.chom.2019.05.005

Kamon, T., Kaneko, H., Itoh, H., Kiriyama, H., Mizuno, Y., Morita, H., Takeda, N., Yamamichi, N., & Komuro, I. (2021). Possible Gender Difference in the Association Between Abdominal Obesity, Chronic Inflammation, and Preclinical Atherosclerosis in the General Population. *International Heart Journal*, *62*(4), 837–842. https://doi.org/10.1536/ihj.20-654

Lin, A., Lacy, M. E., Eaton, C., Correa, A., & Wu, W.-C. (2016). Inflammatory Obesity Phenotypes, Gender Effects, and Subclinical Atherosclerosis in African Americans: The Jackson Heart Study.

Arteriosclerosis, Thrombosis, and Vascular Biology, *36*(12), 2431–2438. https://doi.org/10.1161/ATVBAHA.116.307728

Ling, C., & Rönn, T. (2019). Epigenetics in Human Obesity and Type 2 Diabetes. *Cell Metabolism*, *29*(5), 1028–1044. https://doi.org/10.1016/j.cmet.2019.03.009

Loh, M., Zhou, L., Ng, H. K., & Chambers, J. C. (2019). Epigenetic disturbances in obesity and diabetes: Epidemiological and functional insights. *Molecular Metabolism*, *27S*, S33–S41. https://doi.org/10.1016/j.molmet.2019.06.011

Pagiatakis, C., Musolino, E., Gornati, R., Bernardini, G., & Papait, R. (2021). Epigenetics of aging and disease: A brief overview. *Aging Clinical and Experimental Research*, *33*(4), 737–745. https://doi.org/10.1007/s40520-019-01430-0

Raybould, H. E., & Zumpano, D. L. (2021). Microbial metabolites and the vagal afferent pathway in the control of food intake. *Physiology & Behavior*, *240*, 113555. https://doi.org/10.1016/j.physbeh.2021.113555

Reichelt, A. C. (2016). Adolescent Maturational Transitions in the Prefrontal Cortex and Dopamine Signaling as a Risk Factor for the Development of Obesity and High Fat/High Sugar Diet Induced Cognitive Deficits. *Frontiers in Behavioral Neuroscience*, *10*, 189. https://doi.org/10.3389/fnbeh.2016.00189

Stevens, M., Cheng, J. B., Li, D., Xie, M., Hong, C., Maire, C. L., Ligon, K. L., Hirst, M., Marra, M. A., Costello, J. F., & Wang, T. (2013). Estimating absolute methylation levels at single-CpG resolution from methylation enrichment and restriction enzyme sequencing methods. *Genome Research*, *23*(9), 1541–1553. https://doi.org/10.1101/gr.152231.112

Tian, C., & Hu, G. (2020). Chromatin isolation by RNA purification (ChIRP) and its applications. In *Epigenetics Methods* (pp. 507–521). Elsevier. https://doi.org/10.1016/B978-0-12-819414-0.00025-2

van Dijk, S. J., Tellam, R. L., Morrison, J. L., Muhlhausler, B. S., & Molloy, P. L. (2015). Recent developments on the role of epigenetics in obesity and metabolic disease. *Clinical Epigenetics*, *7*, 66. https://doi.org/10.1186/s13148-015-0101-5

Xu, B., Wang, H., Wright, S., Hyle, J., Zhang, Y., Shao, Y., Niu, M., Fan, Y., Rosikiewicz, W., Djekidel, M. N., Peng, J., Lu, R., & Li, C. (2021). Acute depletion of CTCF rewires genome-wide chromatin accessibility. *Genome Biology*, *22*(1), 244. https://doi.org/10.1186/s13059-021-02466-0