Regulation of metabolic health by dietary histidine in mice

Victoria Flores[1,2,3], Alexandra B. Spicer[4], Michelle Sonsalla[1,2], Nicole E. Richardson[1,2,5], Deyang Yu[1,2,6], Grace E. Sheridan[1,2], Michaela E. Trautman[1,2,3], Reji Babygirija[1,2,7], Eunhae P. Cheng [1,2], Jennifer M. Rojas[8], Shany E. Yang [1,2], Matthew H. Wakai[1,2], Ryan Hubbell[1,2], Ildiko Kasza[9], Jay L. Tomasiewicz[1], Cara L. Green[1,2], Claudia Dantoin[1,2], Caroline M. Alexander[9], Joseph A. Baur[8], Kristen C. Malecki[4], Dudley W. Lamming[1,2,3,5,6,7,10,*]

[1]William S. Middleton Memorial Veterans Hospital, Madison, WI 53705, USA
[2]Department of Medicine, University of Wisconsin-Madison, Madison, WI 53705, USA
[3]Interdepartmental Graduate Program in Nutritional Sciences, University of Wisconsin-Madison, Madison, WI 53706, USA
[4]Department of Population Health Sciences, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI 53726, USA
[5]Endocrinology and Reproductive Physiology Graduate Training Program, University of Wisconsin-Madison, Madison, WI 53706, USA
[6]Molecular and Environmental Toxicology Program, University of Wisconsin-Madison, Madison, WI 53706, USA
[7]Graduate Program in Cellular and Molecular Biology, University of Wisconsin-Madison, Madison, WI 53706, USA
[8]Department of Physiology and Institute for Diabetes, Obesity, and Metabolism, University of Pennsylvania, Philadelphia, PA 19104, USA
[9]McArdle Laboratory for Cancer Research, University of Wisconsin-Madison, Madison, WI 53705, USA
[10]University of Wisconsin Carbone Cancer Center, Madison, WI 53705, USA

***Correspondence and Lead Contact**
Dudley W. Lamming, PhD
Associate Professor of Medicine
University of Wisconsin-Madison
2500 Overlook Terrace
VAH C3127 Research 151
Madison, WI 53705, USA
dlamming@medicine.wisc.edu
Tel: 608-256-1901 x12861
Fax: 608-263-9983;

Abstract

The increasing prevalence of obesity is a serious threat to global health. Low protein (LP) diets are associated with a decreased risk of diabetes in humans, and a low protein diet promotes leanness and glycemic control in both rodents and humans. The effects of a LP diet on glycemic control are mediated by reduced dietary levels of the branched-chain amino acids (BCAAs). However, we have observed that reducing dietary levels of the other six essential amino acids leads to changes in body composition. Here, we find that dietary histidine plays a key role in the response to a LP diet. Specifically reducing dietary levels of histidine by 67% reduces weight gain of young, lean C57BL/6J mice, reducing both adipose and lean mass gain, without altering glucose metabolism. Specifically reducing dietary histidine rapidly reverses diet-induced obesity and hepatic steatosis in diet-induced obese mice, increasing insulin sensitivity; this normalization of metabolic health was associated not with caloric restriction or increased activity, but with increased energy expenditure that surprisingly did not require *Fgf21*. Histidine restriction started in mid-life promoted leanness and glucose tolerance in aged males, but did not affect frailty or lifespan in either sex. Finally, we demonstrate that variation in dietary histidine levels helps to explain body mass index differences in humans. Overall, our findings demonstrate that dietary histidine is a key regulator of weight and body composition in both mice and humans, and suggest that reducing dietary levels of histidine may be a highly translatable option for the treatment of obesity.

Keywords: histidine, amino acids, protein restriction, BMI, body composition, FGF21

Introduction

Obesity is an increasingly prevalent health concern around the world. Approximately 40% of adult Americans are now considered to be obese, and around the world 2 billion people were overweight in 2016 (Ogden *et al.*, 2014). Obesity is a risk factor for many other diseases, including Alzheimer's disease, cancer, cardiovascular disease and diabetes. Although many individuals who are overweight or obese desire to improve their health and reduce the risk of developing these conditions by losing weight, diets that simply reduce caloric intake are very difficult for most people to sustain.

Over the last two decades, it has become clear that calories from different macronutrient groups differ in their ultimate metabolic impact and effects on health and longevity, and that the traditional view of food and obesity – that a calorie is "just a calorie" – is too simple. Multiple studies have found that calories from carbohydrates, fat, and protein all have unique effects on human metabolism (Hall *et al.*, 2015; Fontana *et al.*, 2016). Calories from protein have generally been thought of as beneficial, as dietary protein promotes satiety and displaces carbohydrates and calorie-dense fat; further, some metabolic benefits of dietary protein have been noted in short-term studies (Seino *et al.*, 1983; Gannon *et al.*, 2003; Dong *et al.*, 2013). However, multiple long-term observational studies have found that humans eating a low protein (LP) diet have a reduced incidence of diabetes and other age-related diseases (Linn *et al.*, 2000; Lagiou *et al.*, 2007; Sluijs *et al.*, 2010; Levine *et al.*, 2014; van Nielen *et al.*, 2014). In a short-term randomized clinical study, an LP diet promotes leanness and glycemic control in humans with obesity (Fontana *et al.*, 2016). LP diets also improve metabolic health in rodents, promoting leanness and blood glucose control in the context of both lean and diet-induced obese animals, and increasing

lifespan (Laeger *et al.*, 2014; Solon-Biet *et al.*, 2014; Solon-Biet *et al.*, 2015; Fontana *et al.*, 2016; Maida *et al.*, 2016; Cummings *et al.*, 2018; Richardson *et al.*, 2021).

We hypothesized that that the beneficial effects of an LP diet might be the results of reduced consumption of specific essential amino acids. In initial studies conducted over the last decade, we focused on the branched-chain amino acids (BCAAs; leucine, isoleucine, and valine), which have been linked to insulin-resistant obesity and diabetes in both rodents and humans. We demonstrated that a 67% restriction of all three BCAAs (or isoleucine alone) recapitulates the effects of an LP diet in lean and Western diet-induced obese mice, enhancing leanness and blood glucose control; we have also observed beneficial effects of an LP diet on aged wild-type mice (Fontana *et al.*, 2016; Cummings *et al.*, 2018; Richardson *et al.*, 2021; Yu *et al.*, 2021). Intriguingly though, we observed that a diet with a 67% restriction of the other six essential amino acids – histidine, lysine, methionine, phenylalanine, threonine, and tryptophan – resulted in reduced weight gain and adiposity without improving glucose tolerance (Fontana *et al.*, 2016).

Here, we investigate the role of these other six essential amino acids, examining the effects of reducing each of these amino acids individually. We find that that restriction of either histidine, phenylalanine, or threonine is sufficient to reduce adiposity in young mice, but does not alter blood glucose control. As restriction of dietary threonine has recently been shown to improve metabolic health by another group (Yap *et al.*, 2020), we decided to focus on the how the restriction of histidine or phenylalanine affects the metabolic health of diet-induced obese mice. We find that restricting histidine dramatically reduces adiposity and restores normal body composition to diet-induced obese mice, while restriction of phenylalanine has a smaller but still positive effect on weight and body composition, and that this is associated with increased energy expenditure. Conversely, we find that dietary histidine levels are correlated with weight and

adipose gain during Western diet feeding of young lean mice. Surprisingly, the effects of dietary histidine are independent of the energy balance hormone fibroblast growth factor 21 (FGF21), which has been implicated in the response to protein restriction as well as the restriction of a number of other essential amino acids (Laeger *et al.*, 2014; Yap *et al.*, 2020; Yu *et al.*, 2021). Although restriction of histidine beginning in midlife improves metabolic health in male mice, it does not impact frailty or lifespan in either males or females. Finally, we find that the consumption of dietary protein with increased histidine content is associated with increased body mass index (BMI) in humans. In conclusion, these results demonstrate that dietary histidine is a critical regulator of weight and body composition, and suggest that interventions based on decreasing dietary levels of histidine may be a translatable way to promote and restore metabolic health.

Results

Reducing dietary histidine, phenylalanine, or threonine reduces weight and fat mass gain in lean mice

We determined the metabolic effect of restricting each of the six non-BCAA essential amino acids (AAs) individually. Our AA-defined control (Ctrl AA) diet contained all twenty common AAs; the diet composition reflects that of a natural chow diet in which 21% of calories are derived from protein and which we have utilized in several previous studies (Richardson *et al.*, 2021; Yu *et al.*, 2021). We created a diet series based on this diet, in which either histidine, lysine, methionine, phenylalanine, threonine, or tryptophan was reduced by 67%. All seven diets are isocaloric, with identical levels of fat, and the calories from reduced essential AAs were balanced by increased levels of non-essential AAs to keep the calories derived from AAs constant (**Table S1**).

In our first experimental cohort, we fed C57BL/6J male mice either a Ctrl AA diet or a diet in which either threonine (Low Thr), phenylalanine (Low Phe), or histidine (Low His) was reduced by 67% (**Fig. 1A**). Tracking weight and body composition over three months, we observed significant effects of Low Phe and Low His diets, with mice fed these diets gaining less than half the weight of Ctrl AA-fed mice (**Fig. 1B**). The effect of the Low Phe and Low His diets was due to a reduction in both fat mass and lean mass gain (**Figs. 1C-1D**). In contrast, a Low Thr diet had no significant effect on weight, but had significantly reduced fat mass gain with a numerical increase in lean mass. The overall effect of all three diets was decreased adiposity (**Fig. 1E**).

We used metabolic chambers to evaluate energy balance, analyzing food consumption, spontaneous activity, and energy expenditure. Intriguingly Low Phe-fed and Low His-fed mice had a significant increase in food consumption on both a per mouse and per body weight basis (**Figs. 1F and S1A**). Consistent with the reduced weight gain and fat accretion of these mice, this increased calorie intake was offset by increased energy expenditure, while spontaneous activity was unaltered (**Figs. 1G and S1B-S1C**). The respiratory exchange ratio (RER), which reflects substrate utilization, was increased at night in mice consuming Low Phe or Low His diets, suggesting increased carbohydrate utilization (**Fig. S1D**). Finally, we assessed blood glucose control by conducting glucose and insulin tolerance tests. We observed no significant effects of any of the diets on either glucose tolerance or insulin sensitivity (**Figs. 1H and S1E**).

Reducing lysine, methionine, or tryptophan has minimal effects on weight and body composition

We utilized a second cohort of mice to test the effect of specifically reducing methionine (Low Met), lysine (Low Lys), or tryptophan (Low Trp) by 67% (**Fig. 2A**). We found that these

diets had minimal effects on weight, fat and lean mass accretion, or adiposity, with weight and no other parameters being affected by a Low Trp diet only (**Figs. 2B-2E**). Consistent with these findings, food consumption, energy expenditure, and substrate utilization was not altered in mice fed any of these three diets (**Figs. 2F-2G and S2A-S2D**). We again observed no significant effects of any of the diets on either glucose tolerance or insulin sensitivity (**Figs. 2H and S2E**).

Specifically restricting dietary histidine restores normal body composition to DIO mice, increasing energy expenditure

We chose to focus our efforts on phenylalanine and histidine, as restriction of these two essential amino acids had by far the greatest impact on weight, body composition, and energy balance; additionally, another group had already found beneficial metabolic effects of restricting dietary threonine (Yap *et al.*, 2020). In our previous work, we determined that restriction of dietary protein can restore metabolic health to diet-induced obese (DIO) even while they continue to consume a high-fat, high-sucrose Western Diet (WD) (Cummings *et al.*, 2018; Yu *et al.*, 2021). We therefore decided to examine the metabolic impact of reducing levels of phenylalanine or histidine in mice pre-conditioned with a WD for 12 weeks (**Fig. 3A**).

To specifically address this question, we designed a new series of diets based on an amino acid-defined WD (WD Control AA) that we have previously utilized (Cummings *et al.*, 2018; Richardson *et al.*, 2021); this diet closely matches the macronutrient profile of a widely used naturally sourced Western diet (TD.88137). Briefly, the level of either phenylalanine (WD Low Phe), histidine (WD Low His), or all amino acids (WD Low AA) was reduced by 67% in the context of a WD; all of the WDs were isocaloric with identical levels of fat, and in case of the WD Low Phe and WD Low His diets, the percentage of calories derived from AAs was kept

constant by proportionally adjusting the amount of non-essential AAs (**Table S2**). In parallel, a group of chow-fed mice never exposed to a WD were switched to the Ctrl AA diet.

As we expected based on our previous studies, DIO mice switched to the WD Control AA diet remained obese, lean mice switched to the Ctrl AA diet remained comparatively light and lean, and mice switched to the WD Low AA diet rapidly lost weight and fat mass, with an overall reduction in adiposity (**Fig. 3B-3E**). Surprisingly, DIO mice switched to the WD Low His diet diets progressively lost weight for 3 weeks, similarly to the WD Low AA-fed mice; both the WD Low His and WD Low AA-fed mice then continued to lose weight at a reduced weight for the next 3 weeks, which then stabilized (**Fig. 3B**). The majority of the weight loss of both groups of mice was due to a dramatic decrease in fat mass, whereas lean mass was not as affected, leading to an overall reduction in adiposity (**Fig. 3C-3E**). WD Low Phe-fed mice showed a lesser degree of weight loss, and did not have a statistically significant different in fat mass or adiposity from mice fed the WD Control AA diet (**Fig. 3B-E**).

We next used metabolic chambers to evaluate energy balance, analyzing food consumption, spontaneous activity, and energy expenditure. We observed that mice fed either the WD Low AA and WD Low His diet had higher food consumption than WD Control AA-fed mice on both a per mouse and per body weight basis (**Figs. 3F and S3A**). Consistent with the reduced body weight of animals fed these diets, our previous observations of WD Low AA-fed mice (Cummings *et al.*, 2018; Yu *et al.*, 2021), and our observations in lean Low His-fed mice not exposed to a WD, we found that the WD Low AA-fed and WD Low His-fed mice had increased energy expenditure normalized to body weight (**Figs. 3G and S3B)**. This occurred despite a decrease in activity during the dark cycle that was statistically significant for the WD Low AA-fed mice (**Fig. S3C**). Similar to our findings in lean mice and previous observations of

WD Low AA-fed mice, we observed that RER was increased in mice consuming the WD Low AA and WD Low His diets (**Fig. S3D**). Not surprisingly given the minor beneficial impact of a WD Low Phe diet on weight and body composition, the only effect of a WD Low Phe diet on components of energy balance that reached statistical significance was a small increase in energy expenditure normalized to body weight (**Fig. 3G**).

Protein restriction induces hepatic expression of the energy balance hormone *Fgf21* (Laeger *et al.*, 2014), and many of the metabolic benefits of PR, including increased energy, in C57BL/6J male mice are mediated by FGF21 through induction of uncoupling protein 1 (UCP1) in inguinal white adipose tissue (iWAT) (Hill *et al.*, 2017; Green *et al.*, 2022). In agreement with our observation of increased energy expenditure in WD Low AA-fed mice, we observed increased hepatic expression of *Fgf21* and iWAT expression of *Ucp1* in these animals (**Figs. 3H-3I**). However, neither hepatic *Fgf21* nor iWAT *Ucp1* was elevated in WD Low His-fed mice.

Restricting dietary histidine improves glycemic control and hepatic health of DIO mice

We assessed blood glucose control by conducting intraperitoneal glucose and insulin tolerance tests. We observed that mice consuming the WD Low AA and WD Low His diets had improved glucose tolerance relative not only to WD Control AA-fed mice, but to Ctrl AA-fed mice of a similar weight never exposed to a WD (**Fig. 4A**). Mice consuming the WD Low AA, WD Low His, and WD Low Phe diets all had improved insulin tolerance relative to WD Control AA-fed mice (**Fig. S3E**).

WD feeding induces hepatic steatosis, likely contributing to the negative effects of a WD on glucose tolerance. As expected, WD Ctrl AA fed mice had evident hepatic steatosis and large lipid droplets, whereas the mice switched to the WD Low His diet had decreased liver droplet

size and normal liver histology at the conclusion of the experiment (**Figs. 4B-4D**). As we have previously observed, restriction of all amino acids (WD Low AA diet) did not lead to improved histology or smaller lipid droplets. We quantified hepatic expression of several lipogenic genes as well as the transcription factor *Srebp-1c*. We observed numerical decreases in the mRNA expression of four lipogenic genes, *Acc1*, *Fasn*, *Elovl6*, and *Scd1* in WD Low His-fed mice relative to WD Ctrl AA-fed mice; the decrease was statistically significant in the case of *Scd1*, although this did not likely account for the specific effect of the WD Low His diet since it also occurred with total AA restriction (**Fig. 4E**). Similarly, expression of the transcription factor *Srebp-1c* was also significantly reduced in WD Low His-fed and WD Low AA-fed mice (**Fig. 4E**).

To quantitatively assess how dietary histidine levels impacted hepatic insulin sensitivity and glucose uptake by peripheral tissues, we performed a hyperinsulinemia-euglycemic clamp on DIO mice switched to either a WD Ctrl AA or WD Low His diet (**Fig. 5A–5K**). Mice fed the WD Low His diet required a considerably greater glucose infusion rate (GIR) to achieve euglycemia than mice fed the WD Ctrl AA (**Fig. 5A**). Calculating hepatic glucose production under basal and hyperinsulinemic conditions revealed that there was no difference in hepatic insulin sensitivity between WD Low His-fed mice and WD Ctrl AA-fed mice (**Figs. 5B-5C**). Thus, the greater GIR required for WD Low His-fed mice to achieve euglycemia was entirely the effect of increased peripheral glucose disposal under clamp conditions (**Fig. 5D**). WD Low His-fed mice had significantly increased glucose uptake rates into critical metabolic tissues, including iWAT, brown adipose tissue (BAT), heart, and skeletal muscle (**Figs. 5E-5H**). In contrast, there were no significant differences in glucose uptake into epididymal white adipose tissue (eWAT) or brain (**Figs. 5I-J**).

We considered the possibility that the greater energy expenditure of WD Low His-fed mice and the greater glucose uptake into iWAT and BAT of these animals were linked to increased thermogenesis. We observed a small, statistically significant difference in body temperature, with WD Low His-fed mice having a lower core body temperature than WD Ctrl AA-fed mice (**Fig. 5K**). However, WD Low His-fed mice showed resistance to cold stress, maintaining a significantly higher core body temperature upon exposure to 4°C **(Fig. 5L).**

Benefits of histidine restriction are independent of FGF21

As noted above many of the metabolic benefits of PR, including increased energy, in C57BL/6J male mice are mediated by the hormone FGF21, including increased food consumption and energy expenditure (Hill *et al.*, 2017; Green *et al.*, 2022). While we did not observe a statistically significance increase in *Fgf21* in histidine restricted animals, in previous work we have observed that the induction of FGF21 by a Low BCAA diet was dependent upon how long the animals had been on the diet (Cummings *et al.*, 2018). We therefore decided to directly test the role of *Fgf21* in the response to histidine restriction by comparing the response of wild-type and *Fgf21*$^{-/-}$ mice to a Low His diet.

We placed wild-type (WT) and *Fgf21*$^{-/-}$ (KO) mice on either Ctrl AA or Low His diets, and then analyzed food intake and energy expenditure using metabolic chambers. We observed a significant overall effect of diet on food consumption, with WT and KO mice responding similarly and increasing food consumption when fed a Low His diet (**Fig. 6A**). Energy expenditure likewise showed a significant effect of diet in both the light and dark phases, with both WT and KO mice increasing energy expenditure when fed a Low His diet; there was also an overall main effect of genotype on energy expenditure, with KO mice expending slightly less

energy during the light phase (**Fig. 6B**). Fuel source utilization was also altered by a Low His diet in both WT and KO mice (**Fig. 6C**).

Restriction of histidine starting in midlife improves the metabolic health of males but does not increase lifespan

Restriction of dietary protein and specific amino acids, including methionine and the branched-chain amino acids, has been shown to promote healthspan and longevity in rodents. To examine the effects of reducing dietary histidine starting in midlife, we randomized 16-month-old male and female C57BL/6.Nia mice from the National Institute on Aging (NIA) Aged Mouse Colony to the Ctrl AA or Low His diet. We followed these animals longitudinally, with assessment of metabolic health and frailty as they aged, and determined their survival. While the body composition of aged female mice was not significantly altered by consumption of the Low His diet, we found that there was a significant effect of a Low His diet on the fat mass and lean mass of male mice; the overall effect was one of reduced male adiposity (**Figs. 7A-D**).

Both mice and humans become increasingly frail with age, and we utilized a recently developed mouse frailty index to assess the effect of a Low His diet frailty (Whitehead *et al.*, 2014; Kane *et al.*, 2016). In male mice we observed an overall effect of age on frailty, but in neither sex did a Low His diet result in a significant change in frailty (**Fig. 7E**). We also assessed glucose tolerance as the mice aged, and in 25-month-old male mice, we found that a Low His diet improved glucose tolerance (**Figs. 7F-7G**). Despite the improvements in metabolic health, a Low His diet had no significant effect on the overall survival of either male or female mice when started at 16 months of age (**Figs. 7H-7I**).

Decreased dietary histidine protects from the effects of a Western diet

We next decided to examine whether decreasing dietary histidine can protect from the deleterious effects of a Western diet (**Fig. 8A**). We fed young 6-week-old C57BL/6J mice either an amino acid-defined WD or a WD Low His diet as described above; in parallel a group of mice was fed a Control AA-defined diet. Over the next 12 weeks, we found that mice fed the WD Low His diet were completely protected from the effects of a WD diet on weight and fat mass, while experiencing a transient minor dip in lean mass (**Fig. 8B-C**). WD-fed mice had significantly thicker layer of dermal WAT at the end of 12 weeks than Control AA-fed mice; the WD Low His mice did not show this increase (**Fig. 8D**). The overall effect was a complete protection from the effect of a WD on adiposity (**Fig. 8E**).

The effect of the WD Low His diet on weight was not driven by reduced calorie intake; indeed as we anticipated based on our results above, WD Low His-fed mice actually consumed more calories relative to WD Ctrl AA-fed mice (**Fig. 8F**). Instead, mice fed a WD Low His diet have increased energy expenditure (**Fig. 8G**). WD Low His-fed mice also had improved glucose tolerance relative to WD Ctrl AA-fed mice (**Fig. 8H**).

Increased histidine intake is associated with higher BMI in humans

As reducing dietary histidine has potent beneficial effects on the body composition of mice, we next examined whether dietary levels of histidine are associated with human health. Among a randomly selected, cross-sectional population-based sample of adults (>18 years old), we examined associations between estimated histidine intake relative to total protein and BMI

(kg/m^2) determined from measured weight and height. After adjusting for confounding variables, we discovered that a single percentage point increase in dietary histidine intake relative to total protein—for example, from 3% to 4% of protein—is linked with a 5.66 unit rise in BMI ($p = 0.001$) **(Fig. 8I).** In accordance with our findings in animals, our data show that a greater dietary consumption of protein that contains a larger proportion of histidine is associated with an increased BMI in a general population-based sample. These findings, when seen in the context of our animal investigations, show that dietary protein quality — may also be a key regulator of human weight, adiposity, and metabolic health.

Discussion

Recent studies have demonstrated that protein quality – in particular, the specific amino acid composition of the diet – plays a significant role in mediating the effects of dietary protein on health. Much of the initial research in this area focused on the essential amino acid methionine, restriction of which promotes metabolic health and extends lifespan in rodents (Orentreich *et al.*, 1993; Richie *et al.*, 1994; Miller *et al.*, 2005; Lees *et al.*, 2014; Schmidt *et al.*, 2016; Lees *et al.*, 2017), due to the belief that lower levels of dietary methionine might account for the health benefits of a vegan diet (McCarty *et al.*, 2009; MacArthur *et al.*, 2021). The branched chain amino acids, leucine, isoleucine, and valine, have also been the subject of significant attention, as blood levels of BCAAs in humans are associated with diabetes and insulin resistance (Newgard *et al.*, 2009). Our lab has previously demonstrated that restriction of the three dietary branched-chain amino acids improves metabolic health, fitness, and extends the lifespan of mice (Fontana *et al.*, 2016; Cummings *et al.*, 2018; Richardson *et al.*, 2021; Yu *et al.*, 2021). We have shown that restriction of the BCAA isoleucine is necessary and sufficient for the metabolic benefits of a low protein diet; further, in humans dietary isoleucine levels are

associated with increased body mass index, while the blood level of isoleucine in humans is associated with mortality (Deelen *et al.*, 2019; Yu *et al.*, 2021).

Our previous experiments with BCAAs suggested that one or more of the other essential amino acids – histidine, lysine, methionine, phenylalanine, threonine, and tryptophan – contributed to the effects of a low protein diet on body composition. In this work, we analyzed the effect of restricting each of these single essential amino acids by 67% relative to the Control diet. We observed statistically significant effects of restricting either histidine, phenylalanine, or threonine on fat mass accretion and adiposity, and restriction of either histidine or phenylalanine on body weight. We concentrated our follow-up analysis in diet induced obese mice on phenylalanine and histidine restriction due to the larger effect size of these interventions, as well as the recent exploration of dietary threonine restriction by another group (Yap *et al.*, 2020).

Here, we determined that specifically reducing dietary histidine, without altering energy density, the caloric contribution of amino acids to the diet, or the protein:carbohydrate ratio (Solon-Biet *et al.*, 2014) is sufficient to restore metabolic health. To the best of our knowledge, these studies represent the first examination of a reduced histidine diet in a mouse model of pre-existing diet-induced obesity and type 2 diabetes. Specifically reducing histidine rapidly normalizes the weight of diet-induced obese mice without calorie restriction, even as these mice continue to eat an otherwise high-fat, high-sucrose Western diet. While the effects of histidine restriction on body composition are similar to the effects of restricting BCAAs, they are distinct in that restriction of dietary histidine in lean mice has no effects on glucose tolerance. Further, while in obese mice histidine restriction modestly improves glucose homeostasis, unlike restriction of isoleucine, histidine restriction does not improve hepatic insulin sensitivity; instead,

the benefits of histidine restriction on glucose tolerance and insulin sensitivity closely tracks the changes in weight and decreased adiposity.

The decreased weight and adiposity of histidine-restricted animals was not associated not with decreased caloric intake, but instead with increased calorie intake and increased energy expenditure. In this respect histidine restriction is similar to the effects of protein restriction, methionine restriction, BCAA and isoleucine restriction, as well as threonine and tryptophan restriction, in C57BL/6J male mice (Laeger *et al.*, 2014; Hill *et al.*, 2017; Wanders *et al.*, 2017; Cummings *et al.*, 2018; Yap *et al.*, 2020; Richardson *et al.*, 2021; Green *et al.*, 2022; Hill *et al.*, 2022). However, restriction of protein, BCAAs/isoleucine, and methionine in C57BL/6J male mice activate the FGF21-UCP1 axis, and these effect of these restriction on food intake and energy expenditure require *Fgf21* (Laeger *et al.*, 2014; Hill *et al.*, 2017; Wanders *et al.*, 2017; Yu *et al.*, 2021). In contrast, while histidine restriction increases insulin-stimulated glucose uptake into iWAT and BAT, histidine restriction does not activate the FGF21-UCP1 axis and does not depend on the presence of *Fgf21*; male *Fgf21*$^{-/-}$ mice show the same increase in food consumption and energy expenditure when histidine restricted that wild-type males do. Further research will be required to fully define the mechanisms by which dietary histidine regulates energy balance and body composition.

Histidine is a precursor for the formation of histamine, which regulates allergies and inflammatory responses. Histamine has been shown to act as an anorexigenic agent via the regulation of leptin (Itateyama *et al.*, 2003; Yoshimatsu, 2008), and dietary histidine has been shown to suppress food intake in rats (Kasaoka *et al.*, 2004). These results, as well as recent work showing that genetic inhibition of histamine synthesis has been shown to protect from hepatic steatosis (Kennedy *et al.*, 2018), are consistent with our results that restriction of dietary

histidine increases food consumption and has beneficial effects on hepatic steatosis. Future work could examine if these effects of histidine restriction are the result of changes in levels of histamine.

Interestingly, we found that restriction of methionine, lysine, or tryptophan had only minor effects on metabolic health. The lack of an effect of methionine restriction was the most surprising, as multiple studies have shown methionine restriction extends the lifespan of rodents and improves metabolic health, improving glucose tolerance and reducing adiposity (Orentreich *et al.*, 1993; Miller *et al.*, 2005; Lees *et al.*, 2014). However, the degree of restriction in these studies is typically 80% or more, substantially more than the level of restriction we examined. Further, we designed our diet to include all twenty common amino acids, and the presence of cysteine has been shown to block the effects of methionine restriction (Wanders *et al.*, 2016). Similarly, recent work on tryptophan restriction has utilized a great degree of restriction and a lower absolute level of protein (Yap *et al.*, 2020).

Limitations of our study include that our analysis of the effects of histidine restriction on young and diet-induced obese mice were conducted exclusively in male mice of a single inbred strain. Over the last few years, it has become apparent that both sex and genetic background play an important role in the response to dietary interventions (Mitchell *et al.*, 2016; Green & Lamming, 2021; Roy *et al.*, 2021; Green *et al.*, 2022); our own previous work has shown that protein or BCAA restriction has distinct metabolic and molecular effects when examined in both sexes and different strains of mice (Richardson *et al.*, 2021; Green *et al.*, 2022). In our analysis of histidine restriction as a midlife intervention, we observed distinct effects of a Low His diet on body composition and glucose tolerance in males and females. Future studies should consider the effect of dietary histidine levels on the metabolic health of multiple genetic backgrounds and

both sexes. Finally, our findings in humans are only correlative, and it remains to be determined if and how dietary histidine regulates body composition in humans.

In conclusion, we have found that dietary histidine is a critical modulator of metabolic health and body composition in mice and potentially in humans (**Figure 8I**). Specifically reducing dietary histidine reduces weight and fat mass accretion in young lean mice, and rapidly normalizes body weight, fat mass, and adiposity in diet-induced obese mice without requiring calorie restriction. These metabolic effects likely result from increased energy expenditure. In contrast to other dietary regimens involving the restriction of dietary protein or specific amino acids, restriction of histidine does not increase energy expenditure through the FGF21-UCP1 axis. Further, we find that reduction of dietary histidine in diet-induced obese mice also corrects metabolic abnormalities associated with obesity, including impaired glucose tolerance, impaired insulin sensitivity, and hepatic steatosis. Restriction of dietary histidine also protects young lean mice from Western diet-induced obesity, weight gain, and glucose intolerance. While dietary histidine restriction promotes metabolic health in aged male mice, it does not extend their lifespan. Finally, we find that dietary levels of histidine are correlated with metabolic health in humans, and we linked dietary levels of histidine to higher BMI. Our results highlight the critical importance of dietary histidine levels for obesity and metabolic health of both mice and humans, and suggest that reducing dietary histidine levels may be a novel therapeutic and public health strategy to combat the obesity epidemic.

Materials and Methods

Mouse information

All procedures were performed in conformance with institutional guidelines and were approved by the Institutional Animal Care and Use Committee of the William S. Middleton Memorial Veterans Hospital (Madison, WI, USA) or the University of Pennsylvania. (Philadelphia, PA,

USA). Except as noted otherwise, all the studies described here used male C57BL/6J mice purchased from The Jackson Laboratory (Bar Harbor, ME, USA) and all mice were acclimated to the animal research facility for at least one week before entering studies.

To test the effects of individual amino acid restriction, 10-week-old male mice were placed on control or individual amino acid restricted diets as indicated. To test the metabolic effects of histidine in the context of diet-induced obesity, 6-week-old male C57BL/6J mice were pre-conditioned with WD (TD.88137, Envigo, Madison, WI) for 12 weeks, and another group of mice were fed control chow diet (Purina 5001). Following 12-weeks of WD preconditioning, mice on WD diet were then switched to the amino acid defined Western diets described in Figures, while mice fed a chow diet were switched to an amino acid defined Control diet.

To generate male FGF21 KO mice, we crossed CMV-Cre mice (Schwenk *et al.*, 1995) from the Jackson Laboratory (006054) with mice expressing a floxed allele of *FGF21* (Potthoff *et al.*, 2009) from The Jackson Laboratory (022361), then crossed with C57BL/6J mice to remove CMV-Cre; genotyping was performed as described (Yu *et al.*, 2021). Briefly, PCR was performed using DreamTaq DNA Polymerase (Thermo Scientific, EP0705) according to insert instructions.

All amino acid defined diets were obtained from Envigo, and diet compositions and item numbers are provided in **Supplemental Tables 1 and 2**. Mice were housed in a specific pathogen free (SPF) mouse facility with a 12:12 hour light/dark cycle and with free access to food and water, and were maintained at a temperature of approximately 22°C, except for the cold stress study described below. Animals were group housed in static microisolator cages, except when temporarily housed in a Columbus Instruments Oxymax/CLAMS metabolic chamber system. Group sizes are provided in the figure legends.

In vivo Procedures

For glucose tolerance tests, food was withheld overnight (for 16 hours), and mice were then injected intraperitoneally with glucose (1 g/kg) as described (Yu *et al.*, 2021). Insulin tolerance tests were performed by fasting mice for 16 hours (Figures S1 and S2) or 4 hours (Figure S3) starting at lights on, and then injecting insulin (0.75U/kg) intraperitoneally. Glucose measurements were taken using a Bayer Contour blood glucose meter and test strips. Body composition was assessed using an EchoMRI Body Composition Analyzer (EchoMRI, Houston, TX, USA) (Bellantuono *et al.*, 2020). To assess metabolic physiology (O2, CO2, RER, and food consumption) and spontaneous activity, mice were placed into a Columbus Instruments Oxymax/CLAMS or CLAMS-HC metabolic chamber system (Columbus Instruments, Columbus, OH, USA) and acclimated for approximately 24 hours prior to data collection, and data from a continuous 24 hour period was then selected for analysis (Bellantuono *et al.*, 2020).

Hyperinsulinemic-Euglycemic Clamp and Temperature Studies

All hyperinsulinemic-euglycemic clamp and temperature studies were performed in mice at the Penn Diabetes Research Center Rodent Metabolic Phenotyping Core (University of Pennsylvania); mice were sourced directly from The Jackson Laboratory and diets were obtained

from Envigo (Madison, WI). Mice were preconditioned with Western diet (TD.88137) and then switched to either the WD Ctrl AA diet or WD Low His diet.

5 days after the diet switch, rectal temperature was taken at 22°C. Mice were then placed in a cage pre-equilibrated to 4°C for one hour. Upon removal from the 4°C cage, rectal temperature was taken again.

Hyperinsulinemic-euglycemic clamp studies of obese, Western diet fed animals were performed as follows. Indwelling jugular vein and carotid artery catheters were surgically implanted in the mice for infusion 7 days prior to the clamp study day as previously described (Ayala *et al.*, 2006). Mice were fasted for 5 hours prior to initiation of clamp and acclimated to the containers (plastic bowl with alpha dry). Jugular vein and arterial line are connected to the dual swivel 2 hours prior to the clamp initiation. A [3-^{3}H] glucose infusion was primed (1.5 μCi) and continuously infused for a 90 min equilibration period (0.075 μCi/min). Baseline measurements were determined in arterial blood samples collected at -10 and 0 min (relative to the start of the clamp) for analysis of glucose, [3-^{3}H] glucose specific activity, basal insulin and free fatty acids. The clamp was started at t = 0 min with a primed-continuous infusion of human insulin (2.5 mU/Kg/min; Novolin Regular Insulin), a donor blood infusion at 4.5 uL/min to prevent a 5% drop in the hematocrit, and glucose (D50 mixed with [3-^{3}H] glucose 0.05 μCi/uL) was infused at variable glucose infusion rate (GIR) to maintain euglycemia. The mixing of D50 with [3-^{3}H] glucose is required to maintain the specific activity constant during the clamp period. Arterial blood samples were taken at t=80-120 min for the measurement of [3-^{3}H] glucose specific activity, clamped insulin and free fatty acid levels. 120 minutes after initiation of clamp, ^{14}C-2DG (12μCi) is injected and arterial blood samples obtained at 2, 5, 10, 15 and 25 min to determine Rg, an index of tissue-specific glucose uptake in various tissues. After the final blood sample, animals were injected with a bolus of pentobarbital, and tissues were collected and frozen in liquid nitrogen and stored at -80°C for subsequent analysis.

Processing of samples and calculations: Radioactivity of [3-^{3}H] glucose, [^{14}C]2DG and [^{14}C]2DG-6-phosphate were determined as previously described (Ayala *et al.*, 2007). The glucose turnover rate (total Ra; mg/kg/min) was calculated as the rate of tracer infusion (dpm/min) divided by the corrected plasma glucose specific activity (dpm/mg) per kg body weight of the mouse. Glucose appearance (Ra) and disappearance (Rd) rates were determined using steady-state equations and endogenous glucose production (Ra) was determined by subtracting the GIR from total Ra. Tissue specific glucose disposal (Rg; μmol/100g tissue/min) was calculated as previously described (Ayala *et al.*, 2007). The plasma insulin concentration was determined by mouse ELISA kit (Biomarker core, University of Pennsylvania).

Assays and Kits

Unless noted otherwise, all assays were performed using samples from overnight 16-hour fasted mice. Blood FGF21 levels were assayed by a mouse/rat FGF-21 quantikine ELISA kit (MF2100) from R&D Systems (Minneapolis, MN, USA).

Histology and Quantitative PCR

Tissues were harvested from mice on the indicated diets following overnight (16-hr) fasting. A portion of the liver was embedded in OCT, and then cryosectioned and Oil-Red-O stained by the

UWCCC Experimental Pathology Laboratory. Liver sections were imaged using an EVOS microscope as previously described (Cummings *et al.*, 2018). For quantification of lipid droplet size in liver, 6 independent fields were obtained for tissue from each mouse by investigators blinded to the treatment group and then quantified using NIH ImageJ. Skin was paraformaldehyde-fixed (4%) overnight, and then paraffin-embedded for evaluation (Kasza *et al.*, 2014).

A portion of the liver and iWAT was harvested and snap frozen in liquid nitrogen for subsequent molecular analysis. For quantitative PCR, RNA was extracted from liver or adipose tissue using Tri-reagent (Sigma-Aldrich, St. Louis, MO, USA). 1 mg of RNA was used to generate cDNA using Superscript III (Invitrogen, Carlsbad, CA, USA). Oligo dT primers and primers for real-time PCR were obtained from Integrated DNA Technologies (Coralville, IA, USA). Reactions were run on an Applied Biosystems StepOne Plus machine (Applied Biosystems, Foster City, CA, USA) with Sybr Green PCR Master Mix (Invitrogen, Carlsbad, CA, USA). Actin was used to normalize the results from gene-specific reactions.

SHOW study

We analyzed the association between dietary histidine intake and body mass indexe (BMI) in a sample of 2016-2017 Survey of Health of Wisconsin (SHOW) participants. SHOW is an ongoing population-based health examination survey of non-institutionalized residents of Wisconsin. Detailed survey methods have been previously described (Nieto *et al.*, 2010). Survey components relevant to the current analysis included an in-home interview accompanied by measurements of weight and height, and a self-administered questionnaire including the National Cancer Institute's Diet History Questionnaire from which specific dietary intake variables were derived. The study population included 788 individuals that completed all parts of the survey. Demographic characteristics of the population are described in **Table S3**. All study protocols were approved by the University of Wisconsin Health Sciences Institutional Review Board, and all participants provided written informed consent as part of the initial home visit.

The intake of histidine was estimated from the Diet History Questionnaire II (National Cancer Institute) using Diet*Calc software (National Cancer Institute). The estimated levels of histidine are expressed as the percent (%) of total protein. The primary outcome was body mass index (kg/m2) derived from measured weight (kg) and height (m). Multiple linear regression was performed in SAS 9.4 software (Cary, NC) with BMI as the outcome and percent of total protein from histidine as the predictor of interest. In the model we also adjusted for age, gender, education, income, total caloric intake, and physical activity.

Quantification and statistical analysis

Unless otherwise stated, statistical analysis was conducted using Prism 7/8(GraphPad Software). Tests involving repeated measurements were analyzed with two-way repeated-measures ANOVA, followed by a Tukey-Kramer or Dunnett's post-hoc test as specified. All other comparisons of three or more means were analyzed by one-way ANOVA followed by a Dunnett's or Tukey-Kramer post-hoc test as specified in the figure legends. Additional comparisons, if any, were corrected for multiple comparisons using the Bonferroni method.

Competing Interests

DWL has received funding from, and is a scientific advisory board member of, Aeovian Pharmaceuticals, which seeks to develop novel, selective mTOR inhibitors for the treatment of various diseases.

ACKNOWLEDGEMENTS

We thank Tina Herfel (Envigo) for help with diet design, and Maria Nikodemova for assistance with accessing SHOW data. The Lamming laboratory is supported in part by the NIA (AG056771, AG062328, and AG061635), the NIDDK (DK125859), and startup funds from UW-Madison. The Survey of the Health of Wisconsin is funded by the Wisconsin Partnership Program. VF was supported in part by a Research Supplement to Promote Diversity in Health-Related Research (3R01AG056771-01A1S1). DY was supported in part by a fellowship from the American Heart Association (17PRE33410983). NER was supported in part by a training grant from the UW Institute on Aging (NIA T32 AG000213). CLG was supported in part by Dalio Philanthropies and a Glenn Foundation for Medical Research Postdoctoral Fellowship. M.E.M. is supported in part by a Supplement to Promote Diversity in Health-Related Research (R01AG062328-03S1). R.B. is supported in part by training grant T32DK007665. Clamp studies were performed in the Rodent Metabolic Phenotyping Core of the University of Pennsylvania Diabetes Research Center (P30 DK19525). The Lamming laboratory is supported in part by the U.S. Department of Veterans Affairs (I01-BX004031 and IS1-BX005524), and this work was supported using facilities and resources from the William S. Middleton Memorial Veterans Hospital. The content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH. This work does not represent the views of the Department of Veterans Affairs or the United States Government.

References

Ayala JE, Bracy DP, Julien BM, Rottman JN, Fueger PT & Wasserman DH. (2007). Chronic treatment with sildenafil improves energy balance and insulin action in high fat-fed conscious mice. *Diabetes* **56,** 1025-1033.

Ayala JE, Bracy DP, McGuinness OP & Wasserman DH. (2006). Considerations in the design of hyperinsulinemic-euglycemic clamps in the conscious mouse. *Diabetes* **55,** 390-397.

Bellantuono I, de Cabo R, Ehninger D, Di Germanio C, Lawrie A, Miller J, Mitchell SJ, Navas-Enamorado I, Potter PK, Tchkonia T, Trejo JL & Lamming DW. (2020). A toolbox for the longitudinal assessment of healthspan in aging mice. *Nature protocols* **15,** 540-574.

Cummings NE, Williams EM, Kasza I, Konon EN, Schaid MD, Schmidt BA, Poudel C, Sherman DS, Yu D, Arriola Apelo SI, Cottrell SE, Geiger G, Barnes ME, Wisinski JA, Fenske RJ, Matkowskyj KA, Kimple

ME, Alexander CM, Merrins MJ & Lamming DW. (2018). Restoration of metabolic health by decreased consumption of branched-chain amino acids. *The Journal of physiology* **596,** 623-645.

Deelen J, Kettunen J, Fischer K, van der Spek A, Trompet S, Kastenmuller G, Boyd A, Zierer J, van den Akker EB, Ala-Korpela M, Amin N, Demirkan A, Ghanbari M, van Heemst D, Ikram MA, van Klinken JB, Mooijaart SP, Peters A, Salomaa V, Sattar N, Spector TD, Tiemeier H, Verhoeven A, Waldenberger M, Wurtz P, Davey Smith G, Metspalu A, Perola M, Menni C, Geleijnse JM, Drenos F, Beekman M, Jukema JW, van Duijn CM & Slagboom PE. (2019). A metabolic profile of all-cause mortality risk identified in an observational study of 44,168 individuals. *Nature communications* **10,** 3346.

Dong JY, Zhang ZL, Wang PY & Qin LQ. (2013). Effects of high-protein diets on body weight, glycaemic control, blood lipids and blood pressure in type 2 diabetes: meta-analysis of randomised controlled trials. *Br J Nutr* **110,** 781-789.

Fontana L, Cummings NE, Arriola Apelo SI, Neuman JC, Kasza I, Schmidt BA, Cava E, Spelta F, Tosti V, Syed FA, Baar EL, Veronese N, Cottrell SE, Fenske RJ, Bertozzi B, Brar HK, Pietka T, Bullock AD, Figenshau RS, Andriole GL, Merrins MJ, Alexander CM, Kimple ME & Lamming DW. (2016). Decreased Consumption of Branched-Chain Amino Acids Improves Metabolic Health. *Cell reports* **16,** 520-530.

Gannon MC, Nuttall FQ, Saeed A, Jordan K & Hoover H. (2003). An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. *Am J Clin Nutr* **78,** 734-741.

Green CL & Lamming DW. (2021). We are more than what we eat. *Nat Metab* **3,** 1144-1145.

Green CL, Pak HH, Richardson NE, Flores V, Yu D, Tomasiewicz JL, Dumas SN, Kredell K, Fan JW, Kirsh C, Chaiyakul K, Murphy ME, Babygirija R, Barrett-Wilt GA, Rabinowitz J, Ong IM, Jang C, Simcox J & Lamming DW. (2022). Sex and genetic background define the metabolic, physiologic, and molecular response to protein restriction. *Cell Metab* **34,** 209-226 e205.

Hall KD, Bemis T, Brychta R, Chen KY, Courville A, Crayner EJ, Goodwin S, Guo J, Howard L, Knuth ND, Miller BV, 3rd, Prado CM, Siervo M, Skarulis MC, Walter M, Walter PJ & Yannai L. (2015). Calorie for Calorie, Dietary Fat Restriction Results in More Body Fat Loss than Carbohydrate Restriction in People with Obesity. *Cell Metab* **22,** 427-436.

Hill CM, Albarado DC, Coco LG, Spann RA, Khan MS, Qualls-Creekmore E, Burk DH, Burke SJ, Collier JJ, Yu S, McDougal DH, Berthoud HR, Munzberg H, Bartke A & Morrison CD. (2022). FGF21 is required for protein restriction to extend lifespan and improve metabolic health in male mice. *Nature communications* **13,** 1897.

Hill CM, Laeger T, Albarado DC, McDougal DH, Berthoud HR, Munzberg H & Morrison CD. (2017). Low protein-induced increases in FGF21 drive UCP1-dependent metabolic but not thermoregulatory endpoints. *Scientific reports* **7,** 8209.

Itateyama E, Chiba S, Sakata T & Yoshimatsu H. (2003). Hypothalamic neuronal histamine in genetically obese animals: its implication of leptin action in the brain. *Exp Biol Med (Maywood)* **228,** 1132-1137.

Kane AE, Hilmer SN, Boyer D, Gavin K, Nines D, Howlett SE, de Cabo R & Mitchell SJ. (2016). Impact of Longevity Interventions on a Validated Mouse Clinical Frailty Index. *J Gerontol A Biol Sci Med Sci* **71,** 333-339.

Kasaoka S, Tsuboyama-Kasaoka N, Kawahara Y, Inoue S, Tsuji M, Ezaki O, Kato H, Tsuchiya T, Okuda H & Nakajima S. (2004). Histidine supplementation suppresses food intake and fat accumulation in rats. *Nutrition* **20,** 991-996.

Kasza I, Suh Y, Wollny D, Clark RJ, Roopra A, Colman RJ, MacDougald OA, Shedd TA, Nelson DW, Yen MI, Yen CL & Alexander CM. (2014). Syndecan-1 is required to maintain intradermal fat and prevent cold stress. *PLoS Genet* **10,** e1004514.

Kennedy L, Hargrove L, Demieville J, Bailey JM, Dar W, Polireddy K, Chen Q, Nevah Rubin MI, Sybenga A, DeMorrow S, Meng F, Stockton L, Alpini G & Francis H. (2018). Knockout of l-Histidine Decarboxylase Prevents Cholangiocyte Damage and Hepatic Fibrosis in Mice Subjected to High-Fat Diet Feeding via Disrupted Histamine/Leptin Signaling. *Am J Pathol* **188,** 600-615.

Laeger T, Henagan TM, Albarado DC, Redman LM, Bray GA, Noland RC, Munzberg H, Hutson SM, Gettys TW, Schwartz MW & Morrison CD. (2014). FGF21 is an endocrine signal of protein restriction. *J Clin Invest* **124,** 3913-3922.

Lagiou P, Sandin S, Weiderpass E, Lagiou A, Mucci L, Trichopoulos D & Adami HO. (2007). Low carbohydrate-high protein diet and mortality in a cohort of Swedish women. *J Intern Med* **261,** 366-374.

Lees EK, Banks R, Cook C, Hill S, Morrice N, Grant L, Mody N & Delibegovic M. (2017). Direct comparison of methionine restriction with leucine restriction on the metabolic health of C57BL/6J mice. *Scientific reports* **7,** 9977.

Lees EK, Krol E, Grant L, Shearer K, Wyse C, Moncur E, Bykowska AS, Mody N, Gettys TW & Delibegovic M. (2014). Methionine restriction restores a younger metabolic phenotype in adult mice with alterations in fibroblast growth factor 21. *Aging Cell* **13,** 817-827.

Levine ME, Suarez JA, Brandhorst S, Balasubramanian P, Cheng CW, Madia F, Fontana L, Mirisola MG, Guevara-Aguirre J, Wan J, Passarino G, Kennedy BK, Wei M, Cohen P, Crimmins EM & Longo VD. (2014). Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab* **19,** 407-417.

Linn T, Santosa B, Gronemeyer D, Aygen S, Scholz N, Busch M & Bretzel RG. (2000). Effect of long-term dietary protein intake on glucose metabolism in humans. *Diabetologia* **43,** 1257-1265.

MacArthur MR, Mitchell SJ, Trevino-Villarreal JH, Grondin Y, Reynolds JS, Kip P, Jung J, Trocha KM, Ozaki CK & Mitchell JR. (2021). Total protein, not amino acid composition, differs in plant-based versus omnivorous dietary patterns and determines metabolic health effects in mice. *Cell Metab* **33,** 1808-1819 e1802.

Maida A, Zota A, Sjoberg KA, Schumacher J, Sijmonsma TP, Pfenninger A, Christensen MM, Gantert T, Fuhrmeister J, Rothermel U, Schmoll D, Heikenwalder M, Iovanna JL, Stemmer K, Kiens B, Herzig S & Rose AJ. (2016). A liver stress-endocrine nexus promotes metabolic integrity during dietary protein dilution. *J Clin Invest* **126,** 3263-3278.

McCarty MF, Barroso-Aranda J & Contreras F. (2009). The low-methionine content of vegan diets may make methionine restriction feasible as a life extension strategy. *Medical hypotheses* **72,** 125-128.

Miller RA, Buehner G, Chang Y, Harper JM, Sigler R & Smith-Wheelock M. (2005). Methionine-deficient diet extends mouse lifespan, slows immune and lens aging, alters glucose, T4, IGF-I and insulin levels, and increases hepatocyte MIF levels and stress resistance. *Aging Cell* **4,** 119-125.

Mitchell SJ, Madrigal-Matute J, Scheibye-Knudsen M, Fang E, Aon M, Gonzalez-Reyes JA, Cortassa S, Kaushik S, Gonzalez-Freire M, Patel B, Wahl D, Ali A, Calvo-Rubio M, Buron MI, Guiterrez V, Ward TM, Palacios HH, Cai H, Frederick DW, Hine C, Broeskamp F, Habering L, Dawson J, Beasley TM, Wan J, Ikeno Y, Hubbard G, Becker KG, Zhang Y, Bohr VA, Longo DL, Navas P, Ferrucci L, Sinclair DA, Cohen P, Egan JM, Mitchell JR, Baur JA, Allison DB, Anson RM, Villalba JM, Madeo F, Cuervo AM, Pearson KJ, Ingram DK, Bernier M & de Cabo R. (2016). Effects of Sex, Strain, and Energy Intake on Hallmarks of Aging in Mice. *Cell Metab* **23,** 1093-1112.

Newgard CB, An J, Bain JR, Muehlbauer MJ, Stevens RD, Lien LF, Haqq AM, Shah SH, Arlotto M, Slentz CA, Rochon J, Gallup D, Ilkayeva O, Wenner BR, Yancy WS, Jr., Eisenson H, Musante G, Surwit RS, Millington DS, Butler MD & Svetkey LP. (2009). A branched-chain amino acid-related metabolic signature that differentiates obese and lean humans and contributes to insulin resistance. *Cell Metab* **9,** 311-326.

Nieto FJ, Peppard PE, Engelman CD, McElroy JA, Galvao LW, Friedman EM, Bersch AJ & Malecki KC. (2010). The Survey of the Health of Wisconsin (SHOW), a novel infrastructure for population health research: rationale and methods. *BMC Public Health* **10,** 785.

Ogden CL, Carroll MD, Kit BK & Flegal KM. (2014). Prevalence of Childhood and Adult Obesity in the United States, 2011–2012. *JAMA* **311,** 806-814.

Orentreich N, Matias JR, DeFelice A & Zimmerman JA. (1993). Low methionine ingestion by rats extends life span. *The Journal of nutrition* **123,** 269-274.

Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, Mohammadi M, Finck BN, Mangelsdorf DJ, Kliewer SA & Burgess SC. (2009). FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. *Proc Natl Acad Sci U S A* **106,** 10853-10858.

Richardson NE, Konon EN, Schuster HS, Mitchell AT, Boyle C, Rodgers AC, Finke M, Haider LR, Yu D, Flores V, Pak HH, Ahmad S, Ahmed S, Radcliff A, Wu J, Williams EM, Abdi L, Sherman DS, Hacker T & Lamming DW. (2021). Lifelong restriction of dietary branched-chain amino acids has sex-specific benefits for frailty and lifespan in mice. *Nat Aging* **1,** 73-86.

Richie JP, Jr., Leutzinger Y, Parthasarathy S, Malloy V, Orentreich N & Zimmerman JA. (1994). Methionine restriction increases blood glutathione and longevity in F344 rats. *FASEB J* **8,** 1302-1307.

Roy S, Sleiman MB, Jha P, Ingels JF, Chapman CJ, McCarty MS, Ziebarth JD, Hook M, Sun A, Zhao W, Huang J, Neuner SM, Wilmott LA, Shapaker TM, Centeno AG, Ashbrook DG, Mulligan MK, Kaczorowski CC, Makowski L, Cui Y, Read RW, Miller RA, Mozhui K, Williams EG, Sen S, Lu L, Auwerx J & Williams RW. (2021). Gene-by-environment modulation of lifespan and weight gain in the murine BXD family. *Nat Metab* **3,** 1217-1227.

Schmidt JA, Rinaldi S, Scalbert A, Ferrari P, Achaintre D, Gunter MJ, Appleby PN, Key TJ & Travis RC. (2016). Plasma concentrations and intakes of amino acids in male meat-eaters, fish-eaters, vegetarians and vegans: a cross-sectional analysis in the EPIC-Oxford cohort. *Eur J Clin Nutr* **70,** 306-312.

Schwenk F, Baron U & Rajewsky K. (1995). A cre-transgenic mouse strain for the ubiquitous deletion of loxP-flanked gene segments including deletion in germ cells. *Nucleic Acids Res* **23,** 5080-5081.

Seino Y, Seino S, Ikeda M, Matsukura S & Imura H. (1983). Beneficial effects of high protein diet in treatment of mild diabetes. *Hum Nutr Appl Nutr* **37 A,** 226-230.

Sluijs I, Beulens JW, van der AD, Spijkerman AM, Grobbee DE & van der Schouw YT. (2010). Dietary intake of total, animal, and vegetable protein and risk of type 2 diabetes in the European Prospective Investigation into Cancer and Nutrition (EPIC)-NL study. *Diabetes Care* **33,** 43-48.

Solon-Biet SM, McMahon AC, Ballard JW, Ruohonen K, Wu LE, Cogger VC, Warren A, Huang X, Pichaud N, Melvin RG, Gokarn R, Khalil M, Turner N, Cooney GJ, Sinclair DA, Raubenheimer D, Le Couteur DG & Simpson SJ. (2014). The ratio of macronutrients, not caloric intake, dictates cardiometabolic health, aging, and longevity in ad libitum-fed mice. *Cell Metab* **19,** 418-430.

Solon-Biet SM, Mitchell SJ, Coogan SC, Cogger VC, Gokarn R, McMahon AC, Raubenheimer D, de Cabo R, Simpson SJ & Le Couteur DG. (2015). Dietary Protein to Carbohydrate Ratio and Caloric Restriction: Comparing Metabolic Outcomes in Mice. *Cell reports* **11,** 1529-1534.

van Nielen M, Feskens EJ, Mensink M, Sluijs I, Molina E, Amiano P, Ardanaz E, Balkau B, Beulens JW, Boeing H, Clavel-Chapelon F, Fagherazzi G, Franks PW, Halkjaer J, Huerta JM, Katzke V, Key TJ, Khaw KT, Krogh V, Kuhn T, Menendez VV, Nilsson P, Overvad K, Palli D, Panico S, Rolandsson O, Romieu I, Sacerdote C, Sanchez MJ, Schulze MB, Spijkerman AM, Tjonneland A, Tumino R, van der AD, Wurtz AM, Zamora-Ros R, Langenberg C, Sharp SJ, Forouhi NG, Riboli E, Wareham NJ & InterAct C. (2014). Dietary protein intake and incidence of type 2 diabetes in Europe: the EPIC-InterAct Case-Cohort Study. *Diabetes Care* **37,** 1854-1862.

Wanders D, Forney LA, Stone KP, Burk DH, Pierse A & Gettys TW. (2017). FGF21 Mediates the Thermogenic and Insulin-Sensitizing Effects of Dietary Methionine Restriction but Not Its Effects on Hepatic Lipid Metabolism. *Diabetes* **66,** 858-867.

Wanders D, Stone KP, Forney LA, Cortez CC, Dille KN, Simon J, Xu M, Hotard EC, Nikonorova IA, Pettit AP, Anthony TG & Gettys TW. (2016). Role of GCN2-Independent Signaling Through a Noncanonical PERK/NRF2 Pathway in the Physiological Responses to Dietary Methionine Restriction. *Diabetes* **65,** 1499-1510.

Whitehead JC, Hildebrand BA, Sun M, Rockwood MR, Rose RA, Rockwood K & Howlett SE. (2014). A clinical frailty index in aging mice: comparisons with frailty index data in humans. *J Gerontol A Biol Sci Med Sci* **69,** 621-632.

Yap YW, Rusu PM, Chan AY, Fam BC, Jungmann A, Solon-Biet SM, Barlow CK, Creek DJ, Huang C, Schittenhelm RB, Morgan B, Schmoll D, Kiens B, Piper MDW, Heikenwalder M, Simpson SJ, Broer S, Andrikopoulos S, Muller OJ & Rose AJ. (2020). Restriction of essential amino acids dictates the systemic metabolic response to dietary protein dilution. *Nature communications* **11,** 2894.

Yoshimatsu H. (2008). Hypothalamic neuronal histamine regulates body weight through the modulation of diurnal feeding rhythm. *Nutrition* **24,** 827-831.

Yu D, Richardson NE, Green CL, Spicer AB, Murphy ME, Flores V, Jang C, Kasza I, Nikodemova M, Wakai MH, Tomasiewicz JL, Yang SE, Miller BR, Pak HH, Brinkman JA, Rojas JM, Quinn WJ, 3rd, Cheng EP, Konon EN, Haider LR, Finke M, Sonsalla M, Alexander CM, Rabinowitz JD, Baur JA, Malecki KC & Lamming DW. (2021). The adverse metabolic effects of branched-chain amino acids are mediated by isoleucine and valine. *Cell Metab* **33,** 905-922 e906.

Figure Legends

Figure 1. Dietary restriction of histidine, phenylalanine or threonine improves body composition.

(A) Experimental scheme. (B-E) Change in body weight (B), fat mass (C), lean mass (D), and body composition (E) of mice consuming the indicated diets for the indicated time (n=8-9 mice per group, *p<0.05, Dunnett's test vs. Ctrl AA following a mixed-effects model (Restricted Maximum Likelihood (REML)). (F-G) Food consumption (F) and energy expenditure (heat) (G) normalized to body weight of mice fed the indicated diets (n=8-9 per group, *p<0.05, Dunnet's test vs. Ctrl AA conducted separately for the light and dark cycles post 2-way RM ANOVA). (H) Glucose tolerance test (n=9/group, *p<0.05 vs. Ctrl AA, Dunnett's test post ANOVA). Data represented as mean ± SEM.

Figure 2. Dietary restriction of lysine, methionine, or tryptophan has no effect on body composition.

(A) Experimental scheme. (B-E) Change in body weight (B), fat mass (C), lean mass (D), and body composition (E) of mice consuming the indicated diets for the indicated time (n=9 mice per group, *p<0.05, Dunnett's test vs. Ctrl AA post 2-way RM ANOVA). (F-G) Food consumption (F) and energy expenditure (heat) (G) normalized to body weight of mice fed the indicated diets (n=8-9 per group, *p<0.05, Dunnet's test vs. Ctrl AA conducted separately for the light and dark cycles post 2-way RM ANOVA). (H) Glucose tolerance test (n=9/group, *p<0.05 vs. Ctrl AA, Dunnett's test post ANOVA). Data represented as mean ± SEM.

Figure 3. Restricting dietary histidine improves the metabolic health of DIO mice.

(A) Experimental scheme. (B-E) Body weight (B), fat mass (C), lean mass (D), and body composition (E) of mice consuming the indicated diets for the indicated time (n=10-12 mice per group, *p<0.05, Dunnett's test vs. WD Ctrl AA post 2-way RM ANOVA). (F-G) Food consumption (F) and energy expenditure (heat) (G) normalized to body weight of mice fed the indicated diets (n=5-8 per group, *p<0.05, Dunnet's test vs. WD Ctrl AA conducted separately for the light and dark cycles post 2-way RM ANOVA). (H-I) *Fgf21* expression in the liver (H) and *Ucp1* expression in the iWAT (I) of mice fed the indicated diets for 9 weeks (n = 6-9 per group, *p<0.05, Dunnett's test vs. WD Ctrl AA post ANOVA). Data represented as mean ± SEM.

Figure 4. Restricting dietary histidine improves glucose tolerance and hepatic steatosis of DIO mice.

(A) Glucose tolerance test after 3 weeks on the indicated diets (n=10-12/group, *p<0.05 vs. WD Ctrl AA, Dunnett's test post ANOVA). (B-D) Representative Oil-Red-O-stained sections of liver of mice after 9 weeks on the indicated diets. Scale bar, 100 µm (B); quantification of lipid droplet area (C) and lipid droplet size (D) (n=6/group, *p<0.05 vs. WD Ctrl AA, Dunnett's test post ANOVA). (E) Lipogenic gene expression after 9 weeks on the indicated diets (n=6/group, n=6/group, *p<0.05 vs. WD Ctrl AA, Dunnett's test post 2-way RM ANOVA). Data represented as mean ± SEM.

Figure 5. Dietary restriction of histidine increases glucose uptake into multiple tissues of DIO mice.

(A-C) Glucose infusion rate (A), basal and clamp hepatic glucose production (B), insulin responsiveness (C) and glucose disposal rate (D) were determined during a hyperinsulinemic-euglycemic clamp in mice preconditioned with a WD for 12 weeks and then switched to a WD Ctrl AA diet or a WD Low His diet for 4 weeks (n = 5/group; (A-C) *p < 0.05, t-test; (D) *p < 0.05, Sidak's test post 2-way ANOVA). (E-J) Glucose uptake into iWAT (E), BAT (F), heart (G), muscle (H), eWAT (I), and brain (J) were determined during a hyperinsulinemic-euglycemic clamp in mice preconditioned with a WD for 12 weeks and then switched to a WD Ctrl AA diet or a WD Low His diet for 4 weeks (n = 5/group; *p < 0.05, t-test). (K-L) Core temperature was determined using a rectal thermometer at room temp (24°C) (K) and after 1 hour at 4°C (L); n = 3/group, *p < 0.05, t-test. WD Ctrl AA clamp and glucose uptake data has been previously published (Yu *et al.*, 2021). Data represented as mean ± SEM.

Figure 6. FGF21 is not required for the metabolic response to histidine restriction.

(A-C) Food consumption normalized to body weight (A), energy expenditure normalized to body weight (B), and RER (C) of mice fed the indicated diets (n=3-8 per group, the overall effect of genotype [GT], diet, and the interaction represent the p value from a two-way ANOVA conducted separately for the light and dark cycles; *p < 0.05, Sidak's test post two-way ANOVA). Data represented as mean ± SEM.

Figure 7. Mid-life histidine improves metabolic health in males but does not impact lifespan. Male and female C57BL/6J.Nia mice were placed on Control AA and Low His diets starting at 16 months of age. (A-D) Fat mass and lean mass were determined at multiple time points (n= n varies by month; maximum n = 9 Ctrl AA females, 10 Low His females, 14 Ctrl AA males, 10 Low His males). (E) Frailty was assessed at 19 and 24 months of age. (A-E) Statistics for the overall effects of diet, age and the interaction represent the P value from a mixed-effects

model (restricted maximum likelihood (REML)), *p<0.05, two-sided Sidak's post hoc test. (F-G) Glucose tolerance was determined in (F) 23 month old females and (G) 25 month old males (n = 7-13/group females and 10-11/group males, *p < 0.05, t-test). (H-I) Kaplan–Meier plots showing the survival of female (H) and male (I) C57BL/6J.Nia mice fed the indicated diets starting at 16 months of age.

Figure 8. Decreased histidine intake prevents diet-induced obesity and glucose intolerance, and is associated with lower BMI in humans.

(A) Experimental scheme. (B-E) Body weight (B), fat mass and lean mass (C), dermal WAT thickness (D), and body composition (E) of mice consuming the indicated diets. (B, C, E) n=12 mice per group, *p<0.05 vs. all other groups, Tukey's test following a mixed-effects model (Restricted Maximum Likelihood (REML). (D) n=6-7/group, *p<0.05 vs. WD Ctrl AA, Dunnett's test post ANOVA. (F) Food consumption (n=6 cages per group, *p<0.05, Sidak's test post 2-way RM ANOVA). (G) Energy expenditure (heat) normalized to body weight of mice fed the indicated diets (n=5-6 mice per group, *p<0.05, Sidak's test post 2-way RM ANOVA). (H) Glucose tolerance test (n=12/group, *p<0.05 vs. WD Ctrl AA, Dunnett's test post ANOVA). (I) Association between BMI and percent of total protein from histidine from the SHOW study (n = 788, shaded area represents 95% CI). Data represented as mean ± SEM.

Figure 1

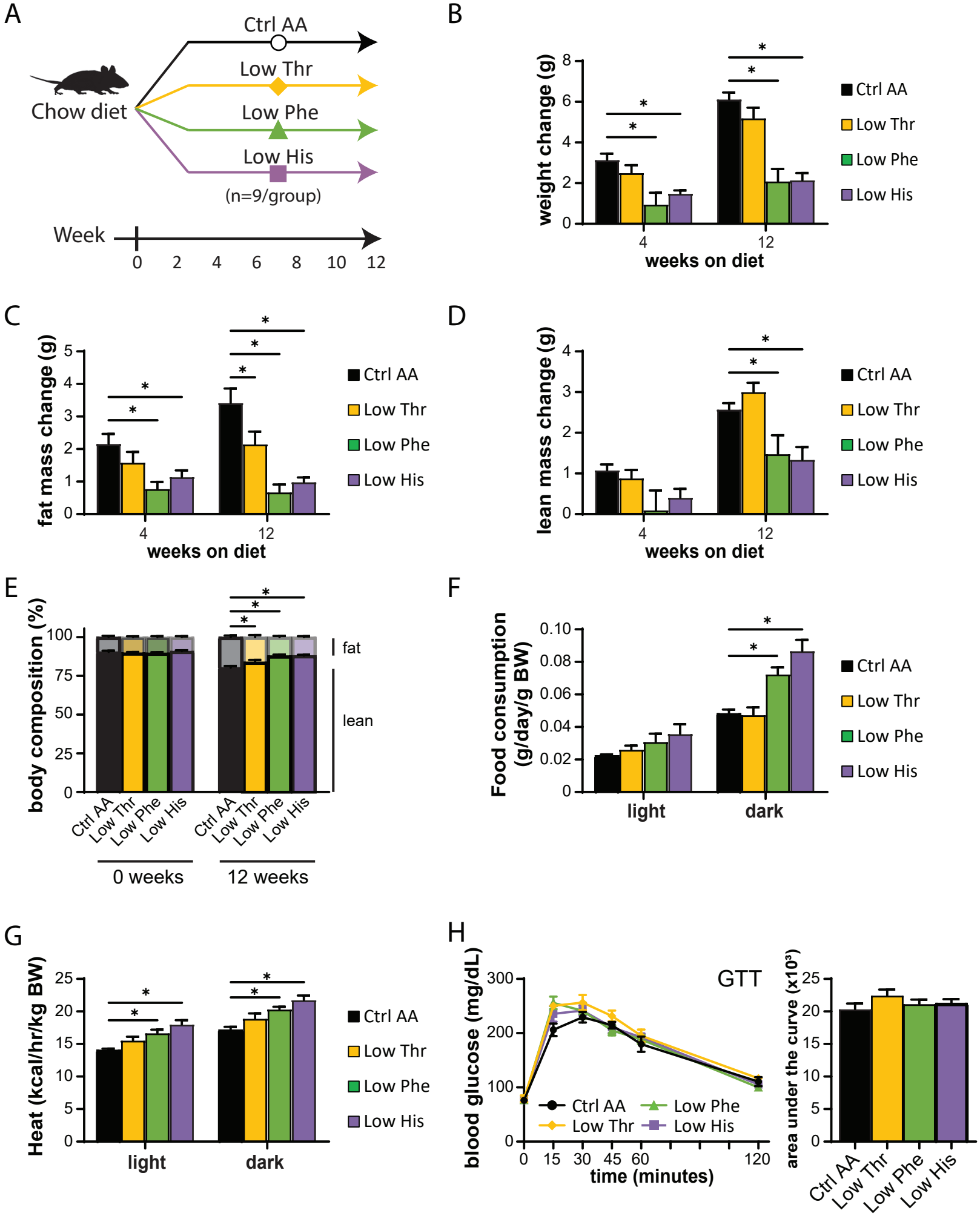

A
Ctrl AA
Low Thr
Low Phe
Low His
Chow diet
(n=9/group)
Week
0 2 4 6 8 10 12
B
weight change (g)
weeks on diet
Ctrl AA
Low Thr
Low Phe
Low His
C
fat mass change (g)
weeks on diet
D
lean mass change (g)
weeks on diet
E
body composition (%)
fat
lean
Ctrl AA
Low Thr
Low Phe
Low His
0 weeks
12 weeks
F
Food consumption (g/day/g BW)
light
dark
G
Heat (kcal/hr/kg BW)
light
dark
H
blood glucose (mg/dL)
GTT
time (minutes)
area under the curve (x10³)

Figure 2

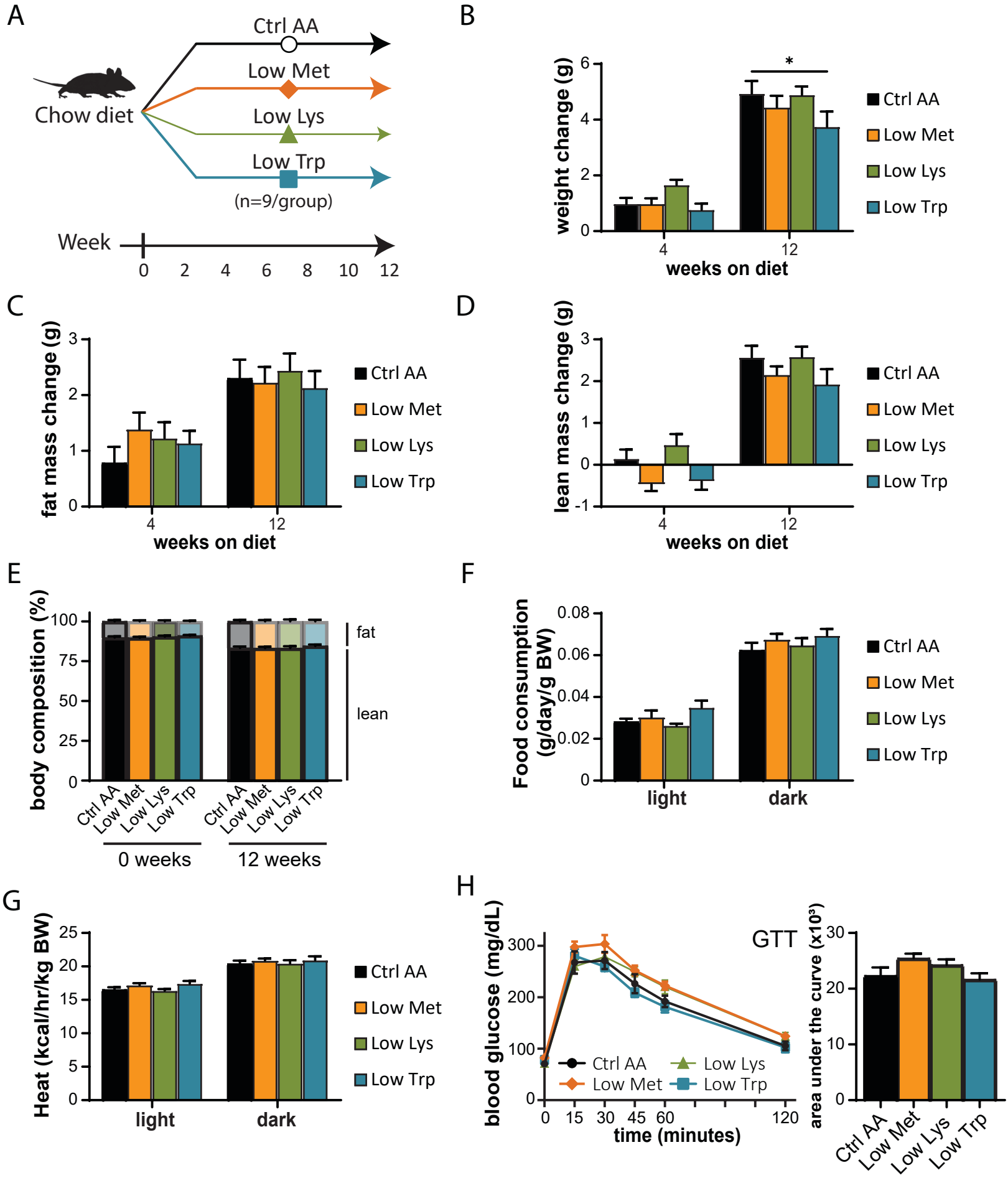

A
Ctrl AA
Low Met
Low Lys
Low Trp
Chow diet
(n=9/group)
Week
0 2 4 6 8 10 12
B
weight change (g)
weeks on diet
Ctrl AA
Low Met
Low Lys
Low Trp
C
fat mass change (g)
weeks on diet
D
lean mass change (g)
weeks on diet
E
body composition (%)
fat
lean
Ctrl AA
Low Met
Low Lys
Low Trp
0 weeks
12 weeks
F
Food consumption (g/day/g BW)
light
dark
G
Heat (kcal/hr/kg BW)
light
dark
H
GTT
blood glucose (mg/dL)
time (minutes)
area under the curve (x10³)

Figure 3

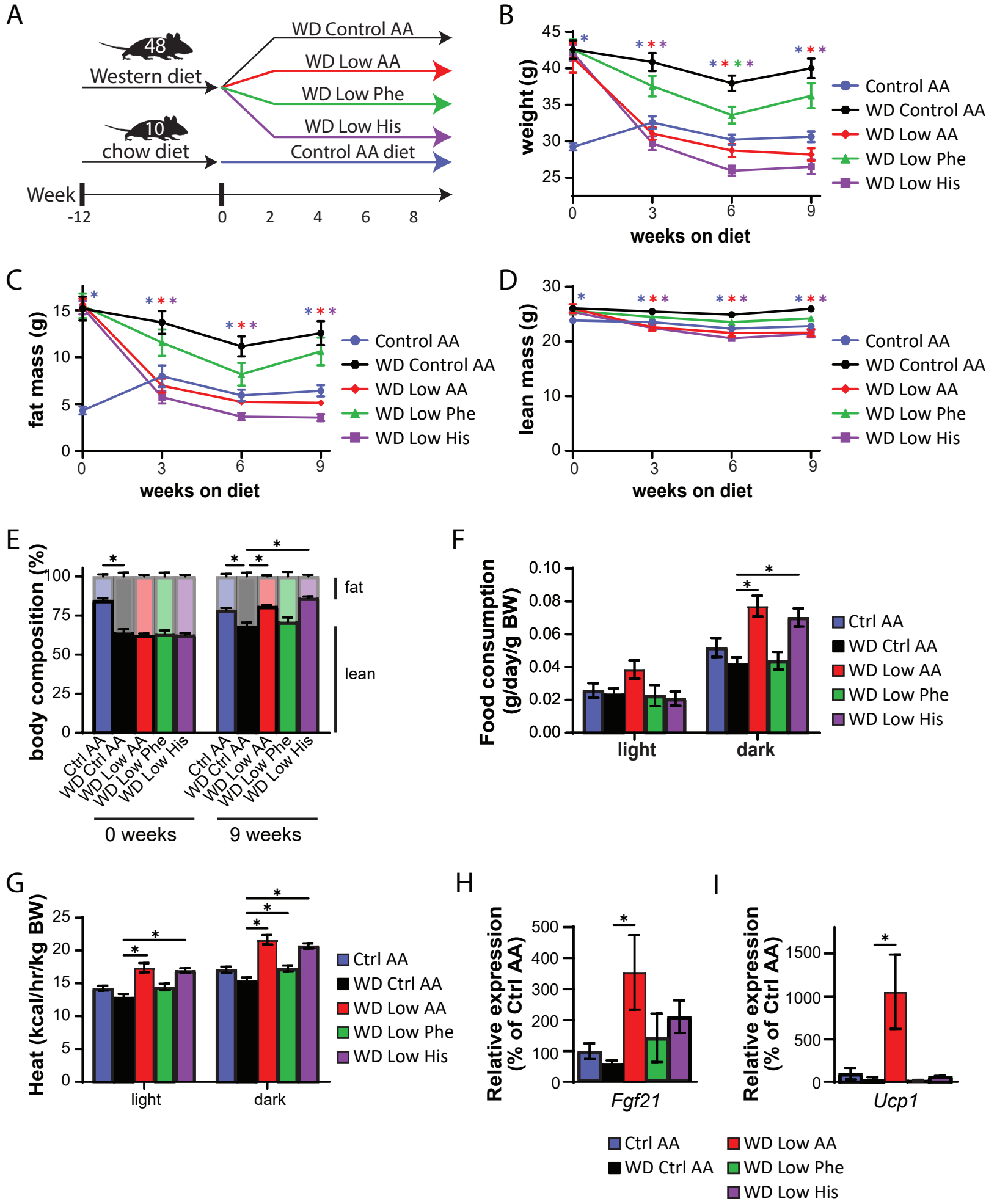

A
48
Western diet
10
chow diet
WD Control AA
WD Low AA
WD Low Phe
WD Low His
Control AA diet
Week
-12
0
2
4
6
8
B
weight (g)
weeks on diet
Control AA
WD Control AA
WD Low AA
WD Low Phe
WD Low His
C
fat mass (g)
weeks on diet
D
lean mass (g)
weeks on diet
E
body composition (%)
fat
lean
Ctrl AA
WD Ctrl AA
WD Low AA
WD Low Phe
WD Low His
0 weeks
9 weeks
F
Food consumption (g/day/g BW)
light
dark
Ctrl AA
WD Ctrl AA
WD Low AA
WD Low Phe
WD Low His
G
Heat (kcal/hr/kg BW)
light
dark
H
Relative expression (% of Ctrl AA)
Fgf21
I
Relative expression (% of Ctrl AA)
Ucp1

Figure 4

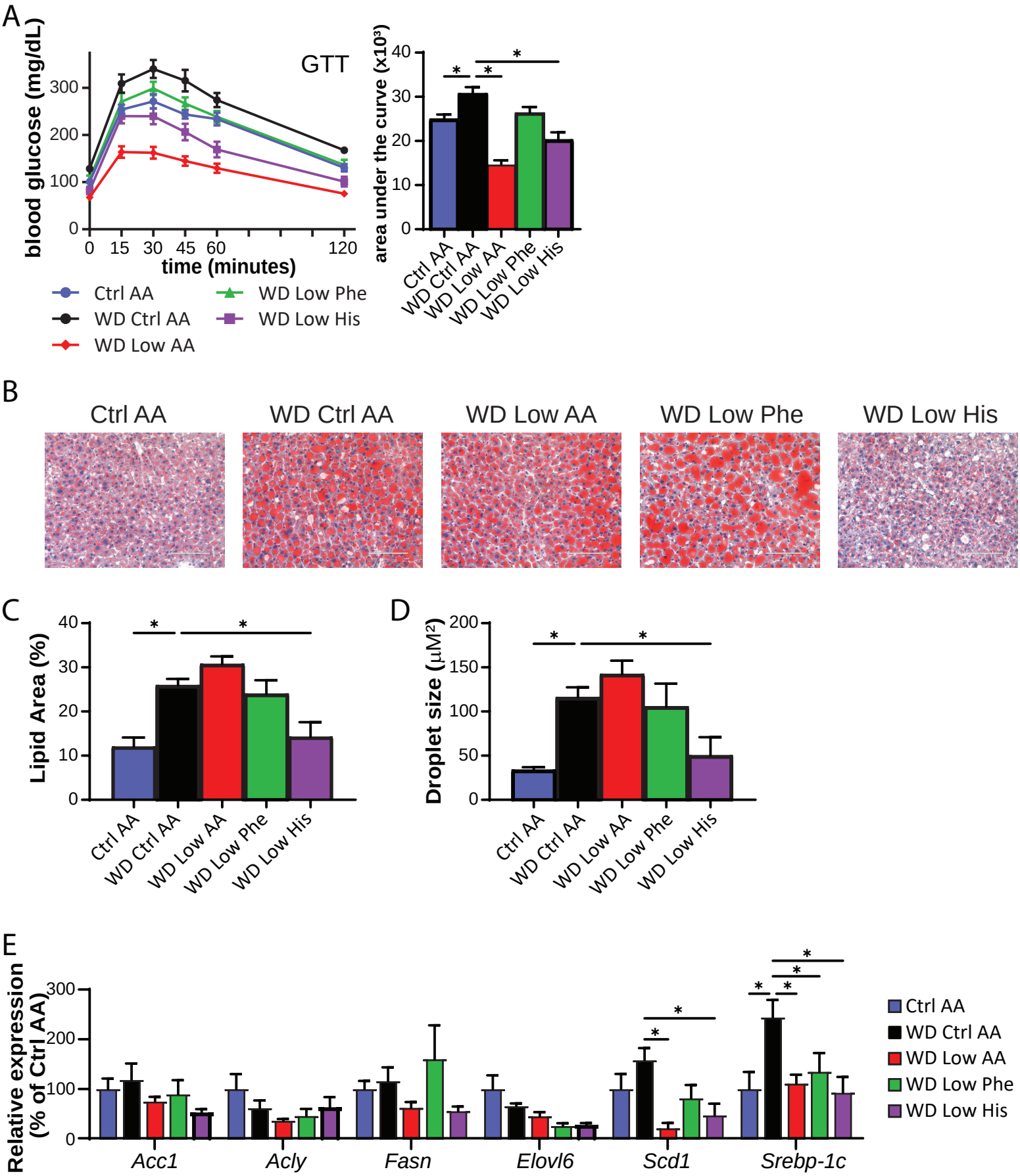

A
GTT
blood glucose (mg/dL)
time (minutes)
0
15
30
45
60
120
100
200
300
Ctrl AA
WD Ctrl AA
WD Low AA
WD Low Phe
WD Low His
area under the curve (x10³)
40
30
20
10
0
*
B
Ctrl AA
WD Ctrl AA
WD Low AA
WD Low Phe
WD Low His
C
Lipid Area (%)
D
Droplet size (μM²)
200
150
100
50
E
Relative expression (% of Ctrl AA)
Acc1
Acly
Fasn
Elovl6
Scd1
Srebp-1c

Figure 5

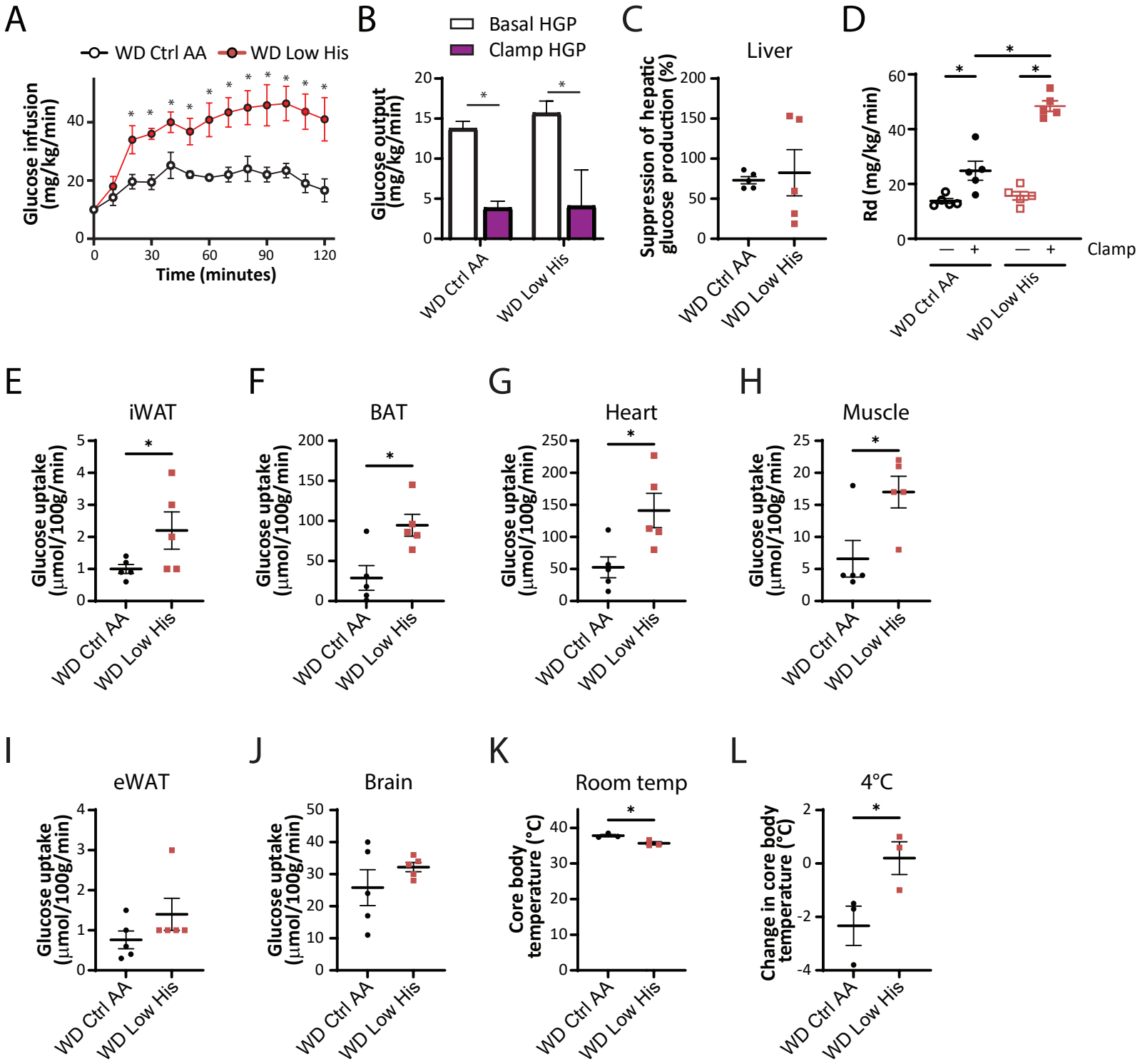

A
WD Ctrl AA
WD Low His
Glucose infusion (mg/kg/min)
Time (minutes)
B
Basal HGP
Clamp HGP
Glucose output (mg/kg/min)
WD Ctrl AA
WD Low His
C
Liver
Suppression of hepatic glucose production (%)
WD Ctrl AA
WD Low His
D
Rd (mg/kg/min)
Clamp
WD Ctrl AA
WD Low His
E
iWAT
Glucose uptake (µmol/100g/min)
WD Ctrl AA
WD Low His
F
BAT
Glucose uptake (µmol/100g/min)
WD Ctrl AA
WD Low His
G
Heart
Glucose uptake (µmol/100g/min)
WD Ctrl AA
WD Low His
H
Muscle
Glucose uptake (µmol/100g/min)
WD Ctrl AA
WD Low His
I
eWAT
Glucose uptake (µmol/100g/min)
WD Ctrl AA
WD Low His
J
Brain
Glucose uptake (µmol/100g/min)
WD Ctrl AA
WD Low His
K
Room temp
Core body temperature (°C)
WD Ctrl AA
WD Low His
L
4°C
Change in core body temperature (°C)
WD Ctrl AA
WD Low His

Figure 6

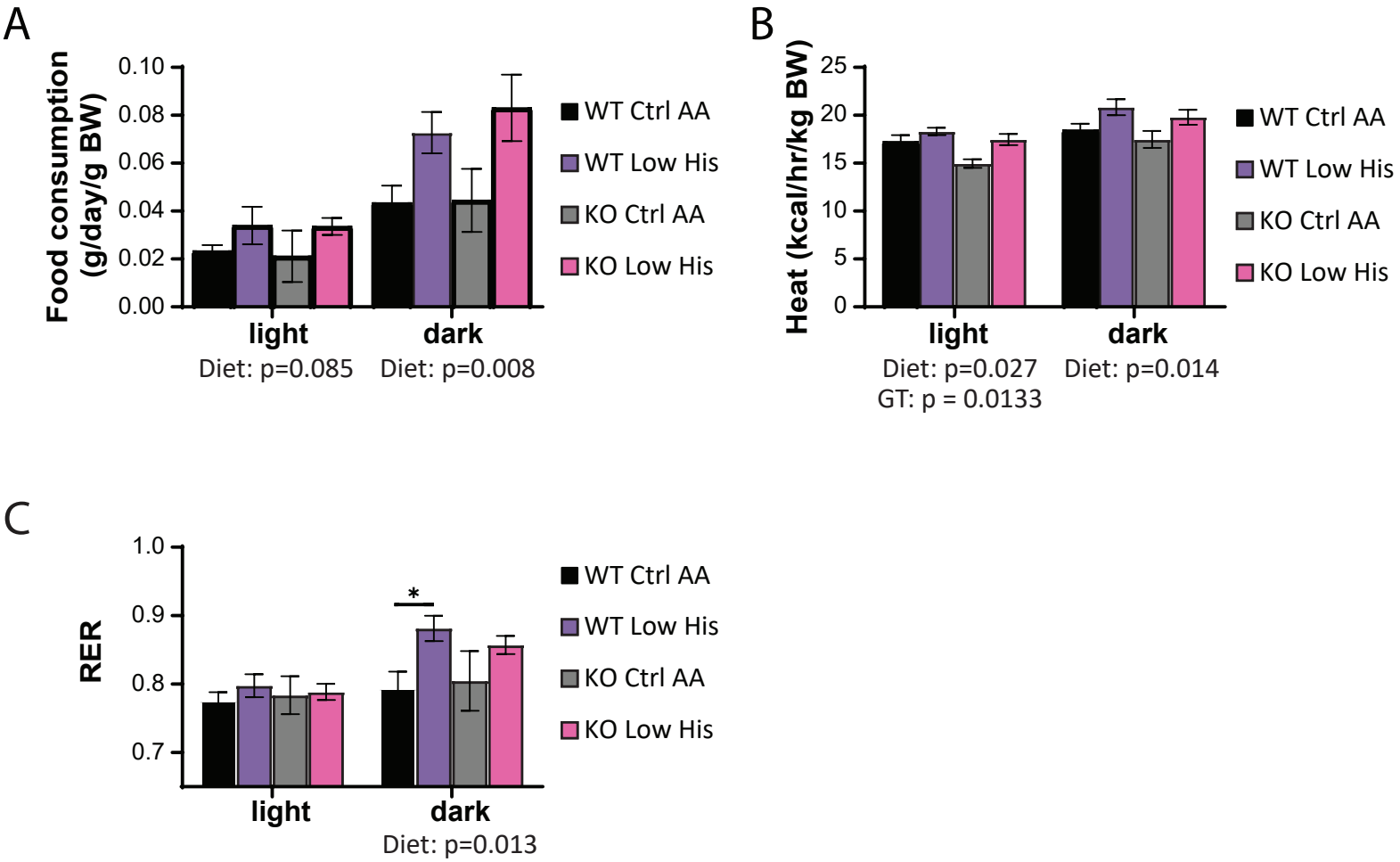

A
Food consumption (g/day/g BW)
0.10
0.08
0.06
0.04
0.02
0.00
light
dark
Diet: p=0.085
Diet: p=0.008
WT Ctrl AA
WT Low His
KO Ctrl AA
KO Low His
B
Heat (kcal/hr/kg BW)
25
20
15
10
5
0
light
dark
Diet: p=0.027
Diet: p=0.014
GT: p = 0.0133
WT Ctrl AA
WT Low His
KO Ctrl AA
KO Low His
C
RER
1.0
0.9
0.8
0.7
*
light
dark
Diet: p=0.013
WT Ctrl AA
WT Low His
KO Ctrl AA
KO Low His

Figure 7

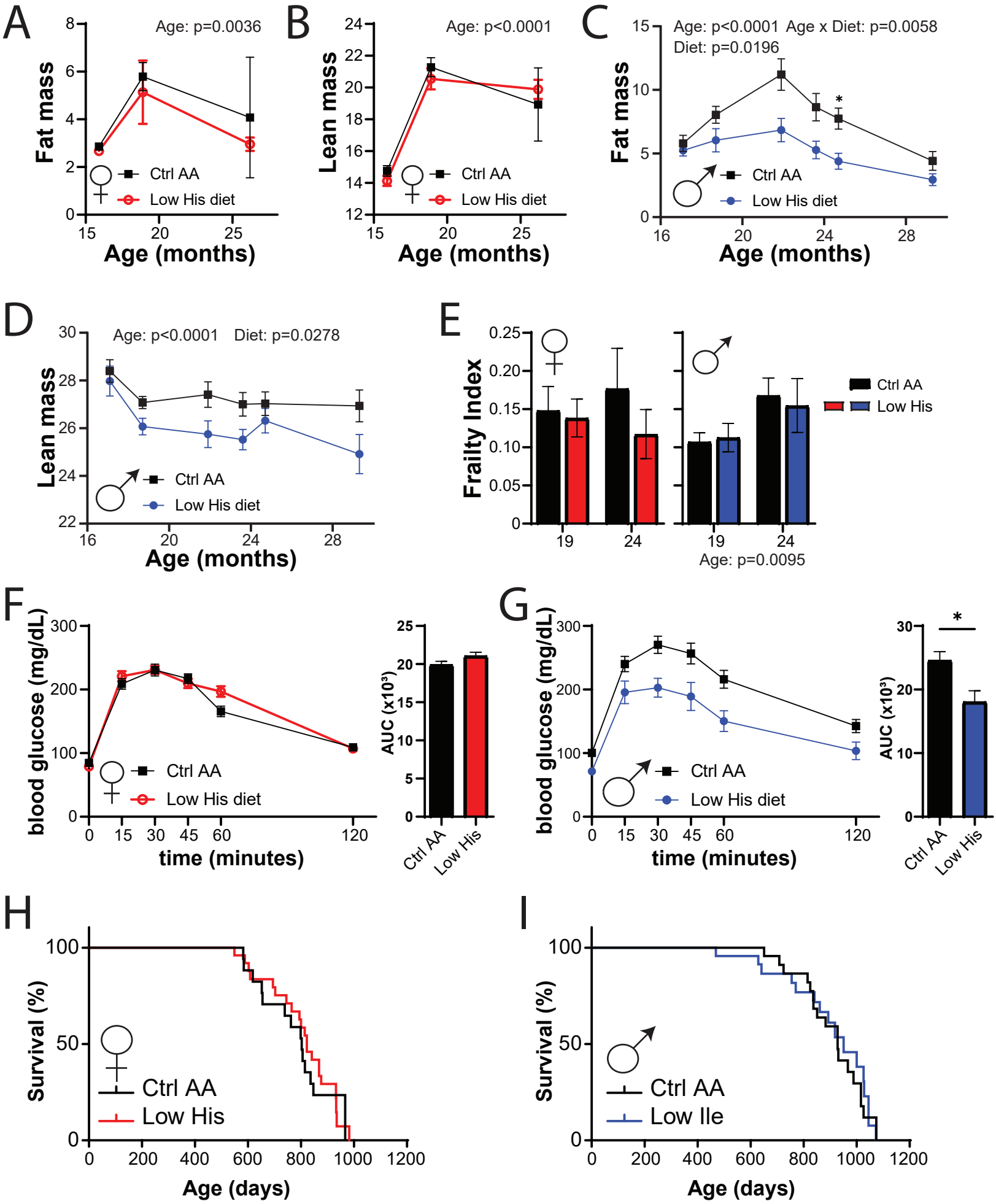
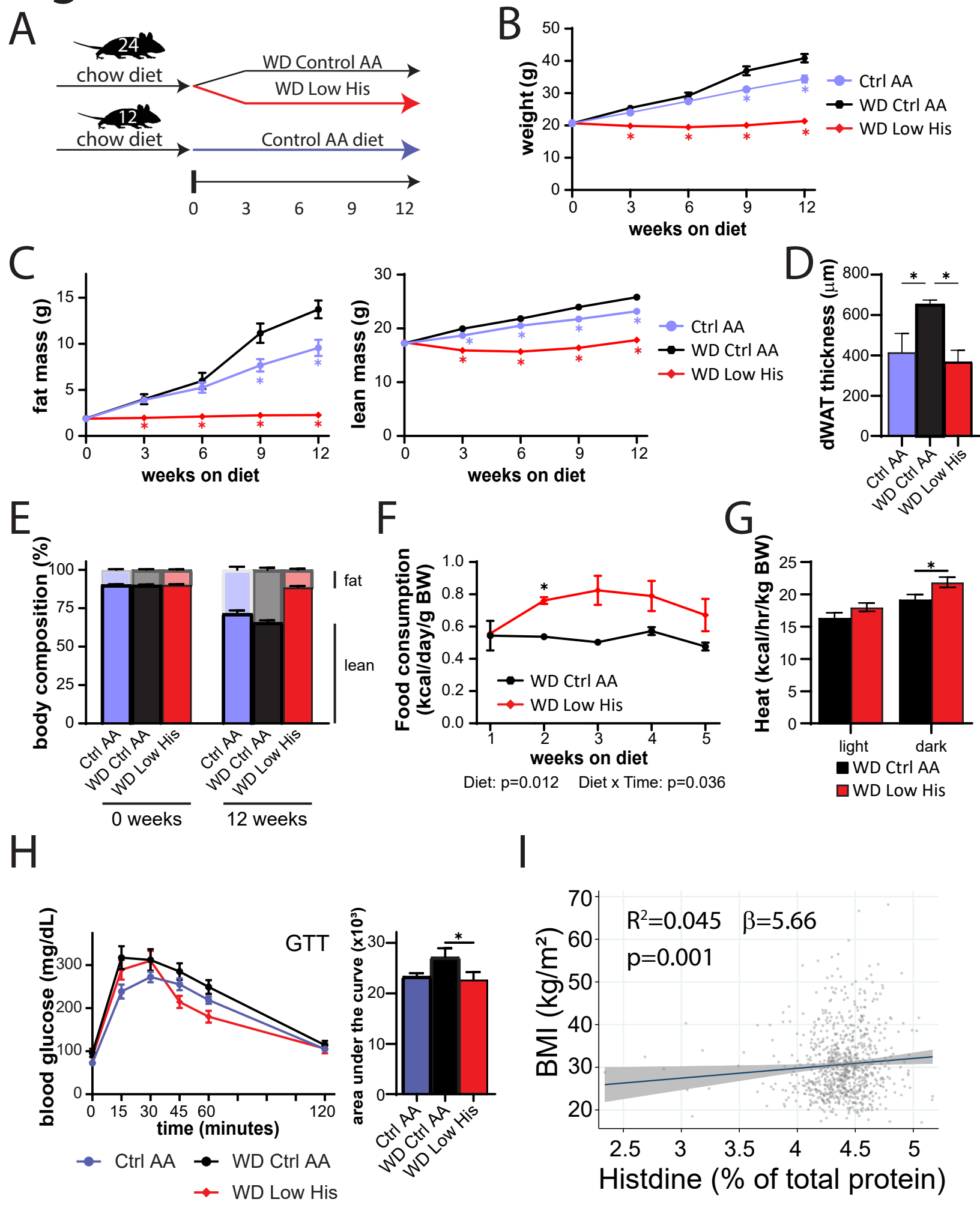

A
Age: p=0.0036
Fat mass
Age (months)
Ctrl AA
Low His diet
B
Age: p<0.0001
Lean mass
Age (months)
Ctrl AA
Low His diet
C
Age: p<0.0001 Age x Diet: p=0.0058
Diet: p=0.0196
Fat mass
Age (months)
Ctrl AA
Low His diet
D
Age: p<0.0001 Diet: p=0.0278
Lean mass
Age (months)
Ctrl AA
Low His diet
E
Frailty Index
Ctrl AA
Low His
Age: p=0.0095
F
blood glucose (mg/dL)
time (minutes)
Ctrl AA
Low His diet
AUC (x10³)
Ctrl AA
Low His
G
blood glucose (mg/dL)
time (minutes)
Ctrl AA
Low His diet
AUC (x10³)
Ctrl AA
Low His
H
Survival (%)
Age (days)
Ctrl AA
Low His
I
Survival (%)
Age (days)
Ctrl AA
Low Ile

Figure 8


A
24
chow diet
WD Control AA
WD Low His
12
chow diet
Control AA diet
0 3 6 9 12
B
weight (g)
weeks on diet
Ctrl AA
WD Ctrl AA
WD Low His
C
fat mass (g)
weeks on diet
lean mass (g)
weeks on diet
Ctrl AA
WD Ctrl AA
WD Low His
D
dWAT thickness (μm)
Ctrl AA
WD Ctrl AA
WD Low His
E
body composition (%)
fat
lean
Ctrl AA
WD Ctrl AA
WD Low His
0 weeks
12 weeks
F
Food consumption (kcal/day/g BW)
WD Ctrl AA
WD Low His
weeks on diet
Diet: p=0.012 Diet x Time: p=0.036
G
Heat (kcal/hr/kg BW)
light
dark
WD Ctrl AA
WD Low His
H
GTT
blood glucose (mg/dL)
time (minutes)
Ctrl AA
WD Ctrl AA
WD Low His
area under the curve (x10³)
Ctrl AA
WD Ctrl AA
WD Low His
I
R²=0.045 β=5.66
p=0.001
BMI (kg/m²)
Histdine (% of total protein)