Title: **Genome-wide analysis of diet and gene interactions in *Drosophila* uncovers the glucose transporter, *CG4607*, as a diet-responsive gene.**

Author list: Deanne Francis[1,2], Shila Ghazanfar [1,4,6], Essi Havula [1,2], James R. Krycer[1,2], Alistair Senior[1,2], Annabel Y. Minard[1,2,5], Thomas Geddes[1,2], Fiona Weiss[1,2], Jean Y.H.Yang [1,4] and David E. James[1,2,3*]

1 Charles Perkins Centre, The University of Sydney, Camperdown, 2006, Australia

2 School of Life and Environmental Sciences, The University of Sydney, Camperdown, 2006, Australia

3 Sydney Medical School, The University of Sydney, Camperdown, 2006, Australia

4 School of Mathematics and Statistics, University of Sydney, Sydney, NSW 2006, Australia.

5 Current Address: Department of Molecular Physiology and Biophysics, Carver College of Medicine, The University of Iowa, Iowa City, IA, 52242

6 Current Address: Cancer Research UK Cambridge Institute, University of Cambridge, Li Ka Shing Centre, Robinson Way, Cambridge, CB2 0RE, United Kingdom

* Corresponding author: David E. James, The University of Sydney, Camperdown, 2006, New South Wales, Australia. Email: david.james@sydney.edu.au

Author contributions: D.F., and D.E.J. conceived the study. D.F., J.R.K and D.E.J participated in experimental design. D.F. performed the experiments and D.F., A.S., E.H. and S.G. analysed data for the experiments. D.F. and D.E.J. wrote the manuscript, and all authors edited the manuscript. D.E.J. supervised the study.

Acknowledgements

The authors would like to thank Jacqueline Stoeckli and Elise Needham for critical feedback, Roel Bevers and Bart Deplancke for useful suggestions and members of the James Lab for helpful discussions. The authors acknowledge the facilities, and the scientific and technical assistance, of the Australian Microscopy & Microanalysis Research Facility at the Charles Perkins Centre, The University of Sydney.

Financial disclosure

The work is supported by National Health and Medical Research (NHMRC) project grants (APP1086851 for D.E.J.) and Diabetes Australia Research Program grants (J.R.K.). J.R.K. is recipient of the Australian Diabetes Society Skip Martin Fellowship. D.E.J. is an NHMRC Senior Career Fellow (APP1117078). The contents of the published material are solely the responsibility of the authors and do not reflect the views of the NHMRC. The authors have no financial conflicts of interest to declare.

Writing assistance

No funded writing assistance was used in the creation of this manuscript.

Ethical disclosure

The authors have nothing to disclose

Data sharing statement

This manuscript does not report the original results or secondary analysis of a clinical trial.

Abstract: It is clear that both genetic and environmental factors contribute to metabolic health. However, they do not act in isolation. Here, we sought to understand how the interaction between genes and diet influence the ability to store and utilise nutrients, a major determinant of metabolic disease. We achieved this by subjecting the *Drosophila* Genetic Reference Panel (DGRP), comprising 200 genetically divergent inbred fly strains, to diets varying in sugar, fat and protein content, and assessing starvation resistance, a proxy for an obesogenic phenotype. We discovered heterogeneity in the response to diet, as strains that were starvation resistant on one diet, were starvation sensitive on another. This indicates that genetics plays a major role in governing the diet-health axis. Furthermore, we discovered a diet-dependent heritability in starvation resistance, where high sugar and high protein diets had a greater genetic contribution to the variation in starvation resistance than those that contained fat. To uncover the genetic underpinnings of this variation in starvation response, we mapped 1,350 diet-responsive SNPs in 585 genes, 356 of which have human orthologues. We validated 39 candidate genes using whole-body knockdown and identified a number of novel genes regulating diet-dependent starvation resistance. One such gene was *CG4607*, a GLUT6/GLUT8 homolog, which was required for glucose tolerance, storage and utilization. A whole-body knock down of CG4607 led to a severe carbohydrate intolerance with the animals dying within 3 days from the start of the feeding. On the other hand, the *CG4607* knockdown animals showed starvation sensitivity on a high protein diet. Overall, this study provides definitive evidence that individuals possess optimal dietary environments based on genetic heterogeneity thus offering strong support for nutrigenomic strategies to map such interactions in humans.

Introduction:

Personalised medicine represents a shift away from the one-size-fits-all approach that has dominated medical practice for the past century. The tailored therapeutic approach is founded on the observation that one treatment does not work for all individuals in a heterogenous population, with differences in disease progression, aetiology, and drug efficacy being apparent among individuals (Ginsburg & Phillips, 2018). The

sequencing of the human genome has allowed the emergence of personalised therapies based on genetic differences between individuals, with considerable success in treating some cancers (Topol, 2014).

Given that overweight and obese individuals currently make up ~13% of the world population (Obesity and overweight), there has been much interest in mapping the heritability of these conditions using genomic approaches. Studies in mono- and di-zygotic twins revealed that a combination of genetic and environmental factors contributed to variance in body weight (Stunkard *et al*; Dubois *et al*, 2012). Despite this evidence, genome-wide association studies (GWAS) of body mass index, insulin resistance and other metabolic traits have identified several causative loci, yet these loci account for only a minority of phenotypic variation [5,8,9]. Studies in mice or humans have demonstrated that individuals display heterogeneous metabolic responses to the same diets (Zeevi *et al*, 2015; Parks *et al*, 2015). Thus, gene-environment interactions likely account for at least part of the missing heritability associated with metabolic diseases (Heianza *et al*, 2017). This emphasises the need to look beyond genetic predisposition alone and encompass the contribution of environmental factors, particularly diet, in assessing individual risk of developing metabolic disease.

A significant impediment to the full potential of personalised medicine is that, although we appreciate that genes and diet contribute to metabolic diseases, we do not yet understand how they interact (Heianza *et al*, 2017). We hypothesise that within a population, individuals respond differently to the same diet as a result of variants in diet-responsive genes. Thus, we are interested in discovering the identity of such 'diet-responsive' genes. This has also been the goal of nutrigenomics, a burgeoning area of nutritional research that investigates how bio-active compounds in food interact with specific genes and pathways (Sales *et al*, 2014; Mead, 2007). While a number of fat-responsive genes were identified in a human study (Maes *et al*), only a small number of genes were identified and other macro-nutrients like sugar and protein were not included. For instance, a high prevalence of obesity and type 2 diabetes in the Greenland Inuit population is linked to mutations in TBC1D4, a protein involved in glucose uptake into muscle and fat. Consequently, the switch from a traditional diet rich in fat and protein to a modern high-carbohydrate diet has drastically affected the metabolic health in Greenland Inuits (Andersen & Hansen, 2018; Manousaki *et al*, 2016).

Studies to identify gene-diet interactions in humans remain a challenge because the environmental variables are difficult to control at a sufficient scale to facilitate genetic mapping. In contrast, the *Drosophila*

fruit fly model system overcomes many of these logistical issues. Importantly, >70% of known human disease genes have fly orthologs (Reiter *et al*, 2001), and genetic tools such as the *Drosophila* genetics reference panel (DGRP) with 200 inbred and fully sequenced lines are available, thereby allowing identification of causal genetic variants (Mackay *et al*, 2012). Here, we combine the high-throughput nature of the *Drosophila* model with the genetic diversity of the DGRP to dissect diet-gene interactions on a population level. In this study, we aimed to identify "diet-responsive" genes and determine the mechanism by which genes in combination with diet control metabolic phenotypes. To do this, we used the DGRP to perform a GWAS to identify SNPs that contribute to variation in response to diets that differ in fat, sugar and protein contents.

Ultimately, knowledge of one's genetic predisposition to particular diets will allow for better control of personal metabolic health. Our study provides strong evidence for diet by gene interactions and uncovers a previously under-appreciated influence of diet on the heritability of starvation resistance. Finally, we provide a rich resource of diet specific genes for further study.

Results

Starvation Resistance of DGRP across 4 different diets

We first sought to identify novel diet-responsive genes in Drosophila. We used survival during starvation as a surrogate for an obesogenic phenotype to screen for dietary effects. This is a powerful and sensitive assay as starvation resistant flies are often replete with fat stores immediately prior to starvation and feeding flies a high sugar diet increases fat stores and prolongs starvation resistance (Bjedov *et al*, 2010; Hoffmann & Harshman, 1999; awdan *et al*, 1998). The diets we selected, (normal food (NF), high carbohydrate diet (HCD), high fat diet (HFD) and high protein diet (HPD) Supplementary table S1), were based on previous studies that explored the effect of different sugar and protein concentrations on starvation resistance in a single strain (Lee & Jang, 2014; Chandegra *et al*, 2017; Skorupa *et al*, 2008). The dietary composition is indicated in table S1: the carbohydrate is sugar, the protein is yeast and the fat was coconut oil. We exposed 3 to 5-day old adult males from 178 DGRP strains to the 4 diets for 10 days and then measured starvation resistance by removing food and assessing survival (Figure 1A). The DGRP has previously been used to examine starvation resistance in flies fed NF (Mackay *et al*, 2012) and there was a strong correlation in starvation resistance across the 178 strains between the two studies (males, NF, Pearson's R= 0.58, Figure 1B), demonstrating the robustness of

the starvation phenotype and the DGRP resource. Interestingly, we found that previously published food intake data [30] was negatively correlated with starvation resistance across genotypes (males, NF, Pearson's R= -0.32, Figure 1B) in both our study and previously published starvation data (Garlapow *et al*, 2015). This is intriguing as it suggests that strains that ate the most were the least resistant to starvation. This could be due to differences in metabolic rate, an increase in hunger cues, nutrient storage capacity or differences in hormonal responses.

Irrespective of strain, flies fed NF, HFD and HCD displayed the greatest variance in starvation resistance, while HPD flies had the least amount of variation across the strains (Figure 1C). We normalised the starvation resistance to NF for each strain and performed hierarchical clustering, indicating clusters of strains that were starvation sensitive to either HCD, HPD or HFD (Figure 1D). Surprisingly these data showed disparity between the responses to different diets, likely driven by genetic background, indicating that there is no single optimal diet where all strains display similar starvation resistance responses (Figure 1D).

We quantified the differential contributions of diet and gene by diet interactions to the variation in starvation resistance among DGRP strains. We determined the broad sense heritability (H^2) of the starvation response using linear-mixed models (LMMs). We found that across the whole data set, after accounting for differences among diets, the H^2 of starvation resistance was 20%. Dietary effects accounted for around 10% of the variance in starvation-resistance, leaving 72% residual (unexplained) variance. A model including a term to estimate genetic variance in dietary effects (random-slopes LMM) had significantly better fit than a model without (likelihood ratio test, L = 11611, d.f. = 14, $p<0.001$) indicating the presence of gene by environment interactions. Within-diet, the H^2 of starvation resistance on NF, HCD, HFD and HPD was 19%, 50%, 20% and 65% respectively (Tables 1 and Supplementary table S2). Thus, across populations certain diets expose genetic diversity more strongly than others and we estimate that genetics plays a particularly strong role in determining starvation resistance in flies fed either HPD or HCD.

Mapping 'diet-responsive genes'

We aimed to identify gene-diet interactions by uncovering SNPs that were associated with diet-responsive starvation resistance, in order to inform follow-up functional analyses of candidate genes. Starvation survival on different diets was normalised to starvation resistance on NF by taking the log2 of the

ratio of mean survival for the diet and the mean survival for NF for each strain of the 178 strains, and these normalised log fold change survival data were used to map diet-responsive SNPs. We first selected SNPs as significantly associated with diet in a multivariate manner by performing multivariate ANOVA testing and selecting SNPs with an associated unadjusted P-value below $1x10^{-4}$. We selected this threshold as a means of increasing the number of true positive SNPs, however at the expense of increasing the rate of false positive SNPs. To enrich the list of candidate SNPs, we selected SNPs for further consideration if they also exhibited a significant and large difference in survival compared to NF in at least one diet, by performing a univariate rank-based Wilcoxon Rank Sum Test, and selecting SNPs with an unadjusted p-value <0.01 as well as an absolute log fold change of at least 0.3 in response to diet (corresponding to a multiplicative factor of survival of at least 2^0.3 = 1.23 of that of NF for increased survival and of at most 2^-0.3 = 0.81 of NF for decreased survival). Using these stringent filters, we identified 1,350 SNPs that were associated with diet-responsive starvation resistance. These SNPs were located within 585 genes (Table S3), 356 of which had human orthologs (Table S3) with a high proportion of the SNPs (>80%) found in non-coding regions (Table S3 and S4). Manhattan plots for each diet revealed the chromosomal distribution of significant diet-responsive SNPs (Fig.2, A-D black dots). Additionally, we performed gene ontology (GO) analysis on the human orthologs of the 'diet-responsive genes' (Table 2) revealing stark differences in enriched biochemical processes between the different diets. For instance, genes associated with enhanced starvation resistance on HFD (UP) were enriched for peptidases that involve proteolytic pathways. In contrast, genes associated with reduced starvation resistance after HFD (down) and HPD were enriched for signal transduction and for HFD, ERK pathway in particular (Table2). Meanwhile, extracellular matrix pathways and pathways linked to sulfotransferases were respectively linked to enhanced and reduced starvation resistance after HCD feeding (Table 2). This suggests that individual diet responsiveness is controlled at the pathway level rather than at the level of individual genes *per se*. These data provide an abundant resource of diet-responsive genes and pathways.

Candidate gene validation

Candidate gene validation

The following criteria were used to select genes for further validation: a more stringent fold change cut-off with respect to diet response (>0.3 fold change); the presence of a human ortholog and an annotated gene function; and some functional link to metabolic homeostasis, as we reasoned that gene-diet interactions in the context of starvation resistance would likely be dominated by genes and/or pathways that control metabolism (Table 3). Using these criteria, we selected 38 genes for further validation using the GAL4-UAS system [23]. We generated whole-body knockdown flies for each gene in the W^{1118} background strain, pre-fed them four diets and monitored starvation with the *Drosophila* activity monitoring system (DAMs). Candidate genes were considered validated when the fold change in survival on each diet (relative to NF) corroborated with the SNP analysis (Table 3,Table S5). Strikingly, of the 38 genes tested, whole body depletion of 9 candidate genes led to lethality, while 21 of the remaining 29 candidates displayed the predicted diet interactions - 58% of these were significant according to a cox hazard multivariate analysis ($p<0.05$, Table 3).

Our SNP analysis combined diet and gene interactions to reveal interesting potential candidates for further study. Among the validated candidate genes, three out of the 22 were previously associated with starvation resistance. *Adenyly cyclase at 76E* (*Ac76E*) which is required for starvation stress resistance in multiple organs (Mattila *et al*, 2009), *spaghetti-squash activator* (*sqa*) a myosin light chain kinase activated by ATG1 to form autophagosome during starvation (Tang *et al*, 2011), and *methuselah-like 3* (*mthl3*) known to enhance starvation resistance in the red flour beetle, T. casteneum. Lipophorin receptor 2 (LpR2), required for the transport and binding of lipid molecules from the plasma membrane(Rodríguez-Vázquez *et al*, 2015; Parra-Peralbo & Culi, 2011) and CG1494, a predicted ABC transporter are both lipid transport proteins(Consortium, 1999). In addition, *Cdc42-interacting protein 4* (*Cip4*) is implicated in phospholipid binding in *Drosophila* and GLUT4 trafficking in mice (Feng *et al*, 2010; Zobel *et al*, 2015) . CCHamide-2 receptor (CCHa-2R) a G-protein coupled receptor and Drosophila ortholog of the bombesin receptor 3 (BRS3) regulates secretion of Drosophila insulin-like peptides in response to nutrient availability (Sano *et al*, 2015). Knockdown of all four genes were associated with reductions in starvation after HFD feeding. Another interesting candidate was happyhour (hppy) which is the Drosophila ortholog of MAP4K3, a serine/threonine kinase that regulates triglyceride homoeostasis and mTOR signalling (Bryk *et al*, 2010). Furthermore, loss of

CG3339 enhanced starvation resistance on HCD, CG3339 is the *Drosophila* ortholog of dynein heavy chain (DNAH9) a protein that regulates minus end directed microtubule trafficking(Consortium, 1999). However, the most striking starvation sensitivity in response to diet was observed for *CG9674* (glutamate synthase) and *CG4607* (GLUT6 /GLUT8 orthologue) knockdown flies fed HCD and HPD, respectively.

Analysis of CG4607, a diet responsive gene

We focused on *CG4607* because depleting this gene led to starvation sensitivity on HPD, it's annotated function as a glucose transporter and its homology to human GLUT6 and GLUT8 [24]. GLUT6 is over expressed in endometrial cancer and is highly expressed in brain, spleen and leukocytes[25]. GLUT8 is highly expressed in the testis, heart, brain, liver, fat, and kidneys and has been shown to respond to insulin[26–28]. Remarkably, flies expressing UAS-*CG4607* ^{KK104152} RNAi in the whole body (*CG4607* ^{KD}) died after only three days of eating HCD (Fig. 3A), while surviving on other diets. DGRP strain 45, which contained the CG4607 SNP, was lethal on HCD (Table S8). To further assess this phenotype, we sought to monitor the behaviour of control and *CG4607* ^{KD} flies while they were eating NF or HCD. We found a striking hyperactivity on HCD that abruptly stopped after 12 h of transitioning to HCD when the flies died (Fig. 3B, C). The hyperactivity was reminiscent of flies during starvation(Markow, 2015) and we speculated that the lethality was due to an inability of the flies to consume HCD, thereby experiencing starvation. However, upon measuring caloric intake in control and *CG4607* ^{KD} flies we observed that knockdown flies consumed 38% more calories of HCD than controls (Fig. 3D). Thus, the observed hyperactivity of *CG4607*^{KD} flies on HCD may be due to a perception of hunger brought about by the loss of glucose sensing, which may increase energy expenditure ultimately leading to lethality due to exhaustion of energy stores.

We investigated the mechanism by which *CG4607* controlled nutrient storage or utilisation in response to HPD. NF pre-fed *CG4607* ^{KD} flies were starvation resistant but became starvation sensitive after becoming low carbohydrate stressed on HPD (Fig. 4A). We posited that the starvation phenotype reflected the levels of energy stores. Fed nutrient levels were similar between *CG4607* ^{KD} and control flies on both NF and HPD with the exception of TAGs on NF and glycogen on HPD where the *CG4607* ^{KD} showed significantly higher levels (Fig. 4B-H). However major differences in nutrient storage levels were observed upon starvation on

both diets. Fasted levels of nutrient storage were higher in *CG4607* ^{KD} flies than in control flies (Fig. 4C, E, H). Fasting of HPD-fed flies resulted in a marked depletion of nutrients in both genotypes with undetectable fasted nutrient levels in control flies. Overall, our data shows that relative to control flies, *CG4607* ^{KD} flies have increased caloric intake and excessive energy stores on NF, which are reduced upon HPD feeding.

Mammalian GLUT6 and GLUT8 both localise to lysosomes in HeLa cells, testis or fibroblasts(Maedera *et al*, 2019; Diril *et al*, 2009). To determine if the localisation of CG4607 was conserved, we expressed CG4607-mRUBY3 in HeLa cells and using immunofluorescence microscopy we observed that, like GLUT6 and GLUT8, CG4607 partially co-localised with lysosomal markers but was absent from the cell surface (Fig.5A, B). Next, we wanted to explore if CG4607 functions like a glucose transporter. To address this, we measured glucose utilisation on a NF diet using a gas trap assay (Francis *et al*, 2019) where we fed flies (^{14}C)-glucose and monitored glucose incorporation into CO_2 and lipids. We observed that *CG4607* ^{KD} flies exhibited lower levels of (^{14}C)O_2 emission and (^{14}C) incorporation into lipids compared to controls (Fig.5C,D), indicating reduced glucose oxidation and utilisation This was independent of food intake that contains (^{14}C)-glucose intake (Fig.5E). Taken together, our data shows that CG4607, a GLUT6/8 orthologue, is a diet-responsive gene that regulates glucose metabolism.

Discussion

The DGRP is a powerful tool for understanding the genetics driving variation of metabolic phenotypes, including starvation resistance(Nelson *et al*, 2016; Unckless *et al*, 2015; Garlapow *et al*, 2015; Mackay *et al*, 2012). Our approach measured starvation resistance in the adult male population after acute exposure to diets that vary in sugar, protein and fat content. Using this method, we screened the DGRP, and our results support the notion that specific individuals possess individual dietary health optima. Additionally, our study revealed that exposure to HCD and HPD elicited a greater genetic contribution to phenotypic variation compared to NF in Drosophila. These data highlight a conserved heterogeneity in the response to diet that underlies the fundamental principles of personalised nutrition. The SNPs we identified provide a resource dataset for further study. Out of 39 candidate diet responsive genes that were selected for more detailed analysis, we validated 2 genes as bona fide diet responsive genes. Knock down of one such gene, *CG4607* a GLUT6/8 homolog, led

to starvation resistance on NF, but this resistance was lost upon HPD feeding. Remarkably, *CG4607* ^{KD} flies died within three days of continuous HCD exposure indicating that this is a potent sugar sensitive gene. Finally, we show that starvation resistance in *CG4607* ^{KD} flies was linked to increased caloric intake and nutrient storage combined with reduced glucose utilisation.

A major finding of this study was the observation that strains fed one diet differed in their starvation response when fed a different diet and to our knowledge this is one of the most definitive studies to show that individuals have optimal diets (Zeevi *et al*, 2015). Genetically isolated populations such as the Greenland Inuits and the Pima Indians provide dramatic examples of the effect of gene-environment interactions on metabolic health. The Greenland Inuits lived relatively healthy on a high fat, low carbohydrate diet, however many developed type 2 diabetes when exposed to high amounts of carbohydrates. Of interest, this population was found to have a higher penetrance of a loss of function mutation in one of the major genes controlling meal induced sugar utilisation, TBC1D4, that is not found in other populations(Manousaki *et al*, 2016; Andersen & Hansen, 2018). In contrast, the Pima Indians of the South Western United States and Mexico subsisted on a high carbohydrate diet, yet with an increased fat intake they rapidly developed the highest incidence of type 2 diabetes of any reported population (Schulz & Chaudhari, 2015). GWAS identified SNPs in Acyl-coenzyme A dehydrogenase 10 (ACAD10) an enzyme required for fatty acid oxidation(Bloom *et al,* 2018) which suggests that defective lipid handling may account for the high incidence of type 2 diabetes in the Pima Indian population. These studies reveal that the mechanisms that underpin diet sensitivity are complex and involve differences in food preference and thus food intake as well as differences in nutrient metabolism (energy storage and utilisation). Another major finding of this study was that gene-diet interactions vary with respect to diet. Since the natural food sources of *Drosophila* are rotting fruits that contain sugar and yeast (Markow, 2015), we postulate that genetic variants involved in the processing, storage and utilization of sugar or protein (from yeast) contribute to differences in starvation resistance. Indeed, changes in sugar and lipid handing enzymes have previously been linked to starvation resistance(Harshman *et al*, 1999). Alternatively, sugar and protein (from yeast) are dominant drivers of food intake with major sensory systems to control intake of these macronutrients and hence these are more likely to be under genetic control(May *et al*, 2019; Chng *et al*, 2017).

This study provides a rich resource of diet responsive genes and pathways. The majority (>80%) of the SNPs in candidate genes were non-coding, a finding that is consistent with previous DGRP (Mackay *et al*, 2012) and human GWAS (Gallagher & Chen-Plotkin, 2018) and the functional consequences of such SNPs remain to be identified. As a proof of principle, we were able to validate candidate genes with intronic SNPs using RNAi knockdown indicating that these types of mutations may control expression of these genes. However, further studies examining transcriptional control are required to validate this conclusion.

We focused on *CG4607*, a validated diet-responsive gene. SNPs in *CG4607* were identified in a GWAS of sleep/activity (Harbison *et al*, 2013) and whose expression is increased in DGRP lines with differential responses to enteric bacterial exposure (Sleiman *et al*, 2015). Whole body depletion of CG4607 resulted in lethality after 3 days on HCD (Fig. 4A) and the lethality was accompanied by hyperactivity and an increase in caloric intake on HCD compared to control flies. Flies typically become hyperactive during starvation, as they tried to forage for food (Yu *et al*, 2016; Yang *et al*, 2015). Thus, our data suggest that death after HCD exposure in *CG4607* ^{KD} flies results from a potential starvation-like phenotype. This is consistent with the role of CG4607 in glucose transport, as *CG4607* would be required for maintaining glucose levels during high sugar diet feeding. Although we see that total sugar levels in whole fly preparations are unchanged between control and *CG4607* ^{KD} flies, they may have higher circulating sugar levels. Furthermore, *CG4607* ^{KD} animals have higher energy stores on NF compared to control flies, which could be due to increased gluconeogenesis, a reaction to a perceived lack of nutrients, thus, mimicking an insulin resistant state. Hence, the presence of excessive sugar due to HCD feeding exacerbates the energy imbalance observed on NF, resulting in rapid lethality.

CG4607 is the closest *Drosophila* orthologue of the human glucose transporters GLUT6 and GLUT8. These transporters are expressed in a range of mammalian tissues (Gorovits *et al*, 2003; Doege *et al*, 2000; Carayannopoulos *et al*, 2000; Lisinski *et al*, 2001; Diril *et al*, 2009). While their function in these tissues remains unclear it is intriguing, in light of our data (Fig 4), that deletion of GLUT8 in mice led to a hyperactivity phenotype (Schmidt *et al*, 2008). Moreover, similar to CG4607, GLUT6 and GLUT8 are targeted to lysosomes and there is no evidence that these transporters function at the plasma membrane, analogous to other facilitative sugar transporters, like GLUTs1-5 (Maedera *et al*, 2019; Lisinski *et al*). Hence,

one possibility is that these transporters subserve their major function on lysosomes. This is intriguing as this is also where the major nutrient sensor mTORC1 is found and mTORC1 activity is regulated by a range of nutrients including glucose (Lee *et al*, 2009; Efeyan & Sabatini, 2013). During nutrient deprivation, the mTOR pathway regulates glycophagy, or the breakdown of cellular glycogen to glucose in autophagic vesicles (Zhao *et al*, 2018; Mony *et al*, 2016) and it is conceivable that CG4607 is required for this process. Our metabolic characterization of *CG4607* ^{KD} flies supports this possibility, as glycogen levels on NF in these flies were higher than the controls after starvation. Similarly, TAG levels in *CG4607* ^{KD} flies were higher after eating NF but were substantially reduced after starvation suggesting that these flies break down more TAG during starvation (Fig. 5 B-H). Thus, our data in *CG4607* ^{KD} flies resembles a lysosomal glycogen storage disease, where lysosomal glycogen cannot be accessed for energy utilisation in the cytosol.

Overall, the ramifications of our findings are substantial for humans because it means that the concept of a 'healthy' diet varies between individuals, thus questioning population-wide nutritional recommendations. While our study provides the basis for a nutrigenomics initiative such an endeavour is likely to require a substantial future investment.

Materials and Methods

Drosophila Stocks and procedures

Stocks: DGRP (Bloomington *Drosophila* Stock Center, Indiana, USA), RNAi reagents (VDRC, Vienna, Austria), *tubulin*-GAL4 and *ubiquitous*-GAL4 (Mattila *et al*, 2015), *CG*-Gal4 (Bloomington # 7011).

DGRP flies were maintained at standard temperature and 12h light/dark cycle. DGRP flies were expanded in bottles before collecting adult males for experiments. 5 replicates of 10 3-5 day old adult males from each strain were collected and passaged onto each diet. Food was changed every other day, and the mortality rate was monitored for the ten days of diet treatment. The diets were well tolerated with a similar lethality on HFD during pre-feeding compared to other diets and did not affect the starvation assay. Afterwards, males were placed into starvation vials with kimwipes and 1 mL of water and monitored every 12 hours for death. The median and mean starvation resistance was analysed using Prism and the R (CRAN, survival and survminer packages).

Gas Trap Assay to measure CO2 and Triglycerides:

The gas trap protocol has been previously described(Markow, 2015).Briefly, 4 replicates of 10 male adult 3-5-day old flies were starved overnight with a Kim wipe and 1mL of water. Flies were placed into 12 well plates containing glucose radiolabelled food and blue dye. We measured glucose oxidation and processed the flies for TAGs and Blue dye content as described.

Blue Dye Extraction and measurements: 4 replicates of 10 flies were collected and homogenized (Reche MM400) in 100uL of water. Samples were briefly spun down and the supernatant was dried down in the Genevac personal evaporator. Dried samples were reconstituted in 50uL of water, vortexed and placed into a 96 well plate for measurement at 628nm in a spectrophotometer. Dilutions of blue dye (Queenie Brand, Coles Supermarket, Australia) were used as a reference. Data was analysed in Excel (Microsoft) and plotted in Prism (Graphpad). Statistical significance was calculated between genotypes using Student's t-test.

Statistical SNP analysis

DGRP SNP genotypes were downloaded from the DGRP Freeze 2 online resource http://dgrp2.gnets.ncsu.edu/data.html for all lines considered in this manuscript. SNPs were filtered so at least five lines contained one of each of the reference and alternate alleles. Lines with missing allele information for a given variant were not considered , resulting in testing across 2,455,135 SNPs. Statistical testing included multivariate analysis of variance (MANOVA) testing , with Wolbachia status as a covariate per variant, with an unadjusted P-value < 1e-4 as significant, as well as Wilcoxon Rank Sum Tests per diet. To assess the effect size, we calculated the median difference in phenotype between 'reference' and 'alternate' allele groups per diet, phenotype being the log2 ratio of survival to NF per line. We selected diet-SNP pairs for further consideration if they were significant for both the MANOVA test and Wilcoxon Rank Sum Test, and had an absolute difference in median phenotypes of at least 0.3.

Gene ontology analysis: Gene ontology and pathway analyses were performed using Fisher's Exact Test in the 'goseq' R package. A total of 6,056 pathways, based on human genes, with at least 10 and at most 500 genes were kept, with a 'gene universe' of 7,677 human genes that are homologues of the fly genes tested.

Pathways were considered significant if FDR-corrected values were below 0.05, per diet and direction of phenotype change.

Validation/Automated starvation resistance (DAMS assay): RNAi knockdown fly lines (see table S5 for reagent ID) were mated to 20 *ubi*-Gal4 females. Sixteen 3-5 day old males were placed on five different diets for ten days. The food was changed every other day for ten days until males were placed into the DAMS apparatus (Trikinetics, inc., USA). The flies were loaded into DAMS tubes containing 2% agar and monitored every 5 minutes for starvation resistance. For activity analysis, 3-5 day old male flies were placed into DAMs tubes with either NF or HCD and monitored for activity for ten days. DAMS data were analysed using the R survminer and rethomics(Geissmann *et al,* 2019) package.

Capillary feeding assay: 10 replicates of 5 3-5 old adult males were placed into vials with water soaked kimwipes and sealed with a rubber stopper with two holes. 5uL capillary tubes with food was placed through one hole and into the vials to allow flies to feed for 24 hours(Lee *et al,* 2008). The diets were composed of yeast (MP Biomedicals cat # 2232731), and sugar (Coles Supermarket, Australia).

Diets: Diets were made up of agar (Sigma) and torula yeast (H.J Langdon & Co, Victoria, Australia), sugar (Coles, Victoria, Australia) and extra virgin coconut oil (Absolute Organics, NSW, Australia).

Triglycerides Assay: 6 to 10 replicates of 6 3-5 day old flies were collected and washed in 4 dilutions of isopropanol to remove excess food. Triglyceride extraction was performed as previously described(FOLCH *et al,* 1957). The lipids were collected after extraction, evaporated under N2 gas and reconstituted with 95% ethanol. Scintillant was added to samples with radioactive tracer instead of ethanol and read on a beta counter (Beckman Coulter). Samples were spun and placed into 96 well plates (Sigma-Aldrich, # CLS9018BC) and incubated with triglyceride reagent (200uL; Thermo Fischer Cat #TR22421) for at 37C for 30min. 1mg/ml glucose was used as a reference. Total absorbance at 340nm was measured in a plate reader (Beckman) and subtracted from a blank before determining the amount of triglyceride using the reference standard curve. All calculations were performed in Excel (Microsoft) and graphed in Prism.

Glucose assay: Glucose was measured from the aqueous phase of the triglyceride extraction (see above) and the methanol: the aqueous mixture was evaporated in a Genevac E2-3 evaporator until a dried pellet was visible. The pellet was reconstituted with water and glucose was measured as described for glycogen.

Glycogen Assay: We collected six replicates of 6 male flies and washed them in several dilutions of isopropanol to remove food. Fasted flies were collected after 24 hours of starvation. Flies were homogenised in 1M Koh for 30 seconds using steel balls and a tissue lyser (Resche MM400). Samples were heated for 30 min at 70 degrees C. Saturated Na_2SO_4 was added following 95% Ethanol for precipitation. The pellet was spun down and then reconstituted in water, heated at 70 degrees and 95% Ethanol was added again. The pellet was spun down, and aminoglycosides were added overnight at 37 degrees. Samples were spun and placed into 96 well plates (Sigma-Aldrich, # CLS9018BC) and incubated with glucose oxidase reagent (200uL; Thermo Fischer, TR15221) for at 37C for 30min. 1mg/ml glucose was used as a reference. Total absorbance at 340nm was measured in a plate reader (Beckman) and subtracted from a blank before determining the amount of glucose using the reference standard curve. All calculations were performed in Excel (Microsoft) and graphed in Prism.

Cell Culture and Immunostaining: HeLa cells were kept in DMEM with 1% glutamax and 10% FCS at 37 degrees and 5% CO2. Cells were transfected with lipofectamine 2000 and split onto coverslips at two $x10^5$ cells/mL. Coverslips were fixed in 4% paraformaldehyde, washed with PBS, and blocked for 30 minutes with 0.02% saponin (Sigma,) and 2% BSA in PBS. Primary antibodies: ms anti-LAMP1(1:100, Developmental Studies Hybridoma Bank 4C CR). Secondary antibodies: (Gt anti-mouse 488 (1:200, Invitrogen)). Coverslips were mounted in mowiol and imaged using a 60X water objective on the A1R confocal (Nikon). Images were processed using Fiji (ImageJ, NIH, Bethesda, MD).

qPCR of knockdown: Three replicates of 10 flies were homogenized in TRIzol™ Reagent (Invitrogen, 15596026) and RNA was precipitated out. cDNA was created (superscript II, Invitrogen 18064014). Tubulin was used as a housekeeping gene and the following primers were used to amplify CG4607:

CG4607 F3 ACTCCCACGCGAAGGAGAA
CG4607 R3 GCTGATTGAGAGTAACTGCCG

The samples were run using the ROCHE Lightcycler 480 II (Roche). The knockdown efficiency was calculated using the delta-delta Ct method (Excel and Graphpad, Prism) and the Ct values were graphed. Significance was calculated using a student's t- test (p<0.0001 ****).

Generation of CG4607-mRuby3: CG4607-mRuby3 construct was created through Gibson cloning. The CG4607 cDNA (#, Drosophila Genome Resource Center, Indiana, USA) was PCR amplified using the following primers:

dCG4607GibF1: GGACTCAGATCTCGAGACAAGATGAAGGGCCAGCAGGAGGAG

dCG4607GibR1: CATGCTGCCttCAGCTGAGGACAATTTCTTTAGGAACACTT

The backbone GLUT4-mRuby3 was PCR amplified to include overhangs using the following primers:

mRuby3_gib_F1: TCCTCAGCTGAAGGCAGCATG

mRuby3_gib_R1: AGCTGAGGATCCCTTGTCTCGAGATCTGAGTCC

PCR products were placed together with Gibson master mix, and the resulting plasmid was sequenced before cell transfection.

Figure Legends:

Figure 1: Inter-strain variation in response to diet. (A) DGRP screen schematic outlines the work flow. Each individually coloured fly represents an individual DGRP line and survival curves represent the measured starvation resistance after exposure of flies to each diet. (B) Pairs plot showing a positive correlation with starvation resistance data from Mackay et al, 2012 and negative correlation with food intake to starvation resistance data on NF from this study. (C) Histograms showing the distribution of the mean starvation resistance of each fly independent of strain on each diet. (D) Heatmap of mean starvation resistance of each DGRP strain relative to its mean survival after NF exposure.

Figure 2: Mapping the SNPs in diet-responsive genes (A) Manhattan plot of selected SNPs based on a multi-variate significance p-value of < 1x 10 -4, filtered by a univariate significance of p< 0.01 and a fold change of >0.3. Highly significant SNPs with a univariate p-value <0.01 and a fold change >0.3 are in black. The y-axis in the Manhattan plot is -log (P-value) for the multivariate test. (B) An upset plot indicating the degree of overlap of the numbers of highly significant

SNPs between each diet and the number of SNPs per diet. (C-E) Manhattan plots of selected SNPs based on a multi-variate significance p-value of < 1x 10 -4, filtered by a univariate significance of p< 0.01 and a fold change of >0.3 for each diet. Highly significant SNPs with a univariate p-value <0.01 and a fold change >0.3 are in black.

Figure 3: Whole body knockdown of *CG4607* is lethal upon HCD feeding. Survival of *ubi*-GAL4>*CG4607* ^{KK104152} flies on NF and HCD. (A) Adult male control and *ubi*-GAL4>*CG4607* ^{KK104152} flies (n=60) were placed on either NF or HCD and manually monitored for lethality every 24 hours. We observed that all *ubi*-GAL4>*CG4607* ^{KK104152} flies were dead within 60 hours after HCD feeding. (B) Actogram of activity and sleep (n=16 flies) wrapped over 24 hours of single housed individual control and *ubi*-GAL4>*CG4607* ^{KK104152} flies in DAMS monitors and beam crosses were monitored every 5 minutes while they ate NF or HCD (C) An inset of B showing a closer view of activity over the first 9 hours. Activity data was analysed using the rethomics package in r(Lee *et al*, 2008). (D) Caloric intake of adult male flies (n=50/genotype) using the CAFE assay. *ubi*-GAL4>*CG4607* ^{KK104152} flies ingested significantly more calories from HCD (p<0.02) than control. Data is mean +/- SD and significance between genotypes was calculated using a t-test , ** p<0.01 (Prism).

Figure 4: *CG4607* interacts with HPD to regulate starvation resistance. (A) A starvation survival plot of 32 individual control and *ubi*-GAL4>*CG4607* ^{KK104152} male flies pre-fed each diet. The survival curves show that *ubi*-GAL4>*CG4607* ^{KK104152} male typically live longer on NF but are starvation sensitive when fed HPD. Data is representative of three independent experiments. Significance is the log-rank test for trend between all genotypes (survminer package in R) (B) Glycogen content was significantly reduced between control flies fed NF and HPD. However, glycogen content was the same between NF and HPD fed *ubi*-GAL4>*CG4607* ^{KK104152} flies (C) Fasted glycogen was reduced in NF fed control flies compared to NF fed *ubi*-GAL4>*CG4607* ^{KK104152} flies, but pre-feeding with HPD reduced glycogen levels of *ubi*-GAL4>*CG4607* ^{KK104152} flies, while starvation after control flies ate HPD was lethal. (D) Glucose content was not significantly different across genotypes. (E) Fasted glucose levels of NF pre-fed flies had similar glucose levels, however, glucose levels were significantly reduced in HPD pre-fed *ubi*-GAL4>*CG4607* ^{KK104152}. (F)Triglyceride levels of *ubi*-GAL4>*CG4607* ^{KK104152} animals were increased on NF feeding, but reduced upon eating HPD. (G) Fasted TAG stores were reduced in control flies pre-fed NF, while NF fed *CG4607*^{KD} flies maintained their TAG stores. (H) HPD pre-fed flies had reduced TAG stores. (all experiments n=36 flies/genotype and representative of two experiments) Significance between genotypes was calculated using a one-way ANOVA , ** p<0.01, *** p<0.001 (Prism).

Figure 5: *CG4607* controls glucose utilisation and uptake. (A,B) Micrographs of fixed and immunostained HeLa cells were transfected with pCMV-CG4607-mRuby3 (red), immunostained with anti-LAMP1 antibody (green) and counterstained with Hoescht (blue). (A, inset (B)) CG4607-mRuby3 colocalises with Lamp1 expressing vesicles. (C) All the following

experiments were performed on n= 40 control and *ubi*-GAL4>*CG4607* ^{KK104152} males. Glucose oxidation is reduced after 4 hours of administrating C14 labelled glucose. (D) C14 incorporation into TAGs is reduced in *CG4607*^{KD} flies. (E) The accumulation of blue dye is not significantly different between control and *CG4607*^{KD} flies during the assay. The data is from two independent experiments. Significance between genotypes was calculated using a student's t-test, ** p<0.01, **** p<0.0001 (Prism).

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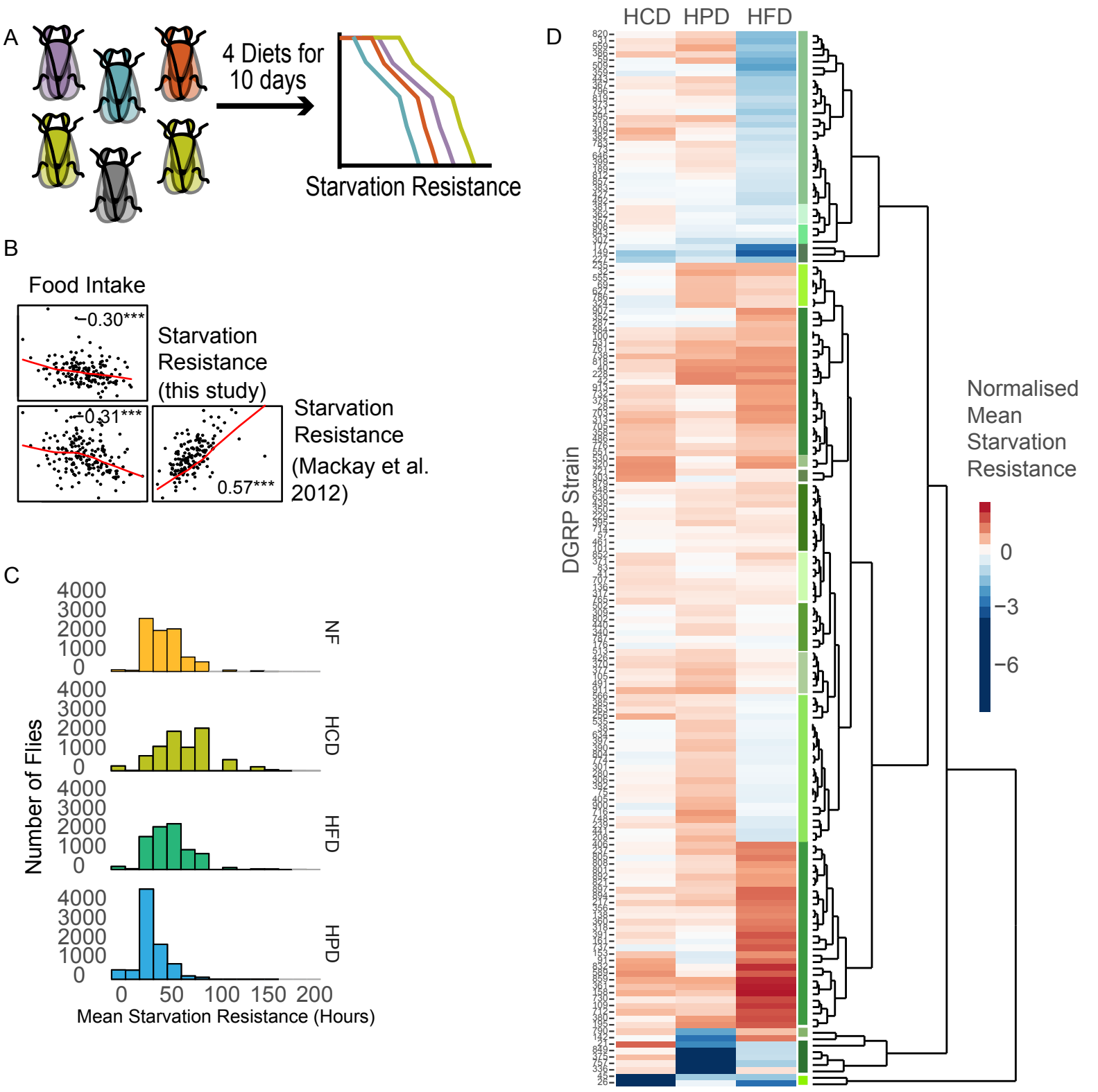
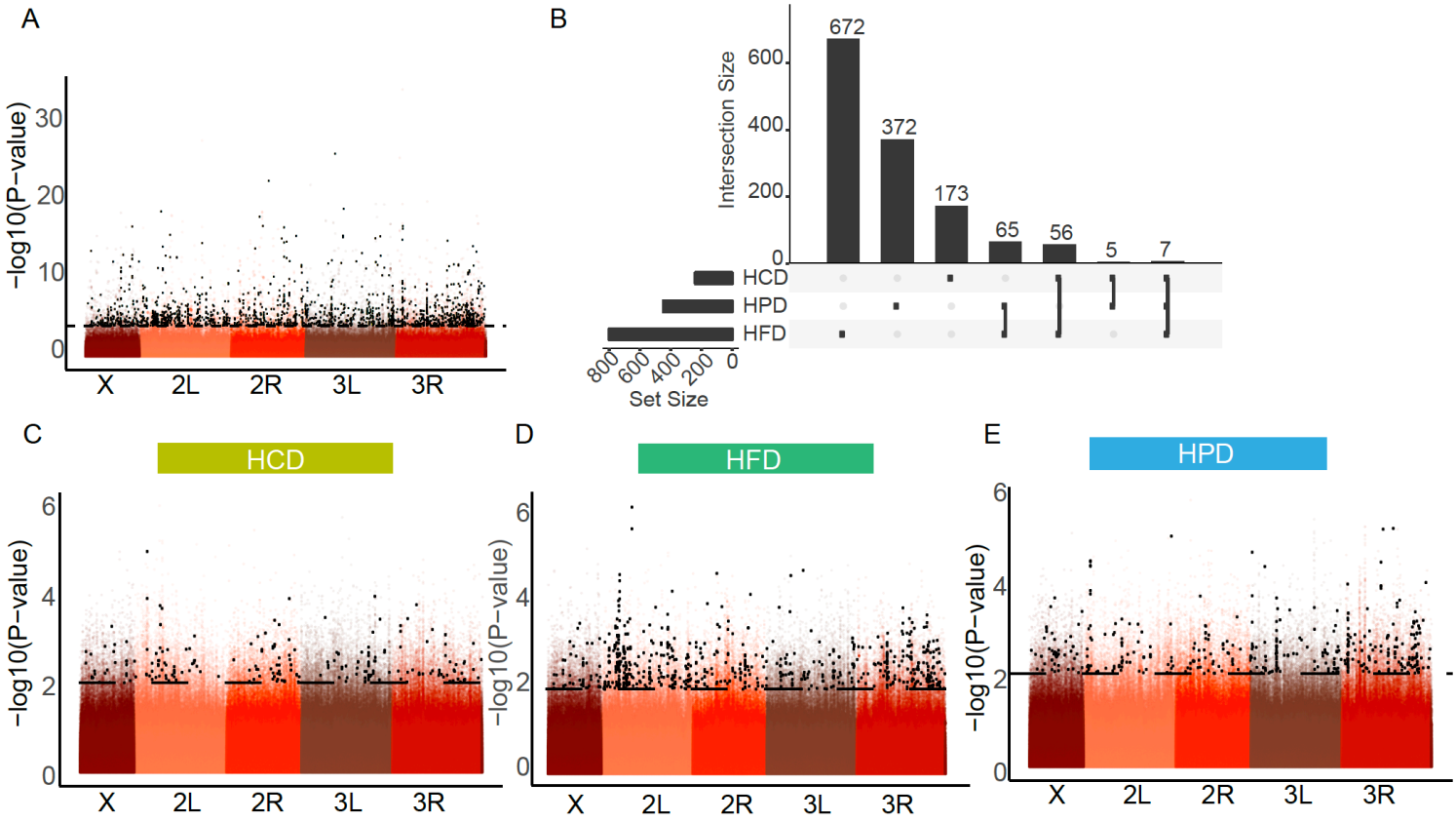
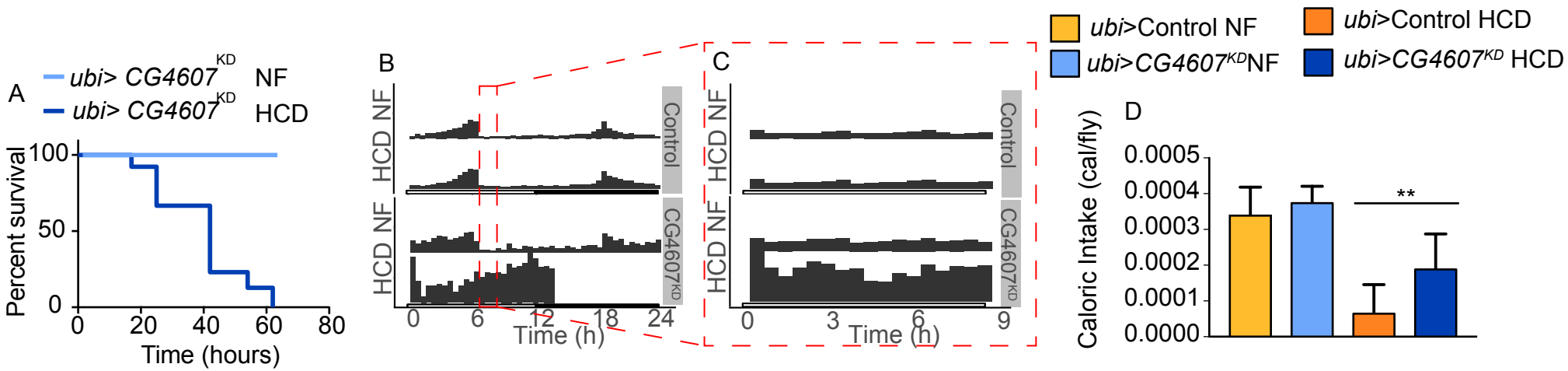
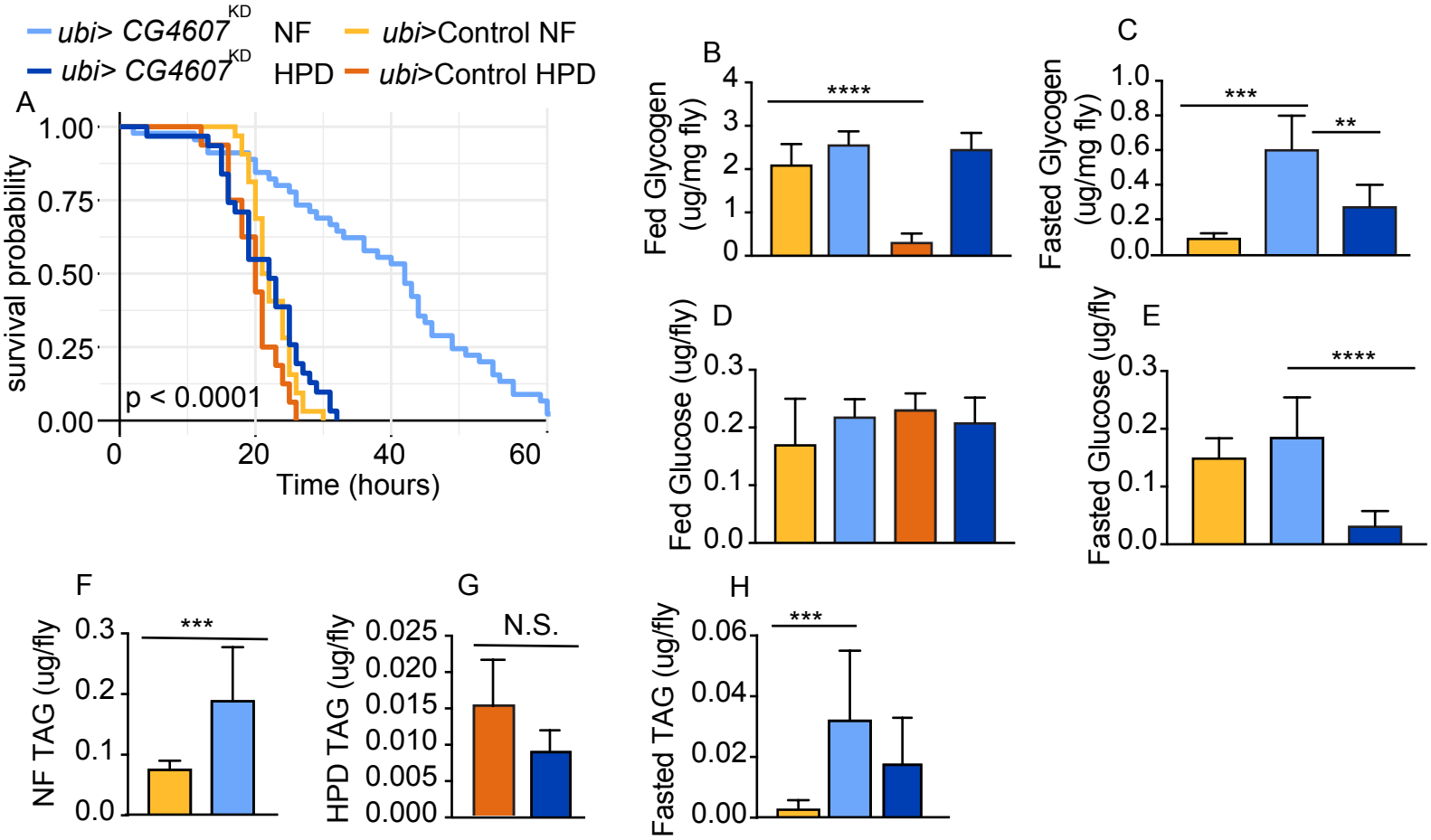

A
4 Diets for
10 days
Starvation Resistance
B
Food Intake
−0.30***
Starvation
Resistance
(this study)
−0.31***
0.57***
Starvation
Resistance
(Mackay et al.
2012)
C
Number of Flies
NF
HCD
HFD
HPD
Mean Starvation Resistance (Hours)
D
HCD
HPD
HFD
DGRP Strain
Normalised
Mean
Starvation
Resistance
0
−3
−6


A
−log10(P−value)
30
20
10
0
X
2L
2R
3L
3R
B
Intersection Size
672
372
173
65
56
5
7
600
400
200
0
HCD
HPD
HFD
800
600
400
200
0
Set Size
C
HCD
D
HFD
E
HPD


A
ubi> CG4607KD NF
ubi> CG4607KD HCD
Percent survival
100
50
0
0
20
40
60
80
Time (hours)
B
HCD NF
HCD NF
Control
CG4607KD
0
6
12
18
24
Time (h)
C
HCD NF
HCD NF
Control
CG4607KD
0
3
6
9
Time (h)
ubi>Control NF
ubi>CG4607KDNF
ubi>Control HCD
ubi>CG4607KD HCD
D
Caloric Intake (cal/fly)
0.0005
0.0004
0.0003
0.0002
0.0001
0.0000
**


ubi> CG4607KD NF
ubi> CG4607KD HPD
ubi>Control NF
ubi>Control HPD
A
survival probability
1.00
0.75
0.50
0.25
0.00
p < 0.0001
0
20
40
60
Time (hours)
B
Fed Glycogen (ug/mg fly)
4
3
2
1
0
****
C
Fasted Glycogen (ug/mg fly)
1.0
0.8
0.6
0.4
0.2
0.0
***
**
D
Fed Glucose (ug/fly)
0.3
0.2
0.1
0.0
E
Fasted Glucose (ug/fly
0.3
0.2
0.1
0.0
****
F
NF TAG (ug/fly
0.3
0.2
0.1
0.0
***
G
HPD TAG (ug/fly
0.025
0.020
0.015
0.010
0.005
0.000
N.S.
H
Fasted TAG (ug/fly
0.06
0.04
0.02
0.00
***

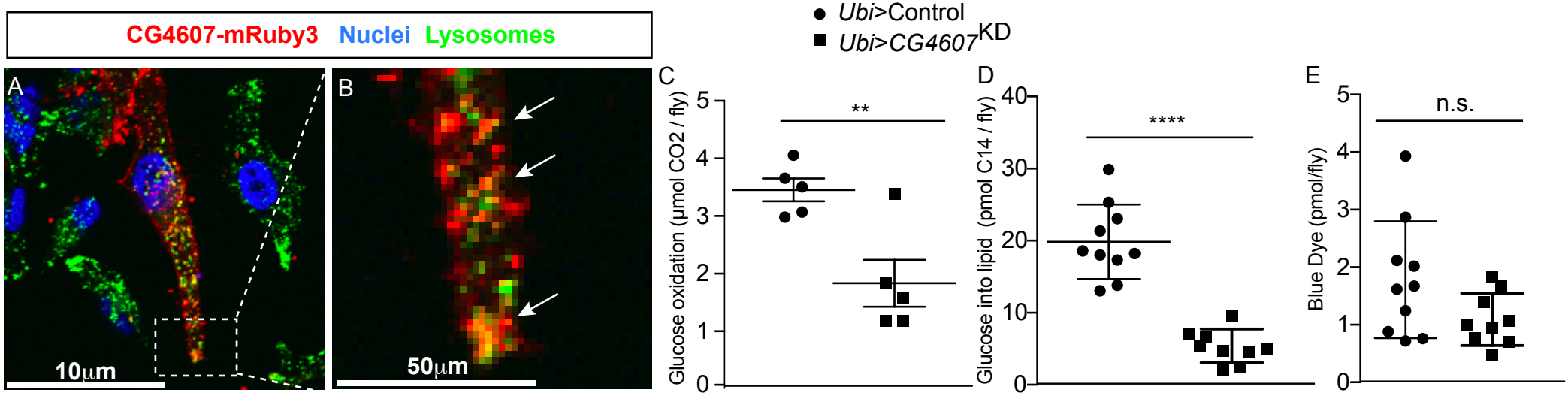

CG4607-mRuby3 Nuclei Lysosomes
A
10μm
B
50μm
Ubi>Control
Ubi>CG4607KD
C
Glucose oxidation (μmol CO2 / fly)
**
D
Glucose into lipid (pmol C14 / fly)
****
E
Blue Dye (pmol/fly)
n.s.

Table 1: Analysis of the mean starvation resistance data on all diets.

Diet	Mean	SEM	Variance (Genetic)	Broad Sense Heritability
NF	51.46	1.15	0.086	19%
HCD	68.88	1.64	0.364	50%
HFD	55.10	1.09	0.089	20%
HPD	36.77	0.97	0.671	65%

A summary table of the mean starvation data, SEM, genetic and environmental variance from the log transformed starvation data across all the DGRP lines on each diet.

Estimates of genetic variance and heritability are derived from linear mixed models of log survival (see supplementary table S2).

Table 2: GO term analysis of human orthologs of diet-responsive genes

HPD					
Up			**Down**		
GO ID	**Term**	**p.Value**	**GO ID**	**Term**	**p.Value**
			GO:0005887	integral component of plasma membrane	5.68E-08
			GO:0004888	transmembrane signaling receptor activity	2.88E-07
			GO:0038023	signaling receptor activity	2.33E-06
			GO:0060089	molecular transducer activity	3.19E-06
HFD					
Up			**Down**		
GO ID	**Term**	**p.Value**	**GO ID**	**Term**	**p.Value**
GO:0004181	metallocarboxypeptidase activity	4.93E-09	GO:0005887	integral component of plasma membrane	6.09E-10
GO:0004180	carboxypeptidase activity	3.28E-08	GO:0004714	transmembrane receptor protein tyrosine kinase activity	9.22E-09
GO:0008235	metalloexopeptidase activity	2.35E-08	GO:0019199	transmembrane receptor protein kinase activity	6.41E-09
GO:0008238	exopeptidase activity	3.50E-07	GO:0038023	signaling receptor activity	1.98E-08
GO:0008237	metallopeptidase activity	3.44E-06	GO:0060089	molecular transducer activity	1.63E-08

			GO:0004888	transmembrane signaling receptor activity	1.01E-07
			GO:0006812	cation transport	5.92E-07
			GO:0070374	positive regulation of ERK1 and ERK2 cascade	1.11E-06
			GO:0022890	inorganic cation transmembrane transporter activity	1.38E-06
			GO:0015370	solute:sodium symporter activity	2.46E-06
			GO:0008146	sulfotransferase activity	3.23E-06
			GO:0046873	metal ion transmembrane transporter activity	4.06E-06
			GO:0070372	regulation of ERK1 and ERK2 cascade	4.17E-06
			GO:2001257	regulation of cation channel activity	4.13E-06
			GO:0015077	monovalent inorganic cation transmembrane transporter activity	5.19E-06
			GO:0008324	cation transmembrane transporter activity	7.58E-06
			GO:0010715	regulation of extracellular matrix disassembly	6.61E-06

			GO:0010975	regulation of neuron projection development	6.99E-06
			GO:0015278	calcium-release channel activity	6.61E-06
			GO:0015294	solute:cation symporter activity	8.00E-06
			GO:0032412	regulation of ion transmembrane transporter activity	6.06E-06
			GO:0034220	ion transmembrane transport	8.52E-06
			GO:0034308	primary alcohol metabolic process	7.75E-06
			GO:0045664	regulation of neuron differentiation	8.52E-06
			GO:0070371	ERK1 and ERK2 cascade	8.66E-06
			GO:0022898	regulation of transmembrane transporter activity	9.72E-06
HCD					
Up			**Down**		
GO ID	**Term**	**p.Value**	**GO ID**	**Term**	**p.Value**
GO:0005581	collagen trimer	3.30E-08	GO:0034035	purine ribonucleoside bisphosphate metabolic process	5.59E-07

GO:0005201	extracellular matrix structural constituent	7.45E-07	GO:0050427	3'-phosphoadenosine 5'-phosphosulfate metabolic process	5.59E-07
GO:0030198	extracellular matrix organization	3.29E-06	GO:0008146	sulfotransferase activity	6.78E-06
GO:0044420	extracellular matrix component	3.88E-06			
GO:0043062	extracellular structure organization	7.39E-06			

Tables show the human orthologs of Drosophila genes associated with a predicted decrease (down) or increase (up) in starvation resistance upon HFD,HPD, or HCD exposure.

Table 3: Validated candidate genes.

Gene/ Ortholog	SNP Class	Manova p-value	Diet	vHCD	vHPD	vHFD	G X D pValue	Function
CG8311/ DOLK	NON_SYNONYMOUS_CODING	3.70E-10	HCD	0.17	-0.67	-0.47	7.70E-01	protein glycosylation
otp/OTP	NON_SYNONYMOUS_CODING	2.40E-07	HFD	0.8	0.1	-0.07	6.60E-01	DNA binding
LpR2/ VLDLR	INTRON	4.20E-06	HFD	0.13	0.31	-0.12	6.00E-01	lipid transport
CG15523 /VPS13B	NON_SYNONYMOUS_CODING	9.00E-05	HFD	0.1	0.42	-0.39	5.30E-01	protein targeting to vacuole
Ac76E/A DCY2	INTRON	3.20E-06	HPD	-0.19	-0.27	-0.17	4.90E-01	response to starvation
sqa/ MYLK3	INTRON	8.20E-09	HFD	0.48	0.22	0.15	1.80E-01	starvation-induced autophagy
mthl3/ ADGRG 7	DOWNSTREAM	6.90E-07	HFD	-0.06	N/A	-0.29	1.20E-01	response to starvation, determination of adult lifespan
kug/FAT 3	NON_SYNONYMOUS_CODING	2.70E-05	HFD	-0.28	0.07	0.42	8.60E-02	member of the cadherin superfamily of transmembrane proteins, which mediate cell-cell adhesion and/or cell-cell communication

CG7289/ KIAA2013	NON_SYNONYMOUS_CODING	9.40E-05	HFD	0.16	-0.27	-0.27	8.40E-02	Protein of unknown function DUF2152
hh/Shh	INTRON	1.80E-05	HFD	-0.04	N/A	-0.43	7.10E-02	segment polarity determination, stem cells maintenance and cell migration
sev/ROS1	NON_SYNONYMOUS_CODING	7.90E-05	HFD	-0.2	-0.09	-0.22	2.40E-02	protein tyrosine kinase activity
hppy/ MAP4K3	INTRON	4.20E-06	HCD	-0.55	N/A	-0.3	2.30E-02	triglyceride homeostasis, regulation of TOR signaling, behavioral response to ethanol
CG1494/ ABCA2-3	NON_SYNONYMOUS_CODING	4.00E-05	HFD	0.41	0.17	-0.04	1.30E-02	lipid transport
Cip4/ TRIP10	INTRON	1.60E-06	HFD	-0.31	0.34	-0.94	1.30E-02	lipid binding
Gyc88E/ GUCY1B1	NON_SYNONYMOUS_CODING	1.10E-06	HPD	0.68	0.02	0.19	1.10E-02	neuronal oxygen detector, activated at reduced oxygen levels
CG3339/ DNAH9	NON_SYNONYMOUS_CODING	3.70E-07	HFD	-0.11	-0.07	0.44	4.10E-03	dynein light chain binding
CG43345 /PLD3-4	NON_SYNONYMOUS_CODING	6.70E-08	HFD	0.42	-0.51	-0.67	1.10E-03	Phospholipase D/Transphosphatidylase;

CCHa2-R/BRS3	INTRON	1.10E-05	HFD	-0.01	0.15	-0.77	2.80E-04	neuropeptide signaling pathway
CG9674/ DPYD	UTR_5_PRIME	5.40E-07	HCD	-1.16	0.19	-0.23	1.50E-04	glutamate synthase (NADH) activity
T3dh/ ADHFE1	NON_SYNONYMOUS_CODING	2.60E-06	HCD	0.1	-0.24	-0.33	1.10E-05	alcohol dehydrogenase (NAD) activity
fd96Ca/ FOXB1	NON_SYNONYMOUS_CODING	9.20E-07	HFD	-0.4	-0.45	-0.99	1.00E-10	regulation of transcription
CG4607/ SLC2A6	UPSTREAM	2.20E-05	HPD	DOA	-1.29	-0.11	0.00E+00	glucose transmembrane transporter activity

a.Gene symbol/human ortholog

b. the significance from the multivariate anova of the diet responsive SNP

c. the diet upon which the gene is predicted to affect starvation resistance

d-f. the fold change in starvation resistance ($SRcontrol_{Diet/NF}$ / $SRKD_{Diet/NF}$)

g. The significance of the gene by diet interaction for each gene and it's fold change on the predicted diet (from d).

h. The functional annotation of the gene from www.flybase.org