Supplementary Information

Human-relevant Levels of Added Sugar Consumption Increase Female Mortality and Lower Male Fitness in Mice

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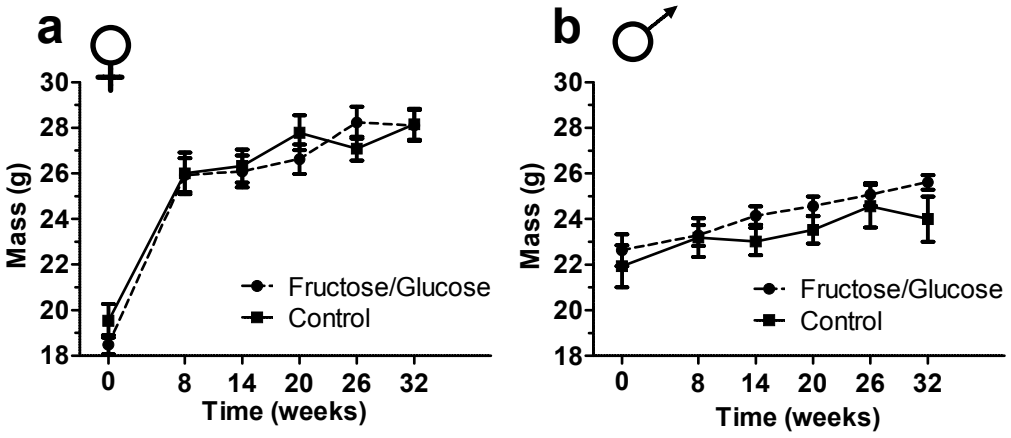
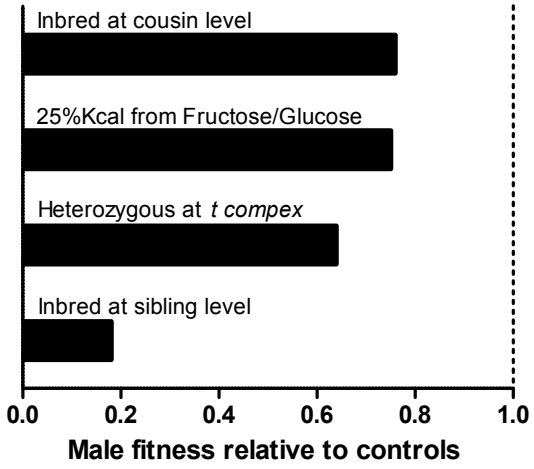
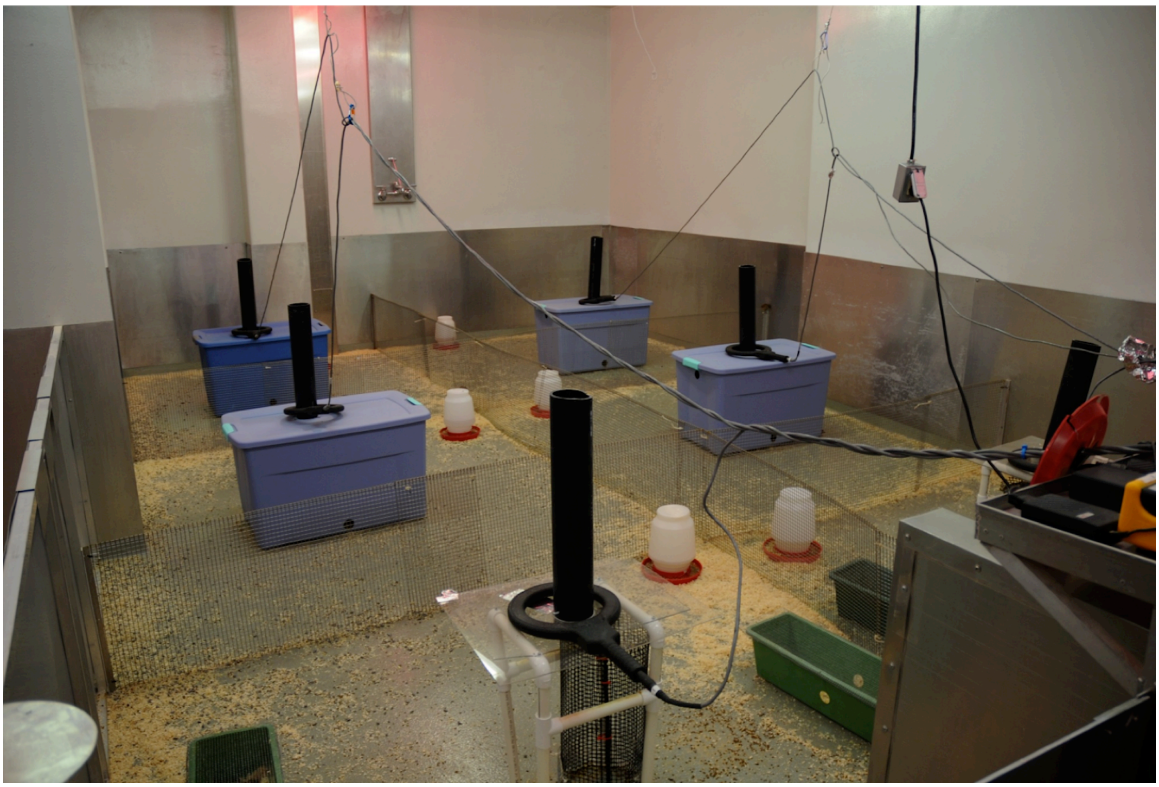


Supplementary Figure S1. Body weight of OPA founders over time by sex. Diet did not impact the weight of either female (*n* = 98 **[a]**) or male (*n* = 58 **[b]**)**,** animals. Founders did gain weight over time with males gaining at decreased rate compared to females (LMM, *t* = 11.52*, t* = -6.01*)*. The large change in female weight between weeks 0 and 8 is due to pregnancy. Lines connect means of surviving population founders at each time point and are based on 713 observations across both sexes; error bars represent standard error.



Supplementary Figure S2. Relative male reproduction costs due to treatments from published OPA studies. Fructose/glucose-fed males experienced similar fitness declines to animals inbred at the cousin level, but not as severe as those bearing the selfish genetic element known as the *t complex* or inbred at the sibling level[13-15].



Supplementary Figure S3. Photograph of an OPA enclosure. Enclosures are approximately 35m^2, and are subdivided into six subsections by hardware cloth. Each subsection has food (black chimneys) and water (poultry waterers) that is associated with a set of nest boxes in either one of four "optimal" territories, which contain nest boxes in enclosed structures (storage tubs) or two "suboptimal" territories with nest boxes in the open (planter boxes with wire lids). PIT tag readers on the ledge of the enclosure are connected to antennae (black "tennis rackets"), which are placed over each feeding station. Photograph courtesy Ben Sutter.

Supplementary Table S1. Mixed model results for competitive ability, reproduction and weight

Male Competitive ability	GLMM with binomial distribution and logit link (Intercept at week 3)			
Random effects	*Variance*	*Std. Deviation*		
Population (Intercept)	0.0023	0.0479		
Fixed effects	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|)*
Intercept	-0.0733	0.1666	-0.440	0.6600
Diet (F/G)	-0.5054	0.2405	-2.102	0.0356*
Time	-0.0084	0.0114	-0.737	0.4610
Diet (F/G)*Time	-0.0092	0.0169	-0.543	0.5869
Female Reproduction	GLMM with Poisson distribution and logarithmic link (Intercept at week 8)			
Random effects	*Variance*	*Std. Deviation*		
Population (Intercept)	0.0458	0.2140		
Population (Slope)	0.0003	0.0183		
Fixed effects	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|)*
Intercept	3.1700	0.1078	29.398	<0.0001***
Diet (F/G)	0.4204	0.0821	5.119	†
Time	0.0174	0.0086	2.032	†
Diet (F/G)*Time	-0.0236	0.0057	-4.157	<0.0001***
Male Reproduction	GLMM with Poisson distribution and logarithmic link (Intercept at week 8)			
Random effects	*Variance*	*Std. Deviation*		
Population (Intercept)	5.047e-05	0.0071		
Fixed effects	*Estimate*	*Std. Error*	*Z value*	*Pr(>|z|)*
Intercept	2.7282	0.0799	34.14	<0.0001***
Diet (F/G)	-0.0881	0.1175	-0.75	†
Time	0.0095	0.0062	1.55	†
Diet (F/G)*Time	-0.0175	0.0083	-2.12	0.0342*
Body Weight	LMM (Intercept at week 0)			
Random effects	*Variance*	*Std. Deviation*		
Individual (Intercept)	5.5835	2.3629		
Population (Intercept)	0.4852	0.6965		
Fixed effects	*Estimate*	*Std. Error*	*t value*	*Significance (|t|>2)*
Intercept	21.6511	0.5929	36.52	Yes
Diet (F/G)	-0.6141	0.7044	-0.87	No
Sex (Male)	0.5194	0.8099	0.64	No
Time	0.2394	0.0208	11.52	Yes
Diet (F/G)*Sex (Male)	0.9643	1.0136	0.95	No
Diet (F/G)*Time	0.0267	0.0268	1.00	No
Sex (Male)* Time	-0.1768	0.0294	-6.01	Yes

*Indicates a GLMM or LMM with a of p value < 0.05., **< 0.01, ***< 0.001.

†Indicates that a p value is not reported as a main effect is within a significant interaction.

Supplementary Table S2. Summary statistics of plasma measures

Plasma Measure	Sex	Mean ± Standard Deviation (n) F/G:Control	Normally Distributed F/G:Control	Unequal Variance	T/U-value	*P* value
Cholesterol mg/dL	Female	141.6 ± 40.58 (8) 116.5 ± 34.41 (9)	Yes:Yes	No	t=1.385	0.1863
	Male	202.2 ± 23.95 (8) 146.9 ± 16.39 (7)	Yes:Yes	No	t=1.850	0.0871
	Combined	171.9 ± 15.59 (16) 129.8 ± 10.08 (16)	Yes:Yes	No	t=2.271	0.0305*
Glucose mg/dL	Female	102.7 ± 21.78 (8) 98.29 ± 21.98 (9)	Yes:No	No	U=36.00	1.0000
	Male	136.4 ± 6.369 (8) 124.3 ± 4.269 (7)	Yes:Yes	No	t=1.536	0.1484
	Combined	119.5 ± 26.02 (16) 109.7 ± 22.04 (16)	Yes:No	No	U=105.0	0.3964
Insulin ng/mL	Female	0.5747 ± 0.1271 (8) 0.3968 ± 0.05674 (9)	Yes:Yes	No	t=1.330	0.2034
	Male	2.076 ± 2.290 (8) 1.283 ± 0.4124 (7)	No:Yes	Yes $F_{7,6}$=30.85 p=0.0005***	U=24.00	0.6943
	Combined	1.325 ± 1.763 (16) 0.7846 ± 0.5382 (16)	No:Yes	Yes $F_{15,15}$=10.73 p<0.0001***	U=103.0	0.3557
Triglycerides	Female	44.18 ± 2.939 (8) 45.92 ± 2.172 (9)	Yes:Yes	No	t=0.4854	0.6344
	Male	47.84 ± 3.261 (8) 48.79 ± 2.565 (7)	Yes:Yes	No	t=0.2237	0.8264
	Combined	46.01 ± 2.173 (16) 47.18 ± 1.643 (16)	Yes:Yes	No	t=0.4290	0.6710
HOMA-IR	Female	3.346 ± 2.547 (8) 2.036 ± 0.8298 (9)	No/Yes	Yes $F_{7,8}$=9.421 p=0.0051**	U=23.00	0.2359
	Male	16.46 ± 19.73 (8) 8.706 ± 3.221 (7)	No:No	Yes $F_{7,6}$=37.50 p=0.0003***	U=26.00	0.8665
	Combined	9.904 ± 15.18 (16) 4.954 ± 4.024 (16)	No:No	Yes $F_{15,15}$=14.23 p< 0.0001***	U=100.0	0.3000

*Indicates t-test, Mann-Whitney U test, or F-test with a p value < 0.05., **< 0.01, ***< 0.001. Normality was assessed with a Shapiro-Wilk test and variance with an F-test.

Supplementary Table S3. Formulation of fructose/glucose diet

Fructose/glucose diet (TD.05668) 25% Kcal from glucose + fructose					
Ingredient	**g/kg**	**% mass**	**Protein g/kg**	**CHO g/kg**	**Fat g/kg**
Wheat, Hard Ground	335.00	33.5	46.57	178.89	6.03
Dextrose, Monohydrate (Cerelose)	111.00	11.1	0	101.18	0
Fructose	101.00	10.1	0	101	0
Corn, Ground	95.00	9.5	7.695	65.74	3.04
Corn Gluten Meal 60	50.00	5	30.35	12.74	1.1
Soybean Meal, 48%	200.00	20	96.6	51	1.8
Dicalcium Phosphate, FG	16.00	1.6	0	0	0
Calcium Carbonate, FG	13.00	1.3	0	0	0
Sodium Chloride NaCl	5.00	0.5	0	0	0
Mineral Mix, TD.80318	1.50	0.15	0.0813	0.6946	0.03212
Vitamin Mix, TD.81125	3.00	0.3	0.0918	0.7844	0.03627
TBHQ (Antioxidant)	0.008	0.0008	0	0	0
Corn Oil	40.00	4	0	0	40
Cellulose (Fiber)	29.49	2.949	0	0	0
Totals (g/kg)	1000	100	181.38	512.02	52.04
Summary data					
	Total		**Protein**	**CHO**	**Fat**
Diet %	*100*		*18.14*	*51.20*	*5.20*
kcal/kg	*3241.96*		*725.53*	*2048.08*	*468.35*
kcal %	*100*		*22.38*	*63.17*	*14.45*

Supplementary Table S4. Formulation of control diet

Control diet (TD.05669)					
Ingredient	**g/kg**	**% mass**	**Protein g/kg**	**CHO g/kg**	**Fat g/kg**
Wheat, Hard Ground	335.00	33.5	46.57	178.89	6.03
Corn Starch	225.00	22.5	0.72	202.5	0.45
Corn, Ground	95.00	9.5	7.70	65.74	3.04
Corn Gluten Meal 60	50.00	5	30.35	12.74	1.1
Soybean Meal, 48%	200.00	20	96.6	51	1.8
Dicalcium Phosphate, FG	16.00	1.6	0	0	0
Calcium Carbonate, FG	13.00	1.3	0	0	0
Sodium Chloride NaCl	5.00	0.5	0	0	0
Mineral Mix, TD.80318	1.50	0.15	0.0813	0.6946	0.0321
Vitamin Mix, TD.81125	3.00	0.3	0.0918	0.7844	0.0363
TBHQ (Antioxidant)	0.008	0.0008	0	0	0
Corn Oil	40.00	4	0	0	40
Cellulose (Fiber)	16.49	1.649	0	0	0
Totals (g/kg)	1000.00	100.00	182.10	512.34	52.49
Summary data					
	Total		**Protein**	**CHO**	**Fat**
Diet %			*18.21*	*51.23*	*5.25*
kcal/kg	*3250.18*		*728.41*	*2049.38*	*472.40*
kcal %			*22.41*	*63.05*	*14.53*

Supplementary Methods

Statistical analysis

Survival

Survivorship of the 156 founders was analyzed by Cox proportional hazard models with males and females assessed separately due to vastly different mortality rates. Day one was defined as when animals entered OPA enclosures. A multivariate model was used to assess the impacts of diet, population, and diet by population. Individuals that survived the duration of the trial or that were removed from the study were censored. In the male data set there were 34 events and 24 censorings, while in the female data set there were 24 events and 74 censorings.

Male competitive ability

To assess the main effects of diet and time (and a time by diet interaction) on male competitive ability, we used a generalized linear mixed model (GLMM) to predict the probability of ownership. As a territory can only be defended or not, we used a binomial distribution with a logit link to assess the probability of ownership. The numbers of territories controlled within populations by each dietary treatment was assessed at multiple time points throughout the study for a total of 140 observations. The number of possible territories in each population is constant at six and territories were occupied (by a mouse of either diet) or unoccupied. Time, diet and their interaction were treated as fixed effects and population was modeled as a random effect with a random intercept calculated for each.

Reproduction

As reproduction data are discrete counts, for each sex we modeled offspring counts over time in a GLMM with a Poisson distribution and a logarithmic link. The model assessed the main effects of diet and time and the interaction on population-level reproduction across the six populations. Reproductive output of each dietary treatment was measured five times (except for one population that was only measured four times) at six-week intervals for a total of 58 observations. Time, diet and their interaction were modeled as fixed effects and population was modeled as a random effect with a random slope and intercept for females and only an intercept for males. The intercept was set at week eight as this was the first time point for which data were available and reproduction at week zero was biologically impossible. Male and female reproduction data were analyzed separately as they were based on separate measurements, with male reproduction being in terms of number of male offspring and female reproduction in terms of total offspring.

Body weight

A linear mixed-effects model (LMM) was used to assess the main effects of diet, sex and time, as well as their respective interactions on the weight of the 156 population founders. As weight data are continuous, a normal distribution was assumed. Diet, sex, time and their interaction were modeled as main fixed effects and individual and population were modeled as random effects with a random intercept. The intercept was set at week zero as this was the first time point for which data were available and made biological sense. Founders were weighed at week zero and surviving individuals were weighed across the five aforementioned pup sweeps for a total of 713 observations. Due

to nested random effects within the model degrees of freedom are not readily calculable and therefore *P* values are not provided. The authors of the statistical package suggest that estimates with $|t| > 2$ are deemed significantly different from zero.

Intraperitoneal glucose tolerance tests (IPGTTs)

The area under the curve (AUC) was calculated for plasma glucose concentrations over time using the trapezoid rule. AUC values were calculated for all individuals prior to OPA entrance and two weeks post release. Male and female data were analyzed separately as sex has been shown to impact glucose tolerance[32]. AUC values were compared across time points and dietary treatments using two-way analysis of variance (ANOVA) with Bonferroni's post tests. Sample prior to OPA entrance was 23 females (15 F/G-fed and 8 controls) and 16 males (8 F/G-fed and 8 controls). While the sample two weeks post entrance had 16 females (8 F/G-fed and 8 controls) and 9 males (5 F/G-fed and 4 controls).

Fasting plasma measures and HOMA-IR

Plasma measures in 17 (8 F/G, 9 control) female mice and 15 male mice (8 F/G, 7 control) were compared between dietary treatments. Sexes were analyzed both separately and combined. Normality of each measurement was assessed with a Shapiro-Wilk normality test. Blood measurements that did not significantly differ from a normal distribution were assessed with t-tests, while those that differed significantly were analyzed with a Mann-Whitney U test. An F-test was used to tests for unequal variance of all blood measurements between dietary groups. Summaries of normality, and F-test results may be found in Supplementary Table S2.

General statistical methods

All mixed-effects models were fit in R using the *glmer* or *lmer* functions of the lme4 library[33,34]. For all mixed-effects models several candidate models for the random effects terms were fit to the data including models estimating both intercept and/or slope for random effects. In all cases the model that explained at least some of the variance with random effects and had the lowest AIC score was selected. Neither the significance of any reported fixed effect nor the magnitude of the effect differed between models. Estimates (and significance) were consistent with those obtained when we ignored either the nested structure and repeated measurements of individuals within populations or repeated measurements of individuals or populations. Proportional hazard models were performed in JMP 9.0.3 and two-way ANOVAs, Mann-Whitney U tests, and t-test were performed in Prism 5.03. All α values are 0.05 and all tests were two-tailed.

Supplementary References

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