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Supplemental information

Sucrose overconsumption impairs AgRP neuron dynamics and promotes palatable food intake

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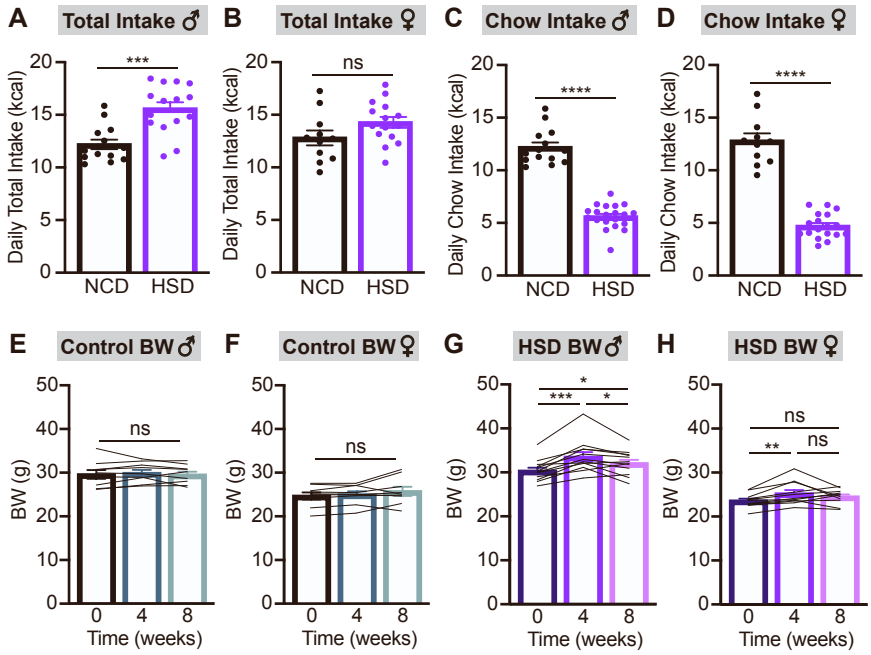

Figure S1. Liquid sucrose availability more robustly impacts caloric intake and body weight in male mice. Related to Figure 1.

(A-D) Total daily caloric intake (A,B) and daily calories from chow (C,D) in control (NCD) and HSD male (A,C) and female (B,D) mice averaged over three weeks. n = 11-20 mice per group ((A) unpaired t-test, p=0.0001; (B) unpaired t-test, p=0.0963; (C) unpaired t-test, p<0.0001; (D) unpaired t-test, p<0.0001).

(E-H) Body weights in NCD male (E) and female (F) and HSD male (G) and female (H) mice at baseline, after four weeks of chow or HSD, and after an additional four weeks of chow. n = 9-12 mice per group ((E) one-way ANOVA, p=0.6269; (F) one-way ANOVA, p=0.1512; (G) one-way ANOVA, p<0.0001; (H) one-way ANOVA, p=0.0397).

Baseline body weight was not significantly different between control and HSD mice of the same sex ((males) unpaired t-test, p=0.5413; (females) unpaired t-test, p=0.2421). (A-H) Dots or lines represent individual mice. Error bars indicate mean ± SEM. T-tests and post-hoc comparisons: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

Figure S2

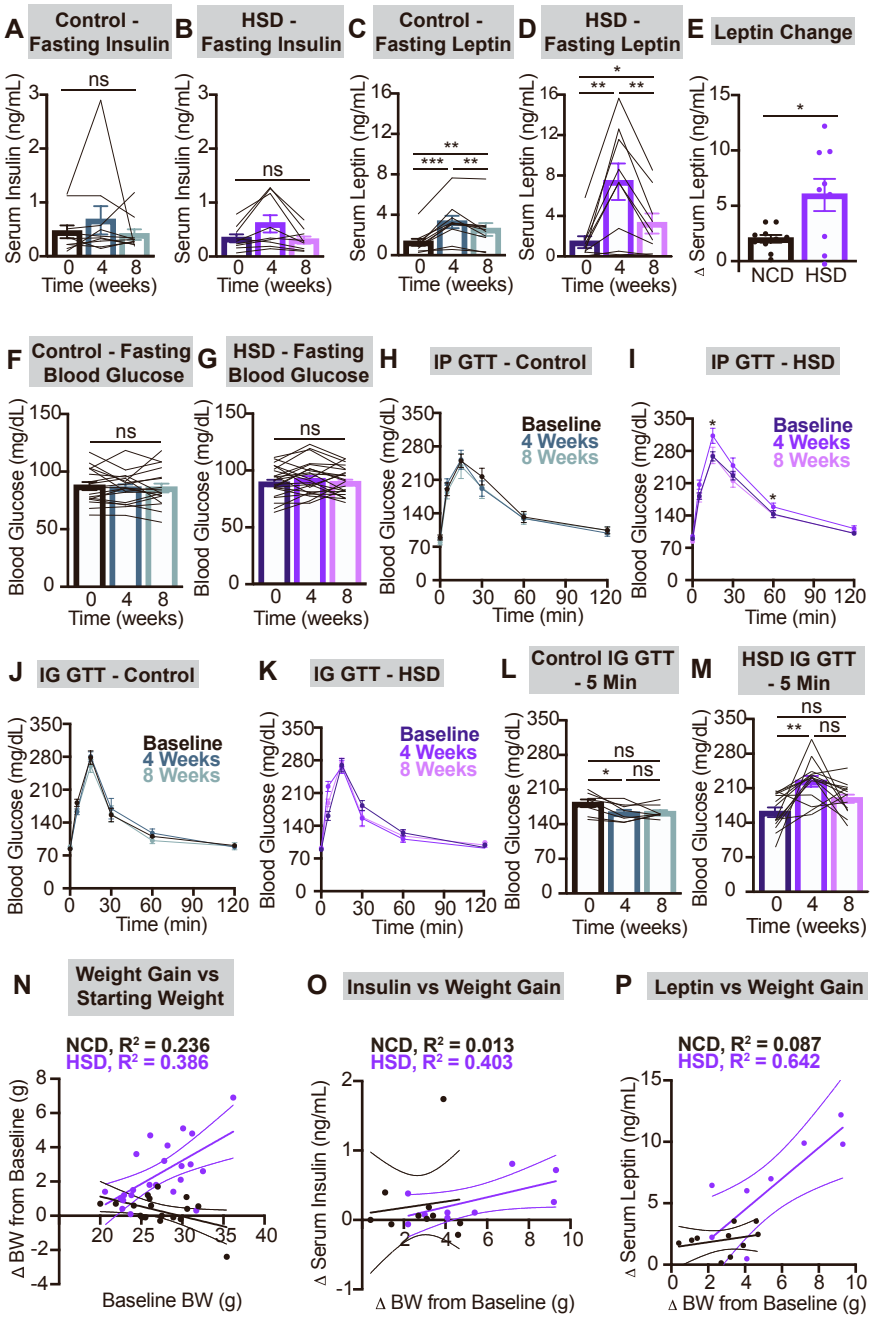
A Control - Fasting Insulin
B HSD - Fasting Insulin
C Control - Fasting Leptin
D HSD - Fasting Leptin
E Leptin Change
Serum Insulin (ng/mL)
Serum Leptin (ng/mL)
Δ Serum Leptin (ng/mL)
Time (weeks)
NCD HSD
ns
F Control - Fasting Blood Glucose
G HSD - Fasting Blood Glucose
H IP GTT - Control
I IP GTT - HSD
Blood Glucose (mg/dL)
Time (min)
Baseline
4 Weeks
8 Weeks
J IG GTT - Control
K IG GTT - HSD
L Control IG GTT - 5 Min
M HSD IG GTT - 5 Min
N Weight Gain vs Starting Weight
O Insulin vs Weight Gain
P Leptin vs Weight Gain
NCD, R² = 0.236
HSD, R² = 0.386
NCD, R² = 0.013
HSD, R² = 0.403
NCD, R² = 0.087
HSD, R² = 0.642
Δ BW from Baseline (g)
Baseline BW (g)
Δ Serum Insulin (ng/mL)
Δ Serum Leptin (ng/mL)

Figure S2. HSD significantly alters glucose homeostasis and increases circulating leptin in proportion with body weight gain. Related to Figure 1.

(A-D) Plasma insulin (A,B) and leptin (C,D) measured following a six-hour fast in NCD (A,C) and HSD (B,D) mice at baseline, after four weeks of chow or HSD, and after an additional four weeks of chow. n = 9-10 mice per group ((A) one-way ANOVA, p=0.3702; (B) one-way ANOVA, p=0.0248; (C) one-way ANOVA, p<0.0001; (D) one-way ANOVA, p=0.0025).

(E) Change in fasting serum leptin in NCD (C) versus HSD (D) mice from baseline to four weeks (unpaired t-test, p=0.0129).

(F,G) Blood glucose measured following a six-hour fast in NCD (F) and HSD (G) mice at baseline, after four weeks of chow or HSD, and after an additional four weeks of chow. n = 18-23 mice per group ((F) one-way ANOVA, p=0.7206; (G) one-way ANOVA, p=0.3704).

(H-K) Blood glucose following 1.5 mg/g glucose delivered intraperitoneally (H,I) and intragastrically (J,K) in NCD (H,J) and HSD (I,K) mice fasted for six hours at baseline, after four weeks of chow or HSD, and after an additional four weeks of chow. GTT = glucose tolerance test. n = 8-16 mice per group ((H) two-way ANOVA, main effect of GTT time point, p<0.0001, main effect of time on diet, p=0.6, interaction, p=0.5418; (I) two-way ANOVA, main effect of GTT time point, p<0.0001, main effect of time on diet, p=0.019, interaction, p=0.2868; (J) two-way ANOVA, main effect of GTT time point, p<0.0001, main effect of time on diet, p=0.3148, interaction, p=0.145; (K) two-way ANOVA, main effect of GTT time point, p<0.0001, main effect of time on diet, p=0.8161, interaction, p=0.0026).

(L,M) Blood glucose values five minutes after intragastric glucose delivery in NCD (L) and HSD (M) mice from (J) and (K), respectively. n = 8-14 mice per group ((L,M) post-hoc testing from 2-way ANOVA in (J,K) are shown).

(N) Baseline weight versus weight gain in NCD (black; Pearson correlation, R^2=0.236, p=0.041) and HSD mice (purple; Pearson correlation, R^2=0.386, p=0.002) after four weeks of chow or HSD, respectively. n = 18-23 mice per group.

(O) Weight gain versus fasting serum insulin change in NCD (black; Pearson correlation, R^2=0.013, p=0.750) and HSD mice (purple; Pearson correlation, R^2=0.403, p=0.0622) after four weeks of chow or HSD, respectively. n = 9-10 mice per group.

(P) Weight gain versus fasting serum leptin change in NCD (black; Pearson correlation, R^2=0.087, p=0.408) and HSD mice (purple; Pearson correlation, R^2=0.642, p=0.009) after four weeks of chow or HSD, respectively. n = 9-10 mice per group.

(A-G,L-P) Dots or lines represent individual mice. (A-M) Error bars indicate mean $\pm$ SEM. (N-P) Error bars indicate mean slope $\pm$ 95% CI. T-tests and post-hoc comparisons: *p<0.05, **p<0.01, and ***p<0.001.

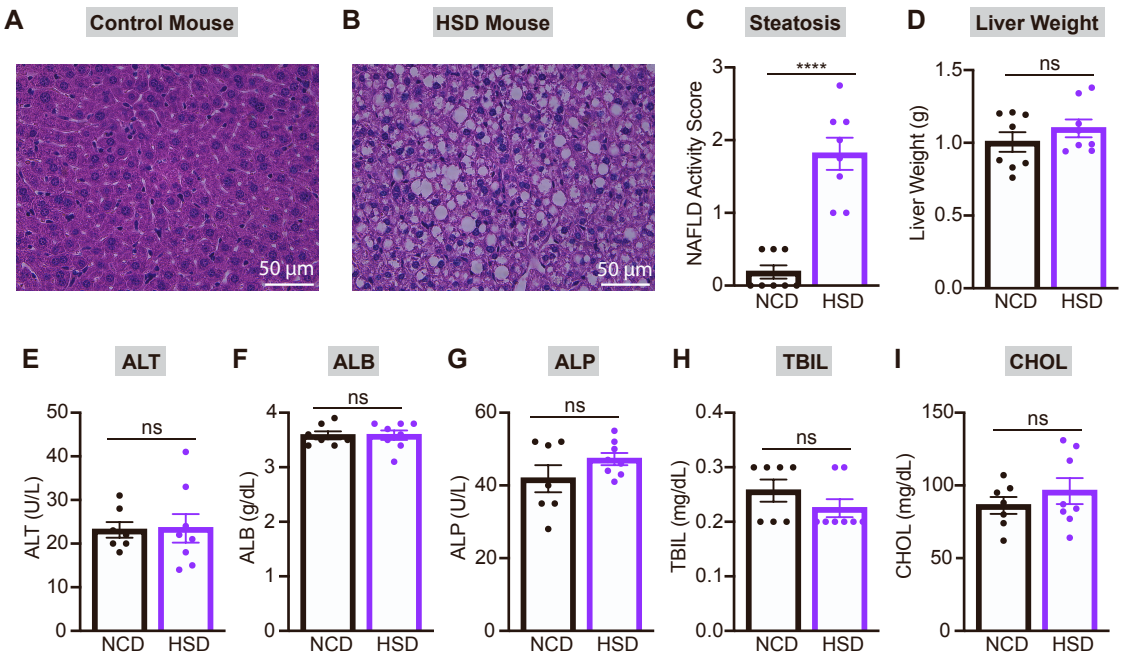

Figure S3. HSD causes hepatic steatosis but does not impair liver function after four weeks. Related to Figure 1.

(A,B) Representative hematoxylin and eosin-stained liver sections from NCD (A) and HSD (B) mice after four weeks of chow or HSD.

(C) Non-alcoholic fatty liver disease (NAFLD) activity score in liver sections from NCD versus HSD mice after 4 weeks of chow or HSD, respectively. n = 8 mice per group (unpaired t-test, $p<0.0001$).

(D) Liver weight in ad libitum-fed NCD and HSD mice after four weeks of chow or HSD, respectively. n = 8 mice per group (unpaired t-test, $p=0.3204$).

(E-I) Alanine transaminase (ALT) (E), albumin (ALB) (F), alkaline phosphatase (ALP) (G), total bilirubin (TBIL) (H), and cholesterol (CHOL) (I) levels in plasma from ad libitum-fed NCD and HSD mice after four weeks of chow or HSD, respectively. n = 7-8 mice per group ((E) unpaired t-test, $p=0.9281$; (F) unpaired t-test, $p=0.988$; (G) unpaired t-test, $p=0.1901$; (H) unpaired t-test, $p=0.2337$; (I) unpaired t-test, $p=0.3868$).

(C-I) Dots represent individual mice. Error bars indicate mean ± SEM. T-tests: ***$p<0.001$.

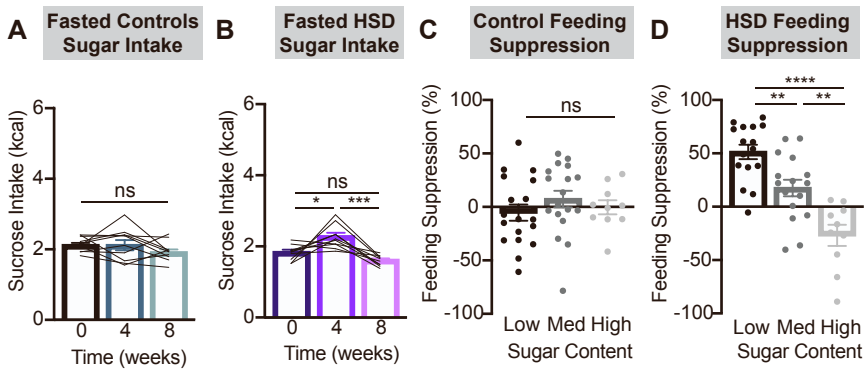

Figure S4. HSD selectively suppresses consumption of low-sugar foods. Related to Figure 2.
(A,B) Two-hour sucrose intake following a six-hour fast in NCD (A) and HSD (B) animals at baseline, after four weeks of chow or HSD, and after an additional four weeks of chow. n = 10 mice per group ((A) one-way ANOVA, p=0.2367; (B) one-way ANOVA, p=0.0009).
(C,D) Extent of feeding suppression after four weeks of NCD (C) or HSD (D) relative to baseline chow (Fig. 2A, B), chocolate (Fig. 2C, D) and liquid sucrose intake (A,B) intake. Feeding suppression = ((intake at baseline - intake after 4 weeks NCD or HSD)/(intake at baseline))*100. Regular chow, chocolate, and 25% sucrose in water represent low-, medium (med)- and high-sugar foods, respectively. n = 10-18 mice per group ((C) one-way ANOVA, p=0.4659; (D) one-way ANOVA, p<0.0001).
(A-D) Dots or lines represent individual mice. Error bars indicate mean ± SEM. Post-hoc comparisons: *p<0.05, **p<0.01, ***p<0.001, and ****p<0.0001.

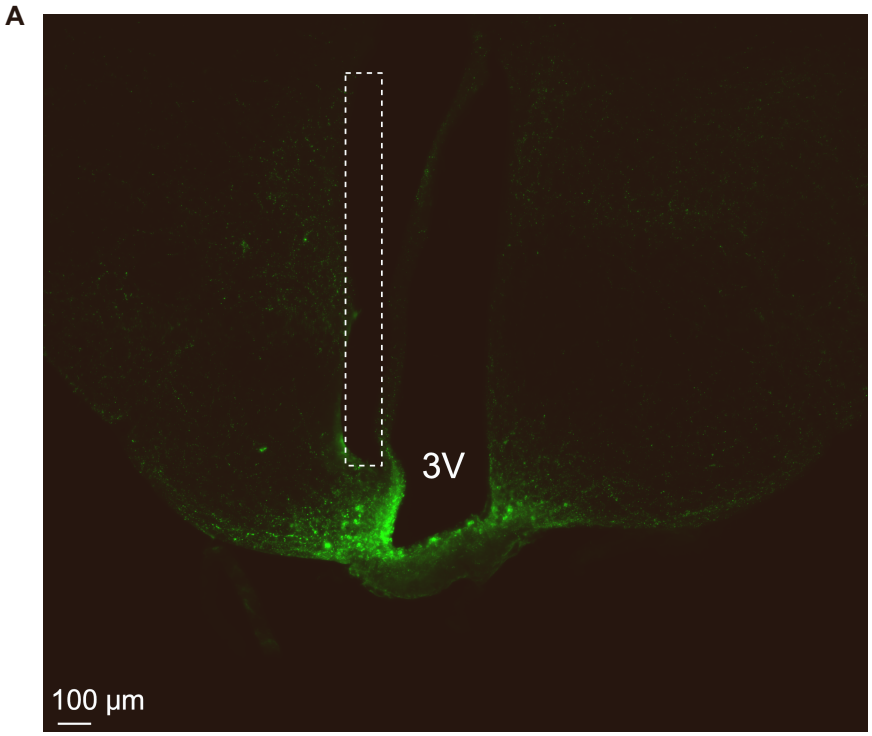

Figure S5. Fiber photometry in AgRP neurons is enabled via injection of an AAV vector expressing Cre-dependent GCaMP6s into the arcuate nucleus of AgRP-Cre mice followed by fiber optic implant insertion. Related to Figures 2, 4-7.

(A) Coronal section from an AgRP-Cre mouse depicting GCaMP expression (green) and path of optical fiber.

3V = third ventricle. Scale bar represents 100 µm. Dashed white line indicates implant insertion site.

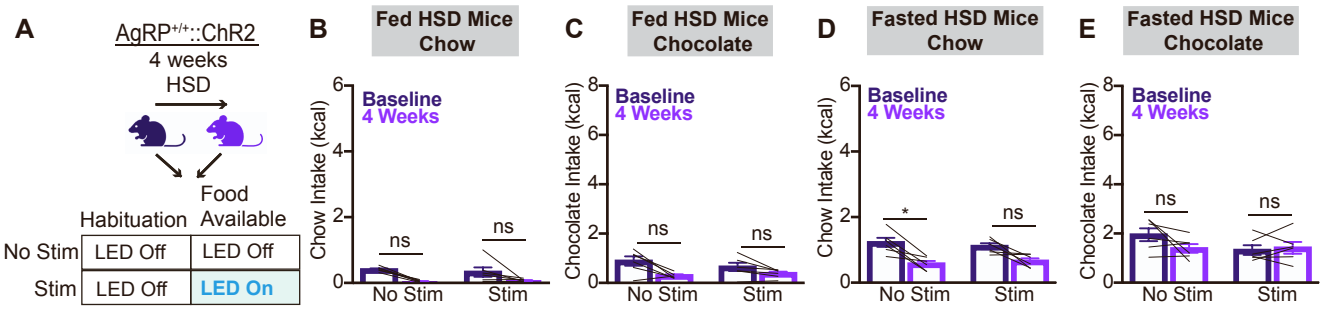

Figure S6. Blue light stimulation does not affect food intake in mice lacking ChR2 expression. Related to Figure 3.

(A) Optogenetic experiment schematic. On separate days, ROSA26-loxStoplox-ChR2-eYFP mice not expressing Cre recombinase and therefore not expressing ChR2 (AgRP+/+::ChR2) equipped for optogenetic AgRP neuron stimulation were assessed under two protocols. Each session involved a 30-minute habituation period without food and without optical stimulation followed by 30 minutes of food availability without (no stim) or with (stim) optical stimulation. Each mouse was tested using both regular chow and chocolate in both the sated and overnight-fasted states. These experiments were performed at baseline and repeated after four weeks of *ad libitum* HSD.

(B,C) Caloric intake of *ad libitum* fed mice given chow (B) or chocolate (C) at baseline and after HSD under “no stim” and “stim” protocols ((B) two-way ANOVA, main effect of no stim vs. stim, p=0.7868, main effect of time on diet, p=0.0042, interaction, p=0.5521; (C) two-way ANOVA, main effect of no stim vs. stim, p=0.6617, main effect of time on diet, p=0.0035, interaction, p=0.2813).

(D,E) Caloric intake of fasted mice given chow (D) or chocolate (E) at baseline and after HSD under “no stim” and “stim” protocols ((D) two-way ANOVA, main effect of no stim vs. stim, p=0.8929, main effect of time on diet, p=0.0032, interaction, p=0.3899; (E) two-way ANOVA, main effect of no stim vs. stim, p=0.1537, main effect of time on diet, p=0.1273, interaction, p=0.2228).

n = 6 mice. (B-E) Lines represent individual mice. Error bars indicate mean ± SEM. Post-hoc comparisons: *p<0.05.

Figure S7

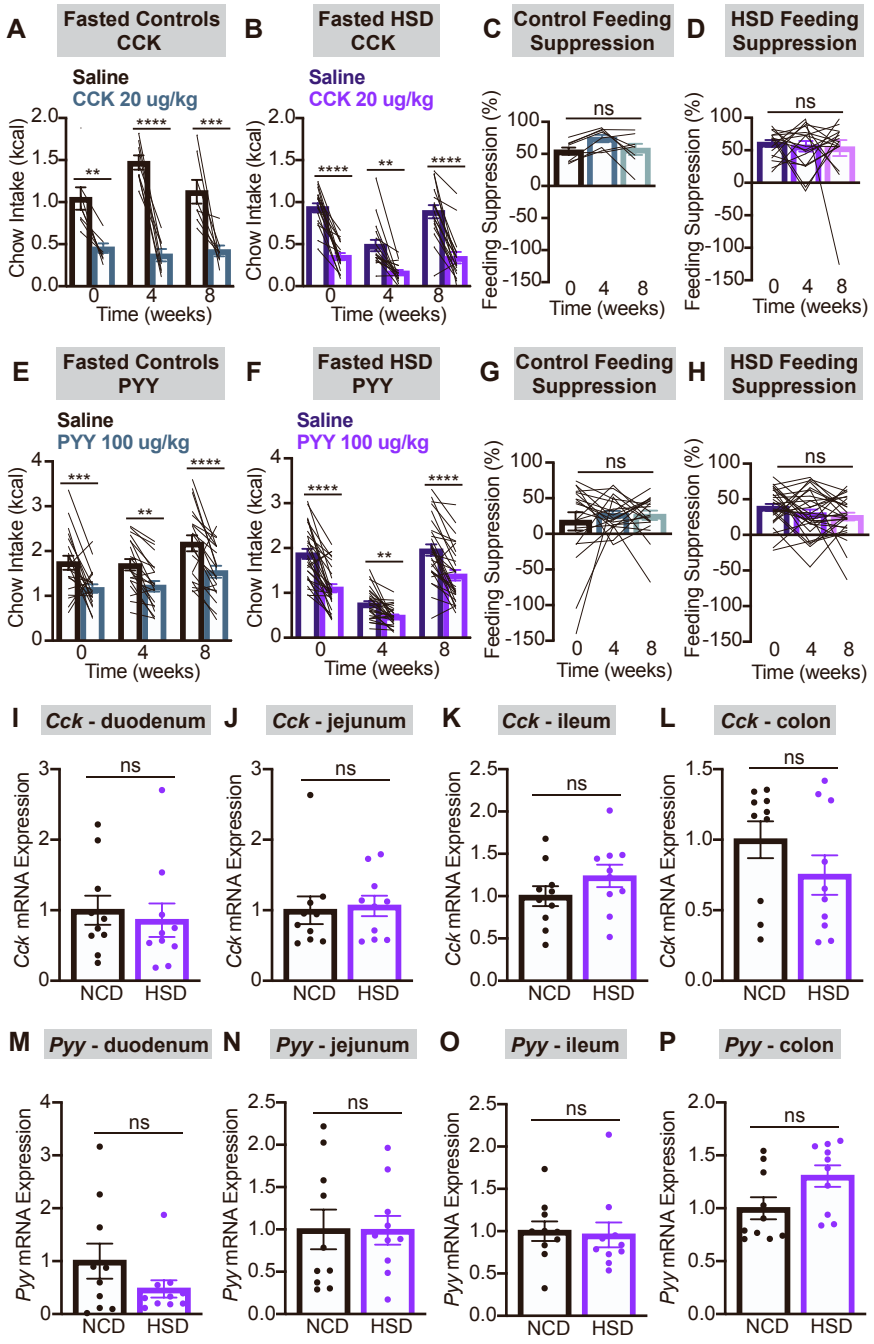
A
Fasted Controls CCK
Saline
CCK 20 ug/kg
Chow Intake (kcal)
Time (weeks)
B
Fasted HSD CCK
Saline
CCK 20 ug/kg
Chow Intake (kcal)
Time (weeks)
C
Control Feeding Suppression
Feeding Suppression (%)
ns
Time (weeks)
D
HSD Feeding Suppression
Feeding Suppression (%)
ns
Time (weeks)
E
Fasted Controls PYY
Saline
PYY 100 ug/kg
Chow Intake (kcal)
Time (weeks)
F
Fasted HSD PYY
Saline
PYY 100 ug/kg
Chow Intake (kcal)
Time (weeks)
G
Control Feeding Suppression
Feeding Suppression (%)
ns
Time (weeks)
H
HSD Feeding Suppression
Feeding Suppression (%)
ns
Time (weeks)
I
Cck - duodenum
Cck mRNA Expression
ns
NCD
HSD
J
Cck - jejunum
Cck mRNA Expression
ns
NCD
HSD
K
Cck - ileum
Cck mRNA Expression
ns
NCD
HSD
L
Cck - colon
Cck mRNA Expression
ns
NCD
HSD
M
Pyy - duodenum
Pyy mRNA Expression
ns
NCD
HSD
N
Pyy - jejunum
Pyy mRNA Expression
ns
NCD
HSD
O
Pyy - ileum
Pyy mRNA Expression
ns
NCD
HSD
P
Pyy - colon
Pyy mRNA Expression
ns
NCD
HSD

Figure S7. HSD does not alter the appetite-suppressing effects of CCK and PYY or the intestinal mRNA expression of *Cck* and *Pyy* in fasted mice. Related to Figure 7.

(A,B) Thirty-minute chow intake following a six-hour fast and intraperitoneal CCK (20 μg/kg) or isovolemic saline as indicated in NCD (A) and HSD (B) animals at baseline, after four weeks of chow or HSD, and after an additional four weeks of chow. n = 8-17 mice per group ((A) two-way ANOVA, main effect of time on diet, p=0.1477, main effect of saline vs. CCK, p<0.0001, interaction, p=0.0270; (B) two-way ANOVA, main effect of time on diet, p<0.0001, main effect of saline vs. CCK, p<0.0001, interaction, p=0.0497).

(C,D) Extent of feeding suppression induced by CCK relative to saline in NCD (C) and HSD (D) mice from (A) and (B), respectively. Feeding suppression = ((intake following saline - intake following CCK)/(intake following saline))*100. ((C) one-way ANOVA, p=0.0919; (D) one-way ANOVA, p=0.8078).

(E,F) Two-hour chow intake following a six-hour fast and intraperitoneal PYY (100 μg/kg) or isovolemic saline as indicated in NCD (E) and HSD (F) animals at baseline, after four weeks of chow or HSD, and after an additional four weeks of chow. n = 20-29 mice per group ((E) two-way ANOVA, main effect of time on diet, p=0.0018, main effect of saline vs. PYY, p<0.0001, interaction, p=0.6463; (F) two-way ANOVA, main effect of time on diet, p<0.0001, main effect of saline vs. PYY, p<0.0001, interaction, p=0.0003).

(G,H) Extent of feeding suppression induced by PYY relative to saline in NCD (G) and HSD (H) mice from (E) and (F), respectively. Feeding suppression = ((intake following saline - intake following PYY)/(intake following saline))*100. ((G) one-way ANOVA, p=0.5918; (H) one-way ANOVA, p=0.1812).

(I-L) Duodenal (I), jejunal (J), ileal (K), and colon (L) *Cck* mRNA expression in NCD and HSD mice after an overnight fast. n = 10 mice per group ((I) unpaired t-test, p=0.6601; (J) unpaired t-test, p=0.8038; (K) unpaired t-test, p=0.2124; (L) unpaired t-test, p=0.2072).

(M-P) Duodenal (M), jejunal (N), ileal (O), and colon (P) *Pyy* mRNA expression in NCD and HSD mice after an overnight fast. n = 10 mice per group ((M) unpaired t-test, p=0.1718; (N) unpaired t-test, p=0.9723; (O) unpaired t-test, p=0.8191; (P) unpaired t-test, p=0.051).

(I-P) Expression presented relative to housekeeping gene *Rplp0*. Dots or lines represent individual mice. Error bars indicate mean ± SEM. Post-hoc comparisons: **p<0.01, ***p<0.001, and ****p<0.0001.

Table S2. Primers used for qRT-PCR. Related to Figure S7 and STAR Methods.

Gene	Forward	Reverse	Amplicon Length (bp)	Source	Species
Rplp0	AGATGCAGCAGATCCGCA	GTTCTTGCCCATCAGCACC	59	Grant Barish, Northwestern University	*Mus musculus*
Cck	CACTGCTAGCGCGATACATCC	GTCCAGGCTCTGCAGGTTC	83	Designed in-house	*Mus musculus*
Pyy	CTGCGCCACTACCTCAAC	CAGAGCTGCGGGGACATC	66	Designed in-house	*Mus musculus*