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# Improving Metabolic Health through Precision Dietetics in Mice

William T. Barrington[1,2], Phillip Wulfridge[3], Ann E. Wells[9], Carolina Mantilla Rojas[1], Selene Y.F. Howe[1], Amie Perry[4], Kunjie Hua[5], Michael A. Pellizzon[10], Kasper D. Hansen[3,6,7], Brynn H. Voy[9], Brian J. Bennett[5], Daniel Pomp[5], Andrew P. Feinberg[3], David W. Threadgill[1,4,8]*

[1]Department of Molecular and Cellular Medicine, Texas A&M Health Science Center, College Station, TX 77843 USA

[2]Department of Biological Sciences, Genetics Program, North Carolina State University, Raleigh, NC 27695 USA

[3]Center for Epigenetics, Institute for Basic Biomedical Sciences, Johns Hopkins University, Baltimore, MD 21205

[4]Department of Veterinary Pathobiology, Texas A&M University, College Station, TX 77843 USA

[5]Department of Genetics, University of North Carolina, Chapel Hill, NC 27559 USA

[6]Department of Biostatistics, Johns Hopkins University, Baltimore, MD 21205, USA

[7]Nathan-McKusick Institute of Genetic Medicine, Johns Hopkins University, Baltimore, MD 21205, USA

[8]Faculty of Nutrition, Faculty of Genetics, and Faculty of Toxicology, Texas A&M University, College Station, TX 77843 USA

[9]Department of Animal Science, University of Tennessee, Knoxville, TN 37996 USA

[10]Research Diets, Inc., 20 Jules Lane, New Brunswick, NJ 08901

***Corresponding Author:** David W. Threadgill, Ph.D., Depts. of Molecular and Cellular Medicine and Veterinary Pathobiology, Texas A&M Health Science Center and Texas A&M University, Reynolds Medical Bldg. Rm. 440, College Station, TX 77843-1114, USA, dwt@tamu.edu, Phone: 979-436-0850, Fax: 979-847-9481.

## ABSTRACT

The incidence of diet-induced metabolic disease has soared the last half-century despite national efforts to improve health through universal dietary recommendations. Studies comparing dietary patterns of populations with health outcomes have historically provided the basis for healthy diet recommendations. However, evidence that population-level diet responses are reliable indicators of responses across individuals is lacking. This study investigated how genetic differences influence health responses to several popular diets in mice, which are similar to humans in genetic composition and propensity to develop metabolic disease, but enable precise genetic and environmental control. We designed four human-comparable mouse diets that are representative of those eaten by historical human populations. Across four genetically distinct inbred mouse strains, we compared the American diet's impact on metabolic health to three alternative diets (Mediterranean, Japanese and Maasai/ketogenic). Furthermore, we investigated metabolomic and epigenetic alterations associated with diet response. Health effects of the diets were highly dependent on genetic background, demonstrating that individualized diet strategies improve health outcomes mice. If similar genetic-dependent diet responses exist in humans, then a personalized, or "precision dietetics," approach to dietary recommendations may yield better health outcomes than the traditional one-size-fits-all approach.

## INTRODUCTION

Over the last half-century, national dietary guidelines have been failed to improve metabolic health in the United States, exemplified by the dramatic increase in metabolic syndrome(COOK *et al.* 2003). National dietary guidelines have largely been built upon epidemiological studies, which show that dietary patterns across populations are strongly correlated with the spectra of diseases(KNOX 1977), and as dietary patterns change, so do disease spectra(KAGAN *et al.* 1974; O'DEA 1992). However, a major limitation of population-level dietary studies is the absence of information on the relationship between individual and population-level responses.

There are clear examples in which genetically related subgroups within a population experience more severe health effects than the population at-large, exemplified by Westernized indigenous populations that have disproportionately high incidences of type II diabetes(O'DEA 1992; SCHULZ *et al.* 2006). The importance of genetic background in diet response is supported by the strong similarity in weight gain within monozygotic twin pairs during long-term overfeeding(BOUCHARD *et al.* 1990). Clinical studies find wide variation across genetically diverse people on the health effects of diet, including weight gain and risk for heart disease(LIU *et al.* 1978; DANSINGER *et al.* 2005; HESSION *et al.* 2009). Some of the variation has been attributed to dietary adherence(TOUBRO AND ASTRUP 1997). Yet, tightly controlled studies find extensive heterogeneity in physiological responses to identical diets(LEVINE *et al.* 1999; ZEEVI *et al.* 2015), demonstrating that innate differences between people contribute to the heterogeneous effects of diets.

Mouse models provide a powerful resource for studying the interaction of genetics with diet. Similar to humans, genetically diverse mice vary in their susceptibility to diet-induced metabolic disease, but enable greater control of genetic and environmental factors. Studies in mice demonstrate that obesity has a strong genetic component and that identical diets affect weight gain differently across strains(West *et al.* 1992; Petro *et al.* 2004; Parks *et al.* 2013). While there are differences in metabolism and metabolic disease between mice and humans(Kennedy *et al.* 2010; Wong *et al.* 2016), a number of research groups have demonstrated the value of mouse studies in unraveling the genetic architecture underlying metabolic responses(Paigen 1995; Almind and Kahn 2004; Cheverud *et al.* 2004; Biddinger *et al.* 2005; Svenson *et al.* 2007; Hill-Baskin *et al.* 2009; Shockley *et al.* 2009; Ussar *et al.* 2015; Sinasac *et al.* 2016).

To explore the impact of the American diet on metabolic health across genetically diverse individuals, we designed a mouse version of the contemporary American diet and compared its metabolic health effects to that of a more typically fed control mouse diet across four inbred strains (A/J, C57BL/6J, FVB/NJ, and NOD/ShiltJ, denoted as A, B6, FVB, and NOD, respectively). The clinical traits assayed were indicative of metabolic syndrome- a cluster of conditions that increase risk of heart disease, stroke and diabetes. We then compared each strain's metabolic health when fed alternative human-relevant diets, including a Mediterranean diet, a Japanese diet, and a ketogenic diet analogous to that consumed by the Maasai, a tribal group in Kenya. In addition, we evaluated the liver metabolome and epigenetic changes underlying differential diet response. The diet (or diets) that was healthiest relative to the American diet was genetic-dependent, demonstrating that health outcomes in mice are improved through individualized dietary strategies, and raising the question whether the development and implementation personalized diet recommendations could also lead to better outcomes in people.

## EXPERIMENTAL PROCEDURES

### Animals and Husbandry

Four-week old A/J, C57BL/6J, FVB/NJ, and NOD/ShiLtJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and acclimated for two weeks. Mice were randomized into diet groups. Two equally sized and identical cohorts of 5 mice per diet, sex and strain (480 total) were studied in 2 locations, North Carolina (NC cohort) and Texas A&M University (TAMU cohort) over 6 months. The NC cohort was housed at the University of North Carolina during the first 4 months for analysis of body composition, metabolic rate, and physical activity. Mice were then transferred to North Carolina State University for the final 2 months for glucose tolerance testing, necropsy, and tissue collection. Mice were housed 5 per cage and maintained at 22°C under a 12-hour light cycle, and maintained and protocols followed in accordance with University of North Carolina, North Carolina State University and Texas A&M University IUCAC guidelines. Mice were euthanized with carbon dioxide and tissues were flash frozen in liquid nitrogen or fixed in formalin.

### Diets

Powdered diets were designed in collaboration with Research Diets, Inc. (New Brunswick, NJ). Traditional Mediterranean (D12052702) and Japanese (D12052703) diets were based on the Food and Agriculture Organization's Food Balance Sheets from Greece and Japan in 1961(NATIONS 2016). The American diet (D12052705) was based on USDA's 2008 Dietary Assessment of Major Food Trends(NCI). The Maasai/ketogenic diet (D12052706) was designed to allow mice to remain in ketosis, formulated with dairy sources as consumed by the Masaai(MANN *et al.* 1965) and with menhaden oil and corn oil added to ensure mice had essential lipids. Purified control mouse diet (D12052701, Research Diets, Inc.) was used as a control diet for comparison to the American diet. Diets were designed to recapitulate human diets as closely as possible, matching macronutrient ratio, fiber content, types of ingredients and fatty acid ratios to the human diets (Tables S1-4). Protein sources used included beef protein to match red meat intake, casein to match dairy intake, soy protein to match soy intake, egg white protein to match egg and white meat intake, and fish protein to match seafood intake. Cornstarch, wheat starch, rice starch, potato starch, sucrose, and fructose were matched accordingly to the types and amounts of starches and sugars in the diets. Soybean oil, corn oil, menhaden oil, sunflower oil, butter, lard, safflower oil, flaxseed oil and olive oil were used to reconstruct lipid profiles.

**Body Composition and Weight**

Body composition (lean and fat mass) was assessed in both cohorts at 12 weeks (EchoMRI™-130 Body Composition Analyzer). Body composition measurements were verified by comparing lean and fat mass measurements to scale weight.

**Food Consumption**

Food consumption was measured in the TAMU cohort at 14 weeks by singly housing in wire bottom cages over a paper filter to collect spilled food. Starting, ending, and spilled food weights were recorded. Two 24-hour periods acclimation periods were followed by two 24-hour testing periods. Each period was separated by 3 days. Diet-by-strain groups had $n$ = 4-10 mice of both males and females, except female NOD mice fed Maasai/ketogenic diet, which were omitted due to distress during the acclimation period.

**Fasted Glucose and Glucose Tolerance Test**

Fasting glucose concentrations were measured following a 6-hour fast in both cohorts after 16 weeks on diet. Glucose (2 g/kg) was administered by oral gavage. Blood glucose levels were measured with a Bionome GM100 glucose monitor (Bionome USA Corp) at 0, 30, 60, 90, and 120 minutes. Area under the curve (from a baseline of 0) was calculated. All diet-strain groups had $n$ = 9 to 20 mice. One American FVB male and one American B6 male did not show a change in blood glucose after gavaging and were omitted. Two NOD ketogenic males had glucose greater than 600 mg/dL (glucometer maximum) while fasting or at the first time point and were omitted.

**Liver Triglycerides**

Following necropsy, liver triglyceride concentration was quantified in both cohorts as previously described(FOLCH *et al.* 1957). Briefly, 50 mg pieces of liver were homogenized in a 2:1 choloroform-methanol solution. After 30 minutes of incubation, a sodium chloride solution was added to the solution and vortexed. The lower phase was decanted and evaporated under nitrogen steam. Each sample was resuspended in a 0.5% Triton X-100/PBS solution. After sonication, samples were incubated at 55°C for 5 minutes. Infinity Triglyceride reagent (Thermo Scientific) was added and samples were incubated for 5 minutes at 37°C. Absorbance at 500 nm was measured and compared to a standard curve to quantify triglyceride concentration.

**Metabolic Rate and Activity**

Mice in the NC cohort were singly housed in Phenomaster Metabolic Chambers (TSE Systems) at 12 weeks. After an 8-hour acclimation period, data collection included two 12-hour night cycles and one 12-hour day cycle. Heat expenditure, oxygen consumption, and drinking volume were calculated per hour and normalized to lean mass, which was assessed prior to testing. Mice that failed to drink more than 0.5 mL were omitted from water intake analysis (n<2 per diet-strain group). Activity was determined by number of laser beam breaks in both vertical and horizontal axes and calculated per hour. Hyperactive mice (defined by activity >100% strain mean) were omitted from activity analysis ($n \leq 1$ per group). Respiratory exchange rate was calculated as an average per hour.

**Liver Histology**

Formalin-fixed, paraffin-embedded right lobe liver samples were sectioned at 5 μm and stained with hematoxylin and eosin. The extent of steatosis was assessed in a blinded fashion by a board-certified veterinarian pathologist using a previously reported scoring system for non-alcoholic fatty liver disease(LIANG *et al.* 2014). Briefly, the scoring system for macrovesicular steatosis, microvesicular steatosis, and cellular hypertrophy was based on the percentage of hepatocytes within the stained section. These parameters utilized the following categories: 0 (< 5% of hepatocytes), 1 (5-33%), 2 (34-66%), and 3 (>66%). Inflammation was evaluated by counting the number of inflammatory foci per field, averaged across of 5 fields of view at 100X magnification. The level of inflammation was assigned using the following categories: 0 (normal, < 0.5), 1 (slight, 0.5-1.0), 2 (moderate, 1.0-2.0), and 3 (severe, >2).

**Blood Lipids and Biochemistry**

Fasted insulin was measured following a 6-hour fast at 18 weeks in both cohorts. Fasted blood samples were not collected from NOD mice in cohort 2, as they showed distress during fasting. Blood was collected via submandibular bleed, placed on ice for at least 30 minutes to allow clotting, then centrifuged at 10 x g for 5 minutes in 1.1 mL Z-

Gel microtubes (Sarstedt). Insulin concentrations were quantified using a Mouse Serum Adipokine Immunoassay ELISA kit (Millipore) on a Bio-Plex 200 System (Bio-Rad).

Alanine aminotransferase (ALT) and cholesterol analysis was performed on serum samples taken at necropsy in both cohorts. Blood was collected via cardiac puncture, chilled for at least 30 minutes, then centrifuged at 10 x g for 5 minutes in 1.1 mL Z-Gel microtubes (Sarstedt). ALT activity was quantified in duplicate using a fluorometric ALT Activity Assay Kit per the manufacturer's instructions (Simga-Aldrich). Total, LDL, and HDL cholesterol concentrations were measured in duplicate using a colorimetric Cholesterol Quantification Kit per the manufacturer's instructions (Sigma-Aldrich).

**Whole Genome Bisulfite Sequencing**

DNA extraction and WGBS were performed on liver samples from 32 males spanning four diet-strain combinations: B6 control mouse diet, B6 American diet, A strain control mouse diet, and A strain American diet (n = 8 per group). Males were used because they had the most divergent diet responses in the B6 strain. Genomic DNA was isolated from liver using the DNeasy Blood & Tissue Kit (Qiagen) and a modified protocol as follows: after tissue lysis and prior to spin column application, 50μg of RNase A (Thermo Scientific) was added to each sample and samples incubated at room temperature for 60 minutes. Samples were eluted in two cycles, in 100μL and 60μL of elution buffer.

WGBS single indexed libraries were generated using NEBNext Ultra DNA library Prep kit for Illumina (New England BioLabs) according to the manufacturer's instructions with modifications. 500ng gDNA was quantified by Qubid dsDNA BR assay

(Invitrogen) and 1% Unmethylated lamda DNA (Promega) was spiked in for monitoring bisulfite conversion efficiency. Samples were fragmented by Covaris S2 or LE220 sonicator to average insert size of 350bp. Size selection was performed using AMPure XP beads and insert sizes of 300-400bp were isolated (0.4x and 0.2x ratios). Samples were bisulfite converted after size selection using EZ DNA Methylation-Gold Kit (Zymo) following the manufacturer's instructions. Amplification was performed after the bisulfite conversion using Kapa Hifi Uracil+ (Kapa Biosystems) polymerase using following cycling conditions: 98°C 45s /8cycles: 98°C 15s, 65°C 30s, 72°C 30s / 72°C 1 min.

Final libraries were run on 2100 Bioanalyzer (Agilent) High-Sensitivity DNA assay. Libraries were quantified by qPCR using the Library Quantification Kit for Illumina sequencing platforms (Kapa Biosystems), using 7900HT Real Time PCR System (Applied Biosystems). Libraries from 12 samples (3 per group) were sequenced on Illumina HiSeq2000 (100bp), with the remainder sequenced on HiSeq2500 (125bp) paired-end single indexed run and 10% PhiX spike-in.

**WGBS Alignment and Methylation Analysis**

Sequencing reads were aligned using the BSmooth *(HANSEN et al. 2012)* bisulfite alignment pipeline (version 0.7.1) and Bowtie 2 version 2.1.0 *(LANGMEAD AND SALZBERG 2012).* Samples from B6 and A strains were aligned to their respective genome builds, obtained from the Collaborative Cross page at UNC Systems Genetics (http://csbio.unc.edu/CCstatus/index.py?run=Pseudo), combined with the genome for lambda phage. BSmooth was used to extract read-level measurements of methylation. To compare CG methylation across strains, the MODtools package *(46)*, which functions similarly to the UCSC Genome Browser's liftOver, was used to convert A genomic coordinates to the B6 build. Following conversion to a common coordinate system, we smoothed the methylation data as previously described *(HANSEN et al. 2012)*.

**Real Time PCR**

Hepatic *Avpr1a* transcript abundance was analyzed in males in both cohorts and in males from the two-week follow up study in which 5 A and B6 mice were fed *ad libitum* American or control mouse diet for two weeks at Texas A&M University. RNA was isolated from liver using a Maxwell 16 LEV simplyRNA kit (Promega). cDNA was generated using a Transcriptor First Strand cDNA Sythesis Kit (Roche). Primer were targeted for *Avpr1a* (5'-CATGTAGATCCACGGGTTGC-3' and 5'-ACACCTTTCTTCATCGTCCAG-3'),and *Rplp0* (5'-CGCTTGTACCCATTHATHATH-3' and 5'-TTATAACCCTGAAGTGCTCGAC-3') (Integrated DNA Technologies). Analysis was performed on a LightCycler 96 Thermocycler (Roche) using LightCycler 480 Sybr Green I Master reaction mix. All samples were run in duplicate and prepared on an EpMotion 5075 automated liquid handling system. Cycling conditions were 95°C for 5 minutes followed by 35 cycles of 95°C for 30 seconds, 55°C for 15 seconds, and 72°C for 60 seconds. A high-resolution melting curve was produced by heating to 95°C for 10 seconds, cooling to 65°C for 60 seconds and 97°C for 1 second, followed by a cooling step of 37°C for 30 seconds. $\Delta C_Q$ expression values were determined by normalizing to *Rplp0* expression.

**Isocaloric Mouse Experiments**

Four week-old C57BL/6J males were purchased from The Jackson Laboratory (Bar Harbor, ME) and acclimated two weeks. Mice were isocalorically fed control mouse diet or American diet for 90 days at Texas A&M University. 10 males were used per group, as males had a more pronounced difference in adiposity between the control diet and the American diet. Mice were housed 5 per cage and fed 11.5 kcal/mouse daily. Prior experiments optimized food quantity to ensure all food was eaten. Mice were weighed and body composition analyzed (EchoMRI™-130 Body Composition Analyzer).

**Metabolomics**

Flash frozen liver samples from the NC cohort were pulverized and 0.025 g was added to 1.3ml of pre-chilled methanol and incubated at -80°C for 15 minutes. Samples were centrifuged for 5 min at 13.2 rpm at 4°C and supernatant removed. 200 ml of 80% methanol:20% water (solvent B) was added and incubated for 15 minutes at -80°C. The sample was centrifuged for 5 minutes at 13.2 rpm at 4°C, and the supernatant added to the same glass vial. 200 ml of solvent B was added and incubated for 15 minutes at -80°C. Samples were centrifuged for 5 minutes at 13.2 rpm at 4°C and supernatant was added to the glass vial. The contents of the glass vial were dried using a nitrogen drying apparatus then 160 µL of sterile water was added—60 µL of $^{13}C$ labeled *E.Coli* was also added to the dried glass vial as an internal standard. Samples, kept at 4°C, were placed in an autosampler tray. 10µL from each sample was injected through a Synergi 2.5 micron Hydro-RP 100, 100 x 2.00 mm LC column (Phenomex) at 25°C. The mass spectrometer (MS) was run in full scan mode, negative ionization mode, adapting a previous protocol(CLASQUIN *et al.* 2012). Samples were analyzed with a resolution of 140,000. A scan window of 85 to 800 m/z (mass-to-charge) was used from 0 to 9 minutes, and a window of 110 to 1000 m/z from 9 to 25 minutes. Solvent A consisted of 97:3 water:methanol, 10 mM tributylamine, and 15 mM acetic acid. Solvent B was methanol. The gradient from 0 to 5 minutes is 0% Solvent B, from 5 to 13 minutes is 20% Solvent

B, from 13 to 15.5 minutes is 55% Solvent B, from 15.5 to 19 minutes is 95% Solvent B, and from 19 to 25 minutes is 0% Solvent B, with a flow rate of 200 µL/min. Raw files generated by Xcalibur were converted to mzML via msconvert(CHAMBERS *et al.* 2012). MAVEN (MELAMUD *et al.* 2010; CLASQUIN *et al.* 2012) was used to correct total ion chromatograms based on retention time for each sample. Identified metabolites were manually chosen and peak abundance was integrated by mass (±5 ppm) and retention time. Unidentified metabolites were chosen using an algorithm with the following settings: minimum peak width, 5; minimum signal/blank ratio, 3 or greater; minimum peak intensity, 10,000; and minimum peak/baseline, 3. Unidentified peaks were filtered manually to remove those that did not meet the above criteria.

**Statistical Analysis**

*Factors contributing to phenotypic variance*

To determine the relative contribution of each factor underlying phenotypic variance, multi-factor ANOVA was performed for each phenotype with the factors strain, diet, strain by diet interaction, sex, and cohort (where applicable). Log transformation was used for data that was not normally distributed (activity, ALT, GTT, insulin, LDL cholesterol, liver triglycerides).

*Comparison of metabolic effects within strains across diet*

To compare effects of the American diet relative to the control diet, two-way or multi-factor ANOVA was performed independently for each strain using the factors diet and sex, and cohort where applicable. Cohen's D effect sizes were calculated using the mean response on American diet minus the mean response on control mouse diet, divided by the pooled standard deviation. The same methods were used to compare effects of

Mediterranean, Japanese, and Maasai/ketogenic diets to the American diet with *p*-values calculated using American diet as a control. Dunnett's Test correction was performed, which corrects for testing multiple comparisons to a control, given that we examined metabolic changes within a strain through diet modification. A *p*-value of 0.05 was used for the significance threshold.

*Methylation Analysis*

Methylation analysis was conducted in R via the bsseq package. The *Avpr1a* result was obtained by searching for differentially methylated regions genome-wide with a t-statistic cutoff of 4.6 and only considering CpGs, which had a coverage of at least 2 in all 31 samples. One sample from the B6 control mouse diet group was excluded from analysis because we observed that its source liver tissue had an abnormal tumor growth. This was confirmed by global hypomethylation, as previously described in human colon cancers*(*REIKVAM *et al. 2011).*

*Metabolomics Analysis*

Metabolites missing 70% or more sample measurements were removed from analysis. Missing values in the remaining metabolites were imputed using k-nearest numbers. Data was assessed for normality using Q-Q plots, residuals, and the Shapiro-Wilks test after each step of the normalization process. Tissue weight and internal standard were treated as covariates. Tissue amount used in the extraction was weighed for each sample. Metabolites measured from the $^{13}C$ *E. Coli* internal standard were matched with their corresponding metabolite; otherwise metabolites measured were matched with a $^{13}C$ metabolite of the same class type. Class types were identified using the Human Metabolome Database(WISHART *et al.* 2013). Once metabolites were adjusted for tissue weight and internal standard, each metabolite was pareto scaled across all mice using the package "MetabolAnalyze"(NYAMUNDANDA GIFT 2010). Each mouse was median normalized across all metabolites and metabolites transformed using cube root. The model assessed in each strain was:

$$Metabolite = diet + sex.$$

Statistical analyses of metabolites were performed using R version 3.1.0 and 3.2.2. The α for all statistical tests was determined to be 0.05. p-values associated with metabolites were adjusted using the Benjamini-Hochberg correction factor.

*Calculation of Health Category Scores and Metabolic Health Index Score*

Health category scores were calculated by multiplying the effect size (Cohen's D) of each alternative diet compared to the American diet for each phenotype in each strain by its confidence level, thereby weighting for both effect size and significance. Phenotypes within each category were designated as positive or negative (below) depending on their association with beneficial or detrimental health effects, respectively, as shown in the model below. To allow for comparison across categories, the category scores were standardized between -1 and +1 across all strain-diet groups within each category.

*body composition score = (lean mass) + (-fat mass) + (-body fat percentage)*

*lipid profile = (HDL cholesterol + (–LDL cholesterol) + (-plasma triglyceride conc.)*

*glucose metabolism = (-glucose tolerance) + (-fasted glucose) + (-fasted insulin)*

*liver health = (-liver triglyceride conc.) + (-ALT)*

To calculate the metabolic health index score, a measure of the cumulative metabolic health effects of a given diet relative to the American diet, we calculated the mean of the health category scores, either with or without inclusion of the body composition score.

**Data Availability**

File S1 contains supplemental data to support the conclusions in this paper.

# RESULTS

## Design of human-comparable mouse diets

We formulated diets based on historical dietary patterns to examine the metabolic effects of diets that span the spectrum of human dietary patterns and are associated with negative (American) or positive (Mediterranean, Japanese, Maasai/ketogenic) epidemiological health outcomes in people. Many studies have compared effects of a control mouse diet (Figure 1a) to a “high fat” or “Western” diet (Figure 1b). Many different high fat diets have been formulated each of which contains varying concentrations of fat and carbohydrate and from different sources than shown in the figure.  It is common to find one or two representative ingredients for each individual nutrient (i.e. casein provides protein, corn starch provides carbohydrate, soybean oil provides fat). To better recapitulate the diversity of ingredients in human diets, we designed diets that match not only macronutrient ratios (i.e. proportions of protein, carbohydrate, and fat on a kcal basis), but also ingredients, including bioactive compounds (e.g. red wine and green tea extracts) (Figure 1d,f), lipid profiles, and fiber content (Tables S1-4). The American diet is representative of contemporary dietary patterns in the United States (Figure 1c) (NCI). The Mediterranean and Japanese diets are representative of traditional eating patterns during the early 1960s, when these populations had among the longest life expectancies and lowest rates of chronic disease(GORDON 1957; MARMOT *et al.* 1975; TRICHOPOULOU AND VASILOPOULOU 2000; KNOOPS *et al.* 2004) (Figure 1d,f). The ketogenic diet is analogous to that of the Maasai tribe, who do not develop heart disease despite eating high levels of fat and cholesterol(MANN *et al.* 1965) (Figure 1e). The ketogenic diet induces ketosis, a physiological state in which the body shifts metabolism to utilize ketones and preserve glucose.

**Sources of phenotypic variation**

Four human-comparable diets (American, Mediterranean, Japanese, and ketogenic) and the control mouse diet were fed *ad libitum* to 10 male and 10 female mice from 4 inbred strains (A, B6, FVB, and NOD). These strains were chosen due to their genetic and phenotypic diversity, with the aim to survey mice with varying behavioral and metabolic profiles(KIRBY *et al.* 2010). The B6 strain is most commonly used in studies and is susceptible to diet induced obesity on a high-fat, high-sugar diet(WEST *et al.* 1992). FVB is more resistant to diet-induced obesity and is highly active, whereas the A strain is resistant to diet-induced obesity despite low levels of activity(BLACK *et al.* 1998; PARKS *et al.* 2013). NOD is metabolically unique from the other strains in its predisposition to develop diabetes (LEITER *et al.* 1987).

Two equally sized cohorts of mice began diets at six weeks and were aged on diets for 12 weeks to allow manifestation of physiological effects before analyzing metabolic traits over an additional 12 weeks (Figure S1). Phenotypes were compared using ANOVA models with diet, strain, the interaction of diet with strain, sex, and cohort as factors. Genetic variation accounted for a considerable proportion of the total phenotypic variation for most phenotypes (Table S5). Sex also contributed significantly to many phenotypes.  For example, sex effects accounted for about half of the variation in body weight and lean weight, 33-42% of variation in metabolic rate and 22% of the variation in water intake. Diet effects contributed to the total phenotypic variation of most traits, including 84% of the variation in respiratory exchange rate (RER), and 20-36% of the variation in plasma triglycerides, cholesterol, and metabolic rate. Cohort effects explained a relatively minor proportion of the total phenotypic variance, although some differences in body composition were observed (Table S5).

Importantly, the interaction of genetic background with diet influenced most metabolic traits, and in some cases accounted for more variation than genetic or dietary effects alone (Table S5). For instance, the genetic interaction with diet accounted for 13% of variation in food intake, whereas the genetic and diet effects each accounted for 11%. Genetic-by-diet interactions were important for water intake and metabolic rate, accounting for 12-15% of the phenotypic variance. While blood lipids were strongly influenced by diet, genetic-by-diet interaction effects contributed considerably, accounting for 10-12% of the variation in plasma triglycerides, and HDL, LDL and total cholesterol. The genetic interaction with diet, where significant, complicates the interpretation of the main effects of genetics and diet, as the effect of genotype can vary across each diet. Together, the results indicate that both genetic and dietary factors contribute in varying degrees to most metabolic traits, and that the genetic interaction with diet plays a key role in influencing metabolic traits.

**Influence of the American diet on activity, metabolic rate and food intake**

To model the potential impact of a precision nutrition approach, we sought to use the American diet as a baseline for comparison to other human diets. However, because the vast majority of mouse studies in any field use a chow diet, we first put the novel American diet into perspective of the control mouse diet (Comparison A), before comparing the American diet to alternative human-relevant diets (Comparison B) (Figure S1).

We examined basic physiological and behavioral parameters and quantified the changes in Cohen's d effect size (*d)*. Physical activity was not affected by diet (Figure 2a). Nonetheless, the American diet increased metabolic rate in the A strain, indicated by increase oxygen consumption (*d* = 0.98) and heat expenditure (*d* = 0.96) (Figure 2b, Figure S2a). Food intake decreased in the NOD strain (*d* = -2.20), but was not significantly altered in other strains (Figure 2c). Water intake increased significantly in A mice (*d* = 1.47) and NOD mice (*d* = 1.37) (Figure 2d). Respiratory exchange rate (RER) was reduced in B6 mice fed American diet (*d* = -1.47), indicative of greater fat oxidation (Figure S2b).

**The American diet induces varying degrees of fat gain across strains**

We compared the body composition of mice fed the American diet relative to those fed the control mouse diet at 12 weeks. The American diet increased body fat to varying degrees across strains with the largest effect was in B6 mice (*d* = 2.18) compared to NOD (*d* = 1.57), FVB (*d* = 1.42), and A mice (*d* = 1.14) (Figure 2e, S2c). This result is consistent with previous research feeding high fat diets(WEST *et al.* 1992; CHEVERUD *et al.* 1999; PARKS *et al.* 2013). Elevated body weight coincided with increased fat mass (Figure 2f, 2k). Interestingly, food intake was poorly correlated with fat gain (Figure 2c, 2e), but this observation is consistent with previous studies(BACKHED *et al.* 2004; PETRO *et al.* 2004; HATORI *et al.* 2012; PARKS *et al.* 2013). To validate this finding, an independent cohort of B6 mice was isocalorically fed American or control mouse diet for 14 weeks. Mice fed American diet weighed 14% more with no change in lean mass but a 74% increase in body fat (Figure S3), emphasizing the importance of factors other than food intake in the accumulation of body fat.

**The American diet negatively impacts blood lipid profiles**

Across strains, mice fed American diet had increased LDL cholesterol with variation in effect size (*d* = 0.82-3.09) (Figure 2g). Concomitantly, HDL cholesterol increased in B6, FVB and NOD strains (*d* = 1.58-1.64) (Figure 2h). Total cholesterol was increased across strains (*d* = 2.01-3.31) (Figure S2e). Plasma triglyceride concentrations decreased in FVB mice (*d* = -2.33), but did not change in other strains (Figure S2f).

**The impact of the American diet on glucose homeostasis differs by strain**

The American diet's effect on glucose homeostasis was evaluated by glucose tolerance test (GTT), and measurement of fasting glucose and insulin. B6 mice, commonly susceptible to glucose intolerance on high fat, high-sugar diets(SURWIT *et al.* 1988), showed glucose intolerance when fed American diet (*d* = 2.35) as demonstrated by increased area under the curve measurements (AUC) (Figure 2i). Similar responses were obeserved in FVB (*d* = 2.69). Consistent with previous research, diet had minimal impact on glucose tolerance in the A strain(SURWIT *et al.* 1988). Glucose tolerance was not significantly altered in the NOD strain, however this may have been due to high interindividual variability in this strain that is genetically predisposed to diabetes(HATTORI *et al.* 1986).

Fasting glucose was increased by the American diet in B6 (*d* = 2.15), FVB (*d* = 1.30) and A (*d* = 0.80) strains (Figure S2g). We did not observe significant alterations to insulin levels (Figure S2h). Previous research has shown increased insulin levels in B6 mice fed a high fat diet, albeit with high variability, requiring larger numbers of mice to detect a significant effect(BURCELIN *et al.* 2002).

**The American diet increases liver triglyceride concentrations**

To determine how liver health is impacted by the American diet, we performed histological examinations, and measured liver triglycerides and serum alanine aminotransferase (ALT), a marker of liver damage. Liver triglyceride concentrations were consistently elevated across strains ($d$ = 2.86-4.05) (Figure 2j). ALT concentrations did not significantly differ from mice fed control diet (Figure S2i). Histological examination revealed increased macrovesicular and microvesicular steatosis in the A, B6 and FVB strains, while only macrovesicular steatosis increased in NOD. NOD mice also exhibited increased hepatic inflammation (Table S6).

**Arginine vasopressin receptor 1A *(Avrp1a*) methylation status is associated with diet response in strains with divergent health effects**

A mechanism by which individuals respond to or are protected from diet-associated changes in metabolic health phenotypes is through modification of the epigenome(STOVER 2011; JANKE *et al.* 2015; BARRETT *et al.* 2016). To examine epigenetic modifications, we performed whole-genome bisulfite sequencing on liver from A and B6 mice fed control diet and American diet. These strains were chosen due to their divergent responses to the American diet, with B6 displaying greater increases in body weight and adiposity and impaired glucose metabolism, while A was comparatively resistant to these phenotypic effects. Changes in methylation patterns at arginine vasopressin receptor 1A (*Avpr1a)* showed a significant contrast between A and B6 mice. B6 fed the American diet were hypermethylated at *Avpr1a* compared to their control diet counterparts (Figure 3a); in contrast, no significant difference was observed in A mice on the two diets, and in fact A strain methylation was roughly equivalent to B6 fed control diet (Figure 3b). Given the observed methylation differences and *Avpr1a'*s known association with metabolic disease in mice and humans(Aoyagi *et al.* 2007; Enhorning *et al.* 2009), we examined *Avpr1a* hepatic transcript abundance, which was greatly reduced in B6 mice fed the American diet (-84%) compared to the other three strain-diet groups (Figure 3c). In a follow-up study, we identified that repression of *Avpr1a* transcription occurs rapidly, as *Avpr1a* transcript abundance was reduced by 54% after two weeks in B6 mice fed American diet (Figure 3d). Genetic variation within this region cannot account for methylation and expression differences, as *Avpr1a* sequences do not vary between the strains(Blake *et al.* 2017).

**Liver metabolite changes vary by strain**

Varying the composition of the diet causes corresponding metabolic shifts at the cellular level. Metabolite levels have been associated with increased risk of metabolic diseases like type 2 diabetes(Huang *et al.* 2013a; Huang *et al.* 2013b; Menni *et al.* 2013; Wang *et al.* 2013). Metabolomic profiling was used to determine the extent to which genetic variation impacted the tissue level metabolic response to the American diet versus the control diet. Profiling was performed in liver because of its primary role in nutrient allocation. Hepatic metabolomes of B6 mice were more affected by the American diet than other strains based on the number of metabolites that differed significantly in abundance between the two diet groups. A total of 16 known metabolites were affected by the American diet in B6, compared to five (FVB), three (NOD) and one (A) in the other strains (Figure 3e-g). Comparable diet-dependent differences across strains were also reflected in the numbers of unknown metabolites (i.e., spectral features that did not map to known compounds in our database) (Figure 3e). Diet-induced changes in metabolite levels were strain-dependent, with 84% of metabolites significantly altered by the American diet in only a single strain (Figure 3f).

**A precision dietetics approach reveals strain-specific effects of diet on physiology and body composition**

Dietary modification is a common initial intervention for patients with metabolic syndrome. While a number of studies have evaluated how obesigenic or atherogenic diets impact mice, studies on human-comparable diets are lacking. To test a precision dietetics approach by evaluating diet responses in the context of genetic background, we compared how physiology and health status differed in A, B6, FVB, and NOD mice fed Mediterranean, Japanese, and ketogenic diets relative to those fed American diet.

Physical activity was highly variable within strain-by-diet groups and was not significantly altered by diet (Figure 4a). Nonetheless, metabolic rate increased in all strains fed ketogenic diet and the magnitude of increase was genetic-dependent (Figures 4b, S4a). Food intake increased in B6 ($d$ = 1.97) and NOD ($d$ = 2.03) mice fed Japanese diet (Figure 4c). All strains except FVB drank more water on ketogenic diet ($d$ = 1.42 to 2.13). RER decreased across strains fed ketogenic diet and increased in all fed Japanese diet (Figure S4b).

Body composition and weight did not significantly differ for any strain fed the Mediterranean diet relative to the American diet (Figure 4e, 4f). Japanese and ketogenic diets yielded similar reductions in body fat A and B6 mice ($d$ = -0.93 to -1.17) (Figures 5a, S4c), whereas FVB mice fed Japanese diet had lower percent body fat ($d$ = -0.94) but not those fed a ketogenic diet (Figure 4e). A reduction in lean weight was unique to A mice fed ketogenic diet (*d* = -1.02) (Figure S4d). Across all diets, caloric intake was poorly correlated with body fat (Figure S5).

To better understand the relationship between percent body fat, metabolic rate, and activity, we plotted the residuals for each phenotype after accounting for sex differences in a subset of mice housed in metabolic cages for three days (Figure 4g). Metabolic rate and percent body fat varied by strain and diet, while activity level did not strongly contribute to shifts in metabolic rate or body fat. The A strain, which is considered resistant to effects of diet(BLACK *et al.* 1998; PARKS *et al.* 2013), had the greatest physiological shift of all strains on the ketogenic diet. Their metabolic rate and percent body fat were similar when fed American, Mediterranean or Japanese diets, but their metabolic rate greatly increased and body fat decreased when fed ketogenic diet.

Variation persisted in some diet-by-strain groups even while accounting for sex differences. This is most evident in the percent body fat of B6 mice. Heterogeneity of diet response has been previously observed in B6 mice(BURCELIN *et al.* 2002; KOZA *et al.* 2006), and indicates that while genetic information can improve prediction of diet response other factors are also influential.

**Effects of Mediterranean, Japanese and ketogenic diets on blood lipid profiles**

The response of blood lipids to diet is one of the most researched topics in biomedical sciences with thousands of studies being performed with varying results(SHEKELLE *et al.* 1981; KRIS-ETHERTON *et al.* 1988; MENTE *et al.* 2009). Utilizing inbred strains of mice clearly demonstrates that dietary effects are dependent on the underlying genetic architecture(WEST *et al.* 1992; CHEVERUD *et al.* 2004; PARKS *et al.* 2013) In our study, the Mediterranean diet markedly reduced LDL cholesterol for A (*d* =

-3.29), FVB (*d* = -2.56) and NOD mice (*d* = -2.97) (Figure 4h) and also decreased HDL cholesterol in A (*d* = -3.48) and B6 (*d* = -1.59) (Figure 4i). The Japanese diet decreased LDL cholesterol in NOD (*d* = -3.04), B6 (*d* = -2.21) and FVB (*d* = -1.69) strains (Figure 4h), and decreased HDL cholesterol only in NOD (*d* = -1.54) (Figure 4i). Plasma triglycerides were elevated in FVB (*d* = 2.20) and A (*d* = 1.87) mice fed Japanese diet (Figure S3f).

The impact of low-carbohydrate diets on blood lipids is controversial. Studies have reported positive or negative effects depending on the age of the participants, obesity status, and duration of the diet (SHARMAN *et al.* 2002; KWITEROVICH *et al.* 2003; DASHTI *et al.* 2006). Our study found beneficial impacts of the ketogenic diet on cholesterol profiles with decreased LDL cholesterol across strains (*d* = -2.21 to -4.72) and increased HDL cholesterol in NOD mice (*d* = 2.49) (Figure 4h,i).

**Influence of Mediterranean, Japanese and ketogenic diets on glucose metabolism**

Impaired glucose homeostasis is common in patients with metabolic syndrome. Glucose tolerance improved in B6 (*d* = -1.47) and FVB (*d* = -1.43) strains fed Japanese diet (Figure 4j). In addition, fasted glucose concentration decreased in B6 mice (*d* = -0.88) (Figure S4g). Fasted insulin concentrations decreased in NOD (*d* = -1.30) but increased in FVB mice fed Mediterranean diet (*d* = 1.14) (Figure 5a, Figure S4h). The ketogenic diet did not improve glucose tolerance in any strain (Figure 4j), but fasted glucose (*d* = -0.89) and insulin (*d* = -1.23) were reduced in B6 mice (Figures 5a, S4g, S4h). In contrast, fasted glucose increased in NOD mice fed a ketogenic diet (*d* = 1.46), while fasted insulin was reduced (*d* = -1.39).

**The effect of diet on liver health differs by strains**

In addition to the effects of diet on the clinical plasma biomarkers of disease, we now appreciate the effects of lipid deposition in the liver on metabolic disease(KOTRONEN AND YKI-JARVINEN 2008; COHEN *et al.* 2011). We found genome-by-diet interactions influence liver phenotypes in mice fed Mediterranean, Japanese, and ketogenic diets. The Mediterranean diet reduced liver triglyceride concentrations in the A ($d$ = -1.20) and FVB ($d$ = -2.25) strains (Figure 4k). The ketogenic diet reduced liver triglycerides in A ($d$ = -1.02) and B6 ($d$ = -1.43). The Japanese diet lowered liver triglyceride concentrations across strains ($d$ = -1.21 to -2.75). Histological examination of A mice fed ketogenic diet revealed that while microvesicular steatosis was reduced ($d$ = -1.57, $p$ = 0.0008), macrovesicular steatosis was not ($p$ = 0.97) (Table S7). Importantly, serum ALT, a marker of liver damage, increased in A mice fed Japanese diet ($d$ = 1.31) (Figure S4i). While each diet benefitted some strains, no single diet was universally beneficial for liver health across strains (Figure 5a).

**Diet alters the liver metabolome in a strain-specific manner**

We compared the liver metabolome of mice fed Mediterranean, Japanese, and ketogenic diets relative to those fed American diet. The Mediterranean diet had little effect on the tissue metabolome across strains (Figure 5b), with Glycerol-3-phosphate in NOD mice being the only known metabolite altered ($d$=-0.78) (Figure 5h). B6 mice were the most sensitive to dietary changes on the Japanese and ketogenic diets (Figure 5b-d). Similar to the comparison between American and control mouse diets (Figure 3f), diet-induced changes were highly strain dependent with 79% of metabolite changes being unique to one strain for Mediterranean diet, 81% for ketogenic diet, and 94% for Japanese diet (Figure 5e-g).

**Categorical health effects of Mediterranean, Japanese, and ketogenic diets**

To provide a more comprehensive view of health effects, we analyzed four major categories of metabolic health (body composition, lipid profile, glucose metabolism, and liver health), by calculating health scores (HS) by combining phenotypes within each category across all strain-diet groups and standardizing the effects on a scale from -1 to +1, with negative numbers indicating worsened health and positive numbers indicating improved health relative to mice fed American diet.

The Mediterranean diet did not improve body composition, but was beneficial for liver health in A (HS = 0.71) and FVB (HS = 0.82) mice (Figure 6). Despite high variability, the FVB Mediterranean group had an improved lipid profile (HS = 0.44). The Mediterranean diet improved glucose metabolism in B6 mice (HS = 0.27) but was detrimental for A mice (HS = -0.28).

The Japanese diet improved body composition across strains (HS = 0.48 to 1.00). It did not consistently improve lipid profiles, but did provide the greatest improvement in glucose metabolism of all diets for B6 (HS = 1.00) and NOD mice (HS = 0.86). It also maximally improved liver health in the FVB (HS = 1.00) and NOD (HS = 1.00) strains, but had detrimental effects in the A strain (HS = -0.36).

The ketogenic diet improved body composition for B6 (HS = 0.93) and A (HS = 0.42) but was detrimental for NOD (HS = -0.28) and FVB (HS = -0.47) (Fig 7). It also improved blood lipid profiles across strains (HS = 0.37 to 1.00). Impacts on glucose metabolism varied, with a strong benefit in B6 mice (HS = 0.62), a mild benefit in A mice (HS = 0.18), no improvement in FVB, and a detrimental effect in NOD (HS = -

0.55). Effects on liver health also varied by strain with benefits in B6 (HS = 0.54) and A (HS = 0.48), but no benefit in FVB or NOD (Figure 6e).

**The healthiest alternative to the American diet depends on genetic background**

The mean of the four health scores (Mean Health Score, MHS) was quantified to provide a measure of collective metabolic health (Figure 7a,b). Only the ketogenic diet improved health for the A strain (MHS = 0.52); both the Japanese (MHS = 0.64) and ketogenic (MHS = 0.61) diets improved health in the B6 strain; and the Japanese diet improved health in FVB (MHS = 0.47) and NOD mice (MHS = 0.65) (Figure 7a,b).

There is an on-going debate over the influence of adiposity on metabolic health, with some suggesting that it is not the accumulation of adipose tissue, but the dysfunction of adipose tissue that causes negative metabolic consequences(GOOSSENS AND BLAAK 2015). The argument is strengthened by the observed lack of metabolic abnormalities in some obese individuals(BLUHER 2013). To determine the metabolic effects of these diets without consideration of adiposity, we calculated MHSs without the body composition parameter (Figure 7c,d). MHS rankings remained relatively consistent with a notable exception that the FVB Mediterranean diet group showed a significant benefit (MHS = 0.36) similar to that of the Japanese diet (MHS = 0.35) (Figure 7d).

## DISCUSSION

In an effort to increase the relevance of rodent findings to people and provide a wider survey of responses to diets, we constructed mouse diets based on dietary patterns of historic populations that better recapitulate human dietary profiles than previous studies. We showed that the American diet caused negative health effects across strains relative to the control diet. However, as in humans(O'DEA 1992; SCHULZ *et al.* 2006), severity of the effects varied across genetic backgrounds. Mice gained fat on the American diet, even though caloric intake did not significantly increase, which is in agreement with previous studies in mice and humans and emphasizes the importance of factors other than caloric intake alone on fat gain and body weight(KEEN *et al.* 1979; WACK AND RODIN 1982; BAECKE *et al.* 1983; KROMHOUT 1983; BRAITMAN *et al.* 1985; ROMIEU *et al.* 1988; NICKLAS *et al.* 1993; PRENTICE AND JEBB 1995; HEINI AND WEINSIER 1997; JARVANDI *et al.* 2011; FORD AND DIETZ 2013; LADABAUM *et al.* 2014). Metabolic rate increased in A mice fed American diet, which may in part explain the strain's resistance to weight gain, although additional research is needed to demonstrate causality.

This study revealed strain-specific, diet-induced epigenetic modification of the vasopressin receptor, *Avpr1a*. The phenotypic changes observed in B6 mice fed the American diet are consistent with the *Avpr1a* knockout mice(AOYAGI *et al.* 2007). Furthermore, people carrying a single-nucleotide polymorphism in the *AVPR1A* gene exhibit phenotypes consistent with both *Avpr1a* knockout mice and B6 mice fed Western diet, including increased incidence of diabetes in those eating a Western-style diet(ENHORNING *et al.* 2009). These data suggest that *AVPR1A* is influential in diet responsiveness.

After comparing the impact of the American diet to a control mouse diet, we investigated how health status differed in mice fed the American diet relative to those fed other human-comparable diets and identified that genome-by-diet interactions were influential for most metabolic phenotypes. Overall, while each strain had a diet or diets that improved health relative to the American diet, no single diet improved health across all genetic backgrounds.

The FVB strain showed beneficial health effects when fed the Mediterranean diet, even though body composition was not improved. This is consistent with human studies that find beneficial metabolic impacts of a Mediterranean diet in some individuals without reducing food intake or body weight(Salas-Salvado *et al.* 2011; Estruch *et al.* 2013). The Japanese diet yielded metabolic benefits in all strains except A, in agreement with the epidemiological evidence showing generally positive health effects of a Japanese diet in people(Gordon 1957; Marmot *et al.* 1975; Marmot and Syme 1976; Kagawa 1978).

The ketogenic diet increased metabolic rate, in agreement with human research suggestive of a “metabolic advantage” of low-carbohydrate diets(Feinman and Fine 2003). Lipid profiles of all strains improved on the ketogenic diet, consistent with the healthy cardiovascular status of the Maasai population(Mann *et al.* 1964; Mann *et al.* 1965). Effects of high fat, low-carbohydrate diets on glucose response in people are controversial with studies having conflicting results(Accurso *et al.* 2008; Delahanty *et al.* 2009). Extrapolating from mouse data, our results suggest that genetic variation may underlie the heterogeneity of diet response, as A and B6 mice fed the ketogenic diet experienced benefits to glucose homeostasis, while FVB and NOD mice did not. The ketogenic diet improved overall health in A and B6 strains but not FVB or NOD strains.

Our findings have potential limitations. While great care was taken to accurately recreate mouse versions of human diets, there are differences including lack of fresh ingredients and spices that may contain additional bioactive compounds. The diets had a baseline vitamin and mineral content, whereas some human diets may lack certain vitamins or minerals. In addition, fiber was limited to one source of insoluble fiber (cellulose) and one source of soluble fiber (inulin) to easily manipulate the levels of these fiber classes, but a complete diet would contain several sources of each fiber class. While our study was not designed to detect the interaction of diet with sex, there is a growing appreciation that these interactions are influential in metabolic health(Bolnick *et al.* 2014; Arnold *et al.* 2017; Link *et al.* 2017; Reue 2017). There are differences in metabolism between humans and mice that could affect outcomes across species, although previous research has demonstrated utility of mouse models in studying metabolic effects in humans(Rohner-Jeanrenaud and Jeanrenaud 1996; von Scheidt *et al.* 2017). This study demonstrates the utility of mouse models to dissect genetic by diet interactions, however studies on diet responsiveness in humans are needed to extend findings to people.

Evaluating diet response in the context of genetic background enabled a much clearer understanding of dietary effects. Nonetheless, some variation persisted within diet-by-strain groups. Additional factors yet to be identified must play a role in diet response. One possibility is that the gut microbiome impacted phenotypes in this study(Backhed *et al.* 2007). Individual-specific epigenetic differences may also have played a role.

This study in mice demonstrates that the health effects of several popular human dietary patterns are dependent on genetic background, adding to a growing appreciation for individual variation in dietary considerations(West *et al.* 1992; Parks *et al.* 2013; Konstantinidou *et al.* 2014; Zeevi *et al.* 2015; Korem *et al.* 2017). Determining the extent to which genetic factors influence diet response in humans is difficult given complex genetic variability and environmental confounders. Even so, the disparate health consequences of a Western-style diet across genetically distinct human populations and the strong concordance of dietary response in monozygotic compared to dizygotic twins suggest that genetics plays an important role. If genetics impacts diet response similarly in people as in mice, then the implementation of personalized dietary recommendations will be important for the mitigation of metabolic disease.

## AUTHOR CONTRIBUTIONS

WTB, DWT, DP, and APF conceived and designed the study; WTB and MAP formulated diets; WTB, DWT, KH, and DP performed mouse experiments; AEW and BHV performed metabolomic analysis; CMR performed cholesterol analysis; SYFH performed adipokine analysis; BJB performed triglyceride analysis; AP performed histological analysis; PW, KDH and APF performed library preparation, sequencing, and epigenetic analysis; WTB, AEW, KDH, and PW performed statistical analysis; WTB and DWT designed the figures and wrote the manuscript; and all authors revised the manuscript.

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## COMPETING FINANCIAL INTERESTS

We do not have any competing financial interests to declare.

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**Figure 1. Diet ingredient profiles and geographic origins.**

(**a**) The purified control mouse diet, used for comparison to the American diet in our study, is typical of those used in mouse research. (**b**) Previous studies have evaluated metabolic effects using Western or high-fat diets. Instead, we designed human-comparable diets representative of the dietary patterns in human populations including (**c**) a contemporary American diet, (**d**) a traditional Mediterranean diet, (**e**) a ketogenic diet analogous to the Maasai diet, (**f**) and a traditional Japanese diet.

**Figure 2. Comparison of metabolic phenotypes in each strain for mice fed American diet relative to the control mouse diet.**

Effect of American diet relative to the control mouse diet in each strain for (**a**) activity (n=9-10), (**b**) heat expenditure (n=9-10), (**c**) food intake (n=4-10), (**d**) water intake (n=7-10), (**e**) body weight (n= 19- 20), (**f**) percent body fat (n = 19-20), (**g**) HDL cholesterol (n = 4-10), (**h**) LDL cholesterol (n = 4-10), (**i**) glucose tolerance test (GTT) (n = 12-20, except NOD American (n = 4)), (**j**) liver triglyceride concentration (n = 13-20). (**k**) Heatmap of health effect size (Cohen's d with higher value indicating improved health and lower value indicating diminished health) for metabolic phenotypes across strains. Data are mean +/- standard error. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ANOVA between means.

**Figure 3. Genetic-by-diet interactions in the methylation status of the *Avpr1a* locus and liver metabolome alterations.**

(**a**) B6 mice fed American diet are hypermethylated relative to those fed the control mouse diet at the *Avpr1a* locus (n=8), $p < 0.0001$ by students t-test. (**b**) American diet feeding did not alter methylation status in the A strain (n=7-8), $p = 0.49$ by students t-test. (**c**) Transcript expression of *Avpr1a* was reduced by 84% in B6 mice fed American diet relative to other strain-diet groups in mice fed diets for 6 months (n = 4-5), $p < 0.0001$ by ANOVA. (**d**) Transcript expression of *Avpr1a* is reduced by 54% in B6 mice fed American diet for two weeks (n=4), $p = 0.0077$ by students t-test. (**e**) Number of liver metabolites, including both known and unknown, significantly altered by American diet relative to control mouse diet. (**f**) Proportion of metabolites significantly changed in all four strains (2%), three strains (4%), two strains (10%), or unique to one strain (84%). (**g**) Heatmap of effect sizes (Cohen's *d*) for known metabolites significantly altered by American diet relative to control mouse diet across strains. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ANOVA between means with Benjamin Hochberg correction factor.

**Figure 4. Comparison of metabolic phenotypes in each strain for mice fed Mediterranean, Japanese, or ketogenic diets relative to American diet.**

Effects of Mediterranean, Japanese and ketogenic diets relative to American diet in each strain are shown for (**a**) activity (n = 8-10), (**b**) heat expenditure (n = 8-10), (**c**) food intake (n = 4-10), (**d**) water intake (n = 7-10), (**e**) body weight (n= 17-20), (**f**) percent body fat (n = 17-20). The influence of activity and metabolic rate on percent body fat varies by strain and diet, as shown for mice in which metabolic rate and activity was measured (**g**). Effects of Mediterranean, Japanese and ketogenic diets relative to American diet in each strain are shown for (**h**) HDL cholesterol (n = 4-10), (**i**) LDL cholesterol (n= 4-10), (**j**) glucose tolerance test (GTT) (n = 9-20), (**k**) and liver triglyceride concentration (n = 11-20). Data are mean +/- standard error. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ANOVA between means with Dunnett's correction to American diet within each strain.

**Figure 5. Comparison of metabolic phenotypes and liver metabolites in mice fed Mediterranean, Japanese, or ketogenic diets relative to American diet.**

(**a**) Heatmap of health effect size (Cohen's d with higher value indicating improved health and lower value indicating diminished health) for metabolic phenotypes across strains. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ANOVA between means with Dunnett's correction to American diet within each strain. Number of known and unknown liver metabolites significantly altered compared to American diet for (**b**) Mediterranean diet, (**c**) Japanese diet, and (**d**) ketogenic diet. Proportion of metabolites significantly changed in all four strains, three strains, two strains, or unique to one strain for (**e**) Mediterranean, (**f**) Japanese, and (**g**) ketogenic diets. (**h**) Heatmap of effect sizes (Cohen's *d*) known metabolites significantly altered relative to American diet across strains, including metabolites that differed between American and control mouse diets (Figure 3g). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ by ANOVA between means with Benjamin Hochberg correction factor.

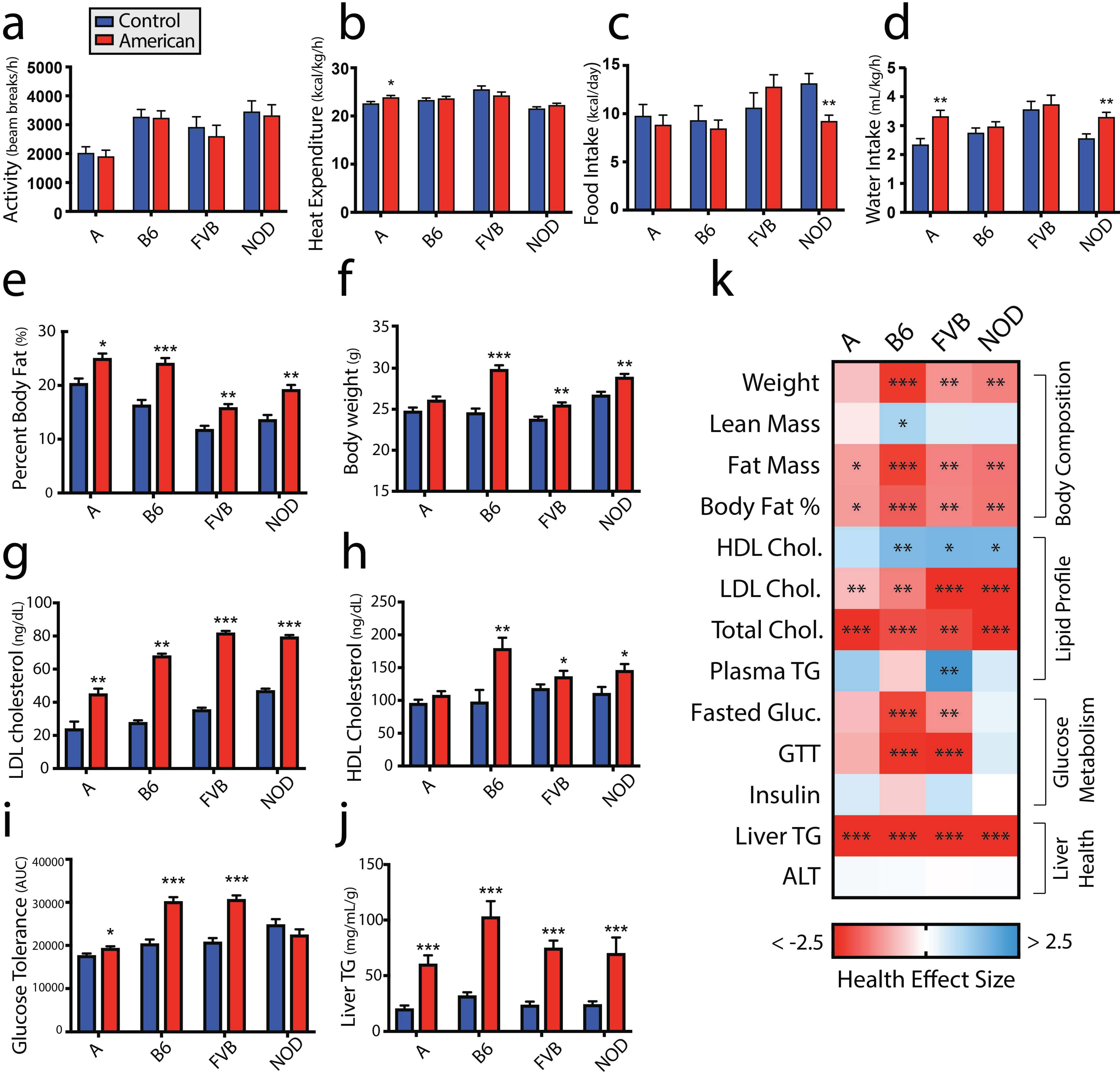
**Figure 6. Scores of four health categories for alternative diets relative to American diet in each strain.**

Health scores indicate cumulative health effects of alternative diets relative to American diet for four categories: body composition (lean mass, fat mass, percent body fat), lipid profile (HDL, LDL, plasma triglycerides), glucose metabolism (fasted glucose, fasted insulin, GTT), and liver health (liver triglycerides, ALT). A positive score represents improved health and a negative score represents diminished health. Scores and 95% confidence intervals are shown for (**a**) A strain, (**b**) B6 strain, (**c**) FVB strain, (**d**) and NOD strain. (**e**) The data is also represented in a heat map for comparison with red showing improved health scores relative to American diet.

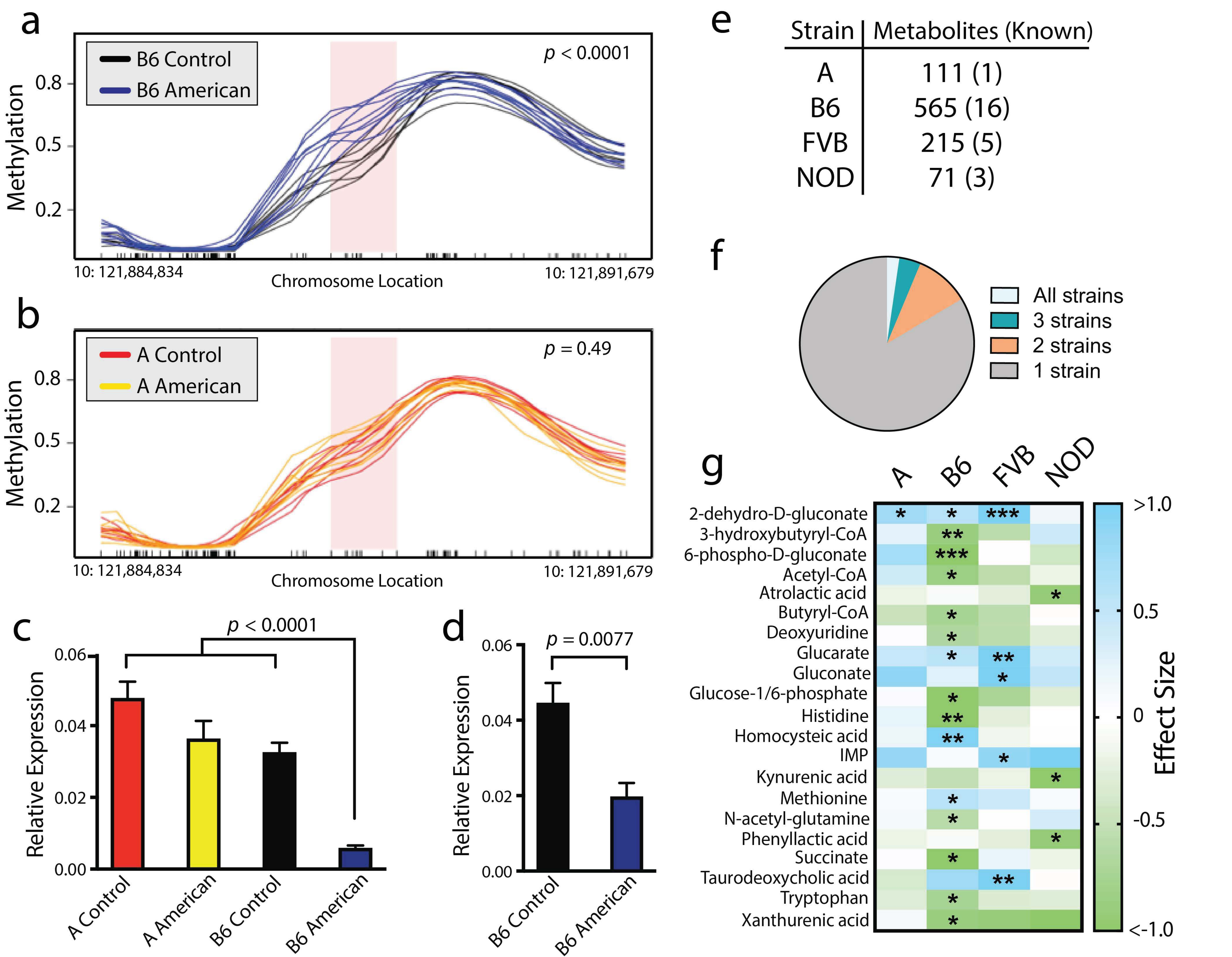
**Figure 7. Mean Health Scores for comparison overall metabolic health relative of mice fed alternative diets relative to American diet in each strain.**

The four health category scores (Figure 6) were averaged to provide a measure of overall metabolic health for each alternative diet relative to American diet. A positive score represents improved health and a negative score represents diminished health. (**a**) Scores were calculated with body composition included and shown with 95% C.I. (**b**) or represented with a heat map. (**c**) Scores are also calculated without body composition parameter and shown with 95% C.I. (**d**) or represented with a heat map.

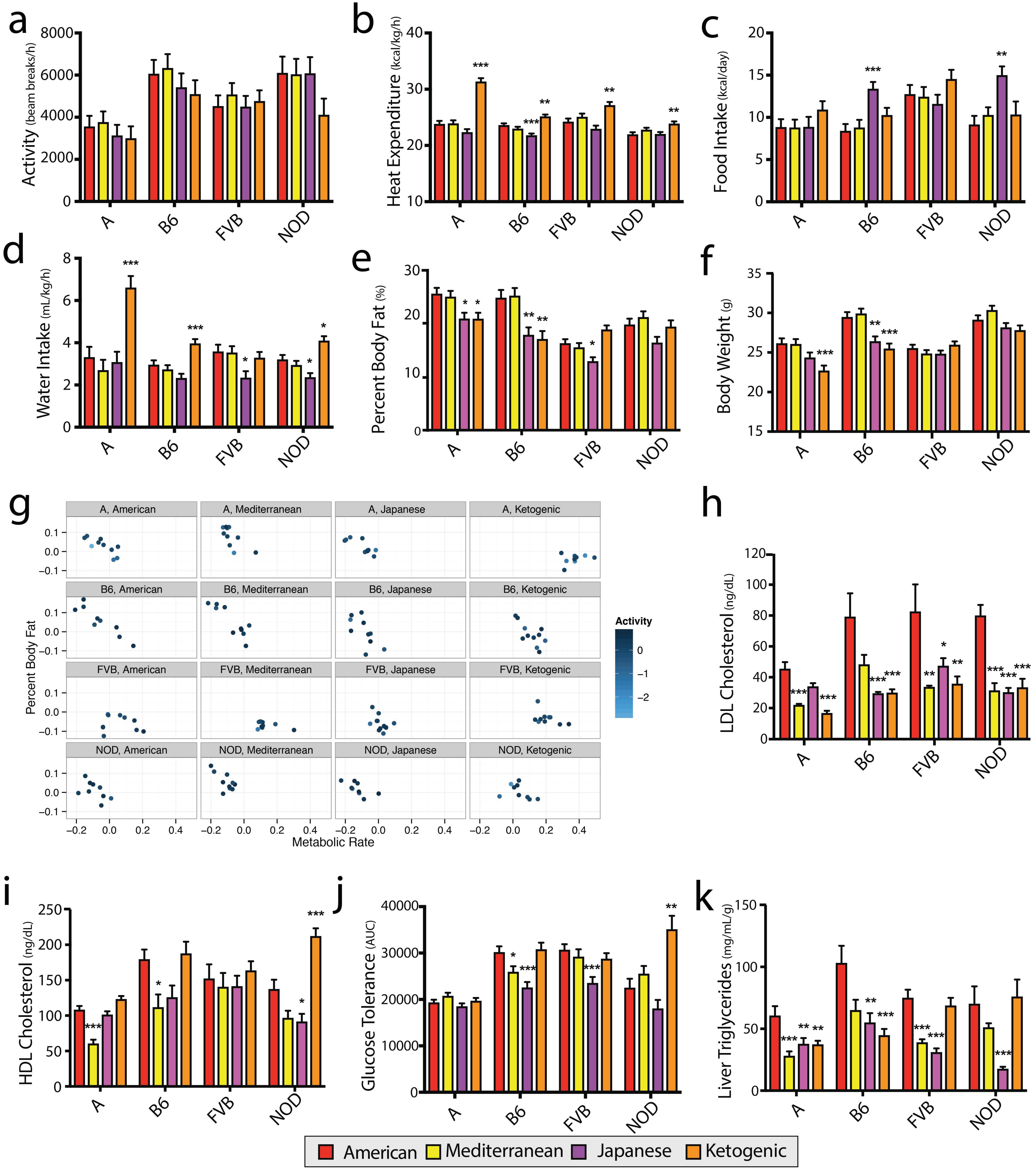
Traditional Mouse Diets
Control Mouse Diet
a
Casein
Corn Starch
Soybean Oil
Western Diet
b
Casein
Corn Starch
Sucrose
Butter
Maltodextrin
Human-Comparable Mouse Diets
American Diet
c
Casein
Fish Protein
Egg White
Beef Protein
Corn Starch
Wheat Starch
Potato Starch
Sucrose
Fructose
Corn Oil
Fish Oil
Butter
Flaxseed Oil
Olive Oil
Mediterranean Diet
d
Casein
Fish Protein
Egg White
Beef Protein
Wheat Starch
Sucrose
Fructose
Fish Oil
Palm Oil
Butter
Flaxseed Oil
Olive Oil
Red Wine Extract
e
Casein
Corn Oil
Fish Oil
Butter
Lard
Ketogenic (Maasai) Diet
f
Soy Protein
Fish Protein
Egg White
Beef Protein
Rice Starch
Sucrose
Fructose
Soybean Oil
Fish Oil
Butter
Olive Oil
Green Tea Extract
Japanese Diet



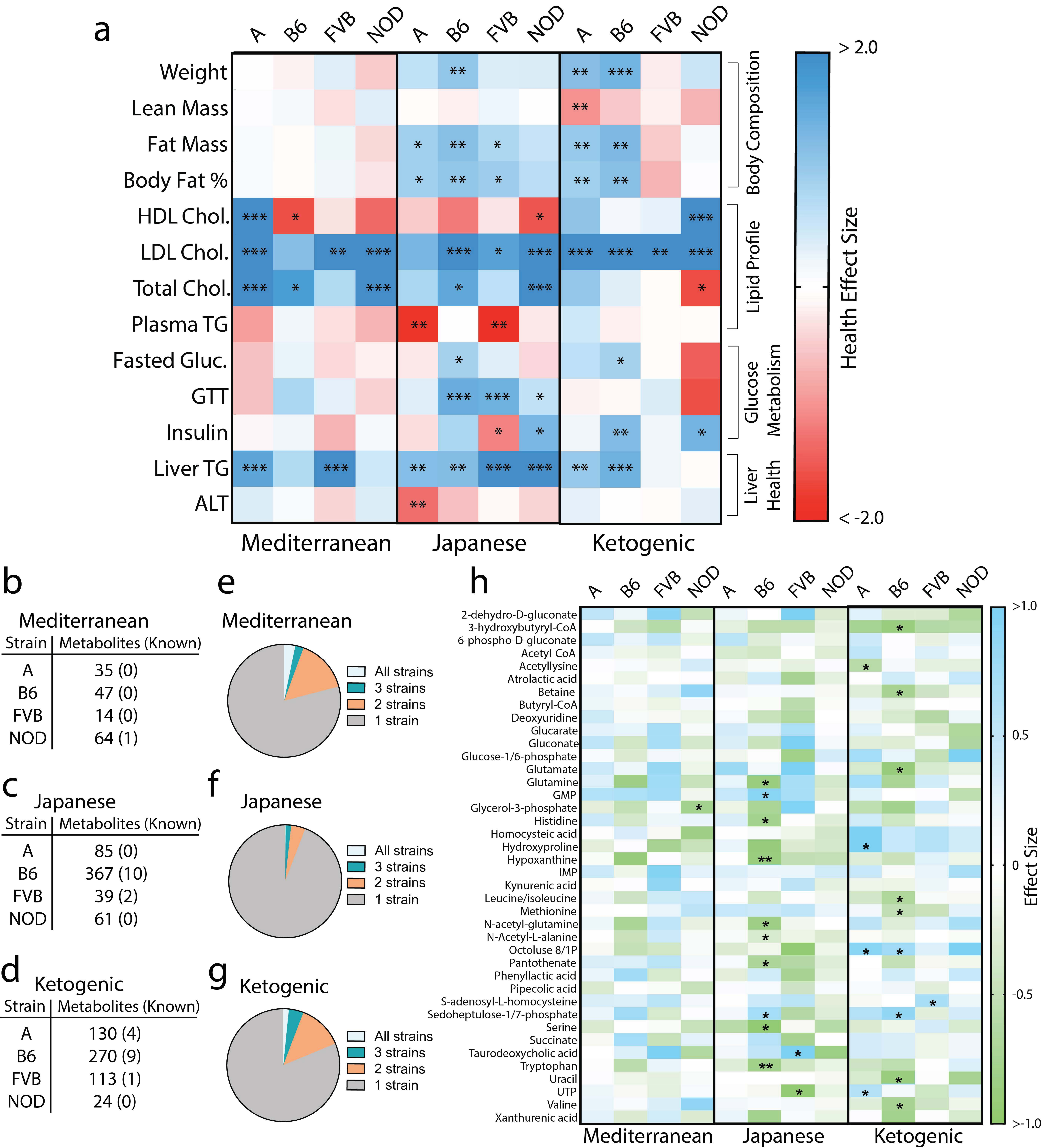
a
Control
American
Activity (beam breaks/h)
b
Heat Expenditure (kcal/kg/h)
c
Food Intake (kcal/day)
d
Water Intake (mL/kg/h)
e
Percent Body Fat (%)
f
Body weight (g)
g
LDL cholesterol (ng/dL)
h
HDL Cholesterol (ng/dL)
i
Glucose Tolerance (AUC)
j
Liver TG (mg/mL/g)
k
A
B6
FVB
NOD
Weight
Lean Mass
Fat Mass
Body Fat %
HDL Chol.
LDL Chol.
Total Chol.
Plasma TG
Fasted Gluc.
GTT
Insulin
Liver TG
ALT
Body Composition
Lipid Profile
Glucose Metabolism
Liver Health
< -2.5
> 2.5
Health Effect Size



a
B6 Control
B6 American
p < 0.0001
Methylation
0.8
0.5
0.2
10: 121,884,834
Chromosome Location
10: 121,891,679
b
A Control
A American
p = 0.49
Methylation
0.8
0.5
0.2
10: 121,884,834
Chromosome Location
10: 121,891,679
c
p < 0.0001
Relative Expression
0.06
0.04
0.02
0.00
A Control
A American
B6 Control
B6 American
d
p = 0.0077
Relative Expression
0.06
0.04
0.02
0.00
B6 Control
B6 American
e
Strain | Metabolites (Known)
A 111 (1)
B6 565 (16)
FVB 215 (5)
NOD 71 (3)
f
All strains
3 strains
2 strains
1 strain
g
A
B6
FVB
NOD
2-dehydro-D-gluconate
3-hydroxybutyryl-CoA
6-phospho-D-gluconate
Acetyl-CoA
Atrolactic acid
Butyryl-CoA
Deoxyuridine
Glucarate
Gluconate
Glucose-1/6-phosphate
Histidine
Homocysteic acid
IMP
Kynurenic acid
Methionine
N-acetyl-glutamine
Phenyllactic acid
Succinate
Taurodeoxycholic acid
Tryptophan
Xanthurenic acid
>1.0
0.5
0
-0.5
<-1.0
Effect Size



a
Activity (beam breaks/h)
b
Heat Expenditure (kcal/kg/h)
c
Food Intake (kcal/day)
d
Water Intake (mL/kg/h)
e
Percent Body Fat (%)
f
Body Weight (g)
g
A, American
A, Mediterranean
A, Japanese
A, Ketogenic
B6, American
B6, Mediterranean
B6, Japanese
B6, Ketogenic
FVB, American
FVB, Mediterranean
FVB, Japanese
FVB, Ketogenic
NOD, American
NOD, Mediterranean
NOD, Japanese
NOD, Ketogenic
Percent Body Fat
Metabolic Rate
Activity
h
LDL Cholesterol (ng/dL)
i
HDL Cholesterol (ng/dL)
j
Glucose Tolerance (AUC)
k
Liver Triglycerides (mg/mL/g)
A
B6
FVB
NOD
American
Mediterranean
Japanese
Ketogenic




b

Mediterranean

| Strain | Metabolites (Known) |
|---|---|
| A | 35 (0) |
| B6 | 47 (0) |
| FVB | 14 (0) |
| NOD | 64 (1) |

c

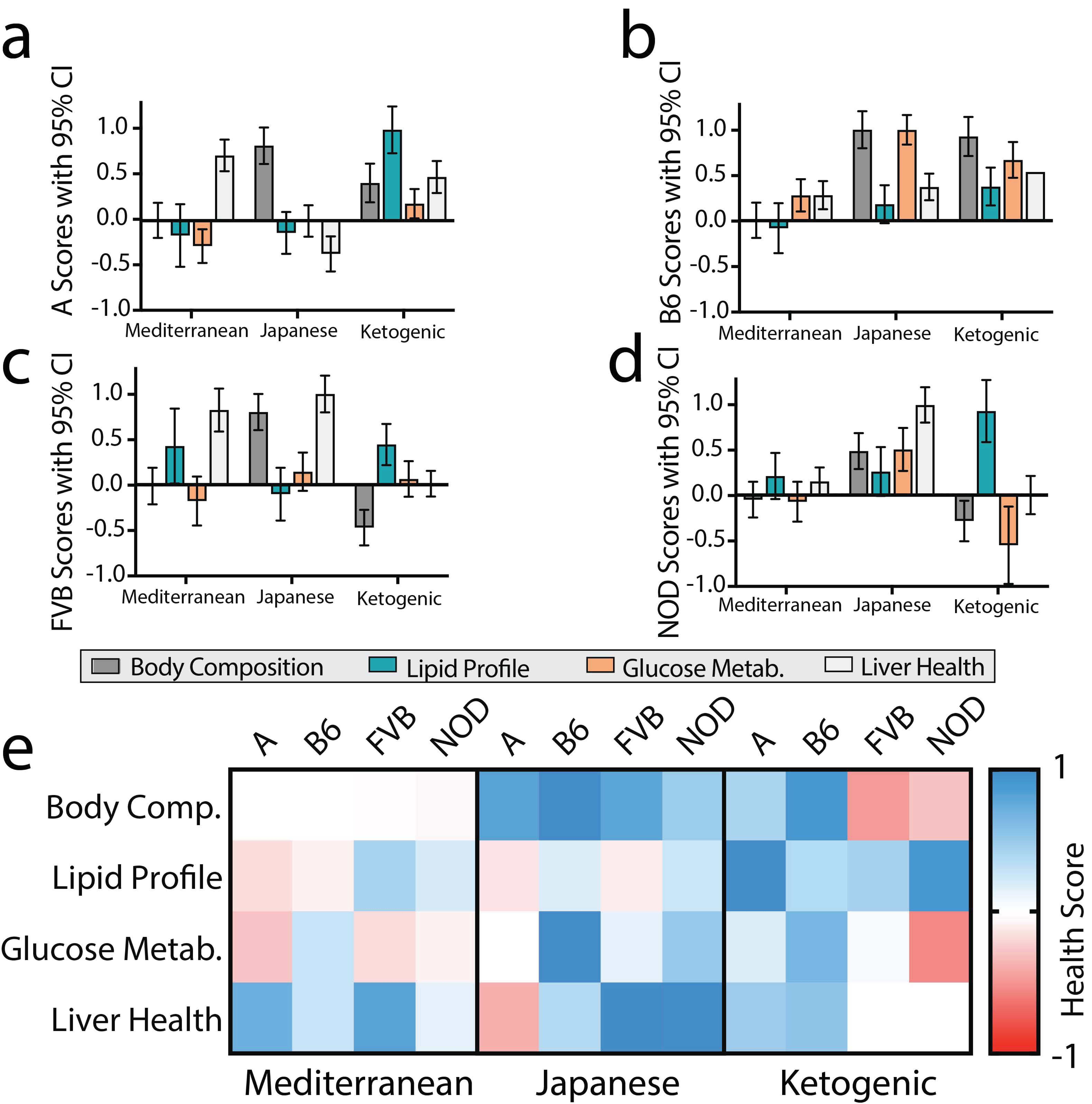
Japanese

| Strain | Metabolites (Known) |
|---|---|
| A | 85 (0) |
| B6 | 367 (10) |
| FVB | 39 (2) |
| NOD | 61 (0) |

d

Ketogenic

| Strain | Metabolites (Known) |
|---|---|
| A | 130 (4) |
| B6 | 270 (9) |
| FVB | 113 (1) |
| NOD | 24 (0) |


a
A Scores with 95% CI
1.0
0.5
0.0
-0.5
-1.0
Mediterranean
Japanese
Ketogenic
b
B6 Scores with 95% CI
c
FVB Scores with 95% CI
d
NOD Scores with 95% CI
Body Composition
Lipid Profile
Glucose Metab.
Liver Health
e
A
B6
FVB
NOD
Body Comp.
Lipid Profile
Glucose Metab.
Liver Health
Mediterranean
Japanese
Ketogenic
1
-1
Health Score

a
b
c
d
Mean Score with 95% C.I.
1.0
0.5
0.0
-0.5
-1.0
A
B6
FVB
NOD
Mediterranean
Japanese
Ketogenic
Health Score
1
-1