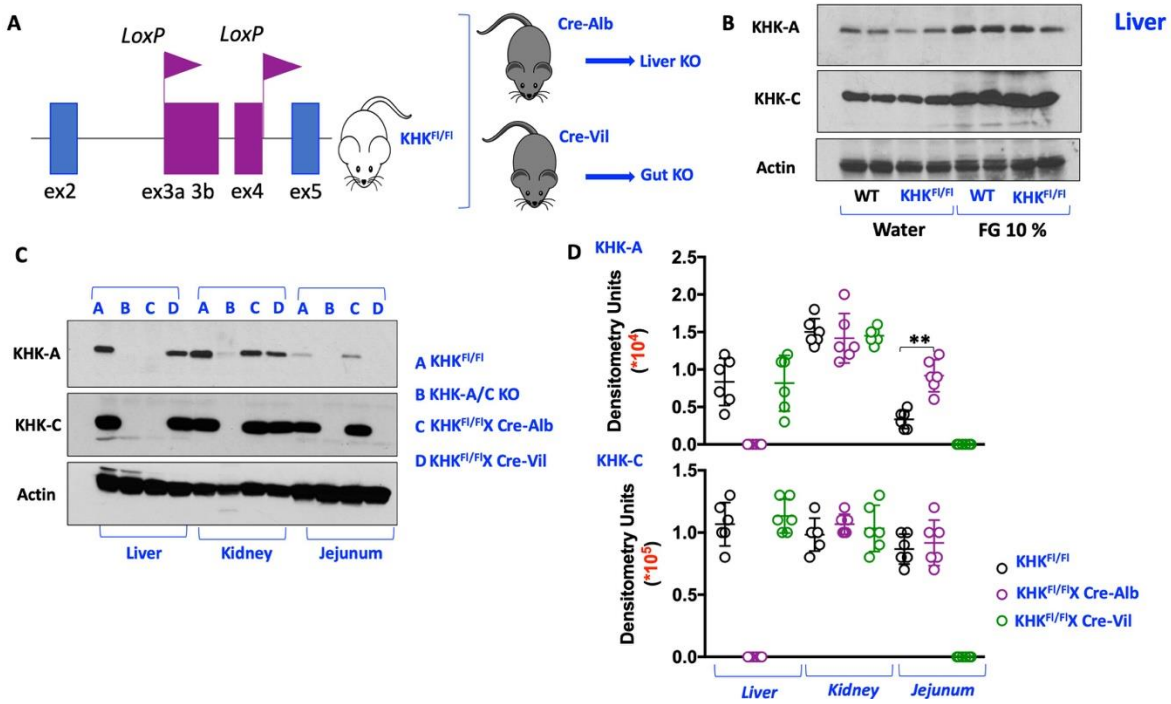
Supplemental Figure 1: Authentication of tissue-specific KHK deficient mice (Related to Figures 2 and 3 and STAR Methods).

A) Schematic depicting the approach to flox out exons 3 and 4 of the *khk* gene in mice ($KHK^{Fl/Fl}$) and the development of specific liver ($KHK^{Fl/Fl}$-Cre*Alb*)and gut ($KHK^{Fl/Fl}$-*CreVil* mice) *KHK* knockout mice.

B) Representative western blot (*n* = 3 total blots) for KHK-A, KHK-C and actin loading control in liver of wild type (WT) and $KHK^{Fl/Fl}$ mice.

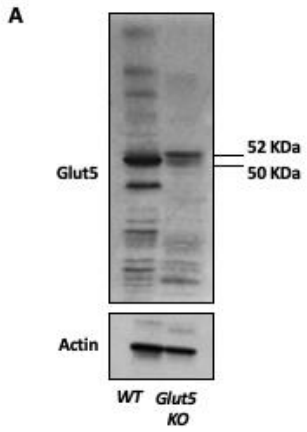
C) Representative western blot (*n* = 3 total blots) for KHK-A, KHK-C and actin loading control in liver, kidney and jejunum of $KHK^{Fl/Fl}$, *KHK-A/C KO,* $KHK^{Fl/Fl}$*XCre-Alb* and $KHK^{Fl/Fl}$-*CreVil* mice.

D) Densitometry for KHK-A and KHK-C (normalized to actin loading control) for 6 different animals in each group. The data in D) were presented as the means ± SEM and analyzed by One Way ANOVA with Tukey post hoc analysis. $^{**}P < 0.01$. *n* = 6 mice per group.



Supplemental Figure 2: Validation of Glut5 antibody in jejunal samples of mice (Related to STAR Methods).

A) Representative western blot (*n* = 2 total blots) against glut5 and actin loading control in jejunal samples of wild type (WT, left) and glut5 knockout (KO, right) mice using antibody from Millipore, 07-1406 lot 2999837). Of note, a nonspecific band is present at a slightly higher size (52 kDa).



Supplemental Table 1: General Parameters in wild type (WT) and fructokinase knockout (KHK-A/C KO) exposed to water control, 10% FG or 30% FG (Related to Figure 2). * *P*<0.05 and ** *P*<0.01 versus respective water controls. One way ANOVA Tukey post hoc analysis, n=6 mice per group.

	WT Water (n=6)	**WT FG 10% (n=6)**	**WT FG 30% (n=6)**	**KHK-A/C KO Water (n=6)**	**KHK-A/C KO FG 10% (n=6)**	**KHK-A/C KO FG 30% (n=6)**	**WT FG 10% vs KHK-A/C KO FG 30% ANOVA**
Body Weight and composition							
Body Weight; 0W (g)	22.3 ± 0.5	23.1 ± 0.7	23.4 ± 0.8	24.2 ± 0.6	22.7 ± 1.3	22.4 ± 1.4	*NS*
Body Weight; 30W (g)	32.2 ± 2.7	**43.5 ± 1.1****	**50.0 ± 1.4****	30.4 ± 1.0	**33.0 ± 1.1****	**33.1 ± 1.2****	*P*<0.01
ΔBody Weight; 30W (g)	8.9 ± 2.2	**20.4 ± 1.7****	**26.6 ± 2.0****	6.2 ± 1.1	**10.3 ± 0.9****	**10.7± 0.9****	*P*<0.01
Average Food Intake (g/day)	3.23 ± 0.3	**2.81 ± 0.3****	**2.65 ± 0.2****	3.15 ± 0.3	**2.71 ± 0.3****	**2.81 ± 0.2***	*NS*
Water/FG Intake (ml/day)	3.1 ± 0.2	**12.1 ± 0.7****	**8.65 ± 1.3****	3.2 ± 0.1	3.7 ± 0.2	3.9 ± 0.2	*P*<0.01
Cumulative Caloric Intake 30W	2105±106	**2846 ± 93****	**3690 ± 225****	2051 ± 121	2077 ± 72	**2886±122****	*NS*
Cumulative Caloric Intake/BW 30W	65.3 ± 13	65.4 ± 26	73.8 ± 31	67.4 ± 16	62.7 ± 16	87.1 ± 21	*NS*
Cumulative Calories from Fructose; 30W	0	**607 ± 45****	**1217 ± 202****	0	**184 ± 14****	**592 ±35****	*NS*
Cumulative Calories from Fructose/BW 30W	0	**13.9 ± 3****	**24.2 ± 7****	0	**5.6 ± 2****	**17.8 ± 4****	*NS*
Liver Weight; 30 W (g)	1.44 ± 0.25	**2.35±0.15****	**3.13± 0.35****	1.35± 0.32	1.46±0.26	1.40±0.25	*P*<0.01
Epididymal Fat Weight; 30W (g)	0.86 ± 0.19	**2.35±0.27****	**2.88 ± 0.14***	1.05 ± 0.20	1.26 ± 0.30	1.22± 0.32	*P*<0.01
Biochemical Blood Analysis							
AST (IU/L)	44 ± 6	**117 ± 13****	**149 ± 14****	39 ± 5	45 ± 6	39 ± 5	*P*<0.01
ALT (IU/L)	25 ± 4	**66 ± 12****	**89 ± 16****	21 ± 6	26 ± 5	22 ± 7	*P*<0.01

Serum Triglycerides (mg/dl)	56.6 ± 6.5	62.2 ± 7.1	**71.1 ± 10.1***	43.3 ± 5.7	40.5 ± 6.0	51.5 ± 6.3	*NS*
Fasting Serum Glucose (mg/dl)	96 ± 6.2	104 ± 3.5	136 ± 12.2**	91 ± 4.2	98 ± 5.0	98.5 ± 2.5	*NS*
Fasting Insulin (ng/ml)	2.23 ± 0.4	**5.88 ± 1.1****	**9.03 ± 2.1****	2.33 ± 0.4	2.43 ± 0.7	2.53 ± 0.2	*P*<0.01
HOMA-IR	1.3 ± 0.2	**3.7 ± 1.02****	**7.5 ± 2.4****	1.3 ± 0.2	1.5 ± 0.2	1.5 ± 0.3	*P*<0.01
OGTT (AUC/10)	412 ± 55	**656 ± 35****	**766 ± 43****	388 ± 23	422 ± 55	395 ± 15	*P*<0.01
Fasting Leptin (ng/ml)	4.72± 0.5	**9.98 ± 1.6****	**15.32 ± 2.9****	4.65 ± 0.5	5.31 ± 0.4	4.85 ± 0.7	*P*<0.01
Serum Creatinine (mg/dl)	0.13± 0.03	0.09 ± 0.02	0.18 ± 0.03	0.12 ± 0.02	0.13 ± 0.04	0.15± 0.03	*NS*
Serum BUN (mg/dl)	21.2 ± 3.5	13.3 ± 3.5**	24.8 ± 2.5	26.5 ± 2.8	28.2 ± 4.5	25.5 ± 3.8	*P*<0.01
FGF21 (pg/ml)	396 ± 72	**5386 ± 617****	**6915 ± 727****	286 ± 45	**421 ± 42***	**1107 ± 160****	*P*<0.01
Liver Analysis							
Triglycerides (mg/g)	43 ± 13	**145 ± 23****	**193 ± 12****	44 ± 6	40 ± 10	33 ± 12	*P*<0.01
Fgf21 mRNA (AU)	0.93 ± 0.3	**7.65 ± 0.8****	**8.26 ± 1.1****	0.43 ± 0.1	0.51 ± 0.1	1.06 ± 0.4	*P*<0.01

Supplemental Table 2: General Parameters in *KHK*$^{Fl/Fl}$ and gut-specific fructokinase knockout (*KHK*$^{Fl/Fl}$*XCre-Vil*) exposed to water control, 10% FG or 30% FG (related to Figure 3). * *P*<0.05 and ** *P*<0.01 versus respective water controls. One way ANOVA Tukey post hoc analysis, n=6 mice per group except for portal vein AUC (n=3).

	***KHK*$^{Fl/Fl}$ Water (n=6)**	***KHK*$^{Fl/Fl}$ FG 10% (n=6)**	***KHK*$^{Fl/Fl}$ FG 30% (n=6)**	***KHK*$^{Fl/Fl}$*XCre-Vil* Water (n=6)**	***KHK*$^{Fl/Fl}$*XCre-Vil* FG 10% (n=6)**	***KHK*$^{Fl/Fl}$*XCre-Vil* FG 30% (n=6)**	***KHK*$^{Fl/Fl}$ FG 10% vs *KHK*$^{Fl/Fl}$*XCre-Vil* FG 30% ANOVA**
Body Weight and composition							
Body Weight; 0W (g)	23.1 ± 0.4	23.4 ± 0.6	22.5 ± 0.6	23.6 ± 0.3	22.9 ± 0.4	22.8 ± 0.6	*NS*
Body Weight; 30W (g)	33.6 ± 1.9	**46.3 ± 3.5****	**51.3 ± 2.7****	33.4 ± 1.1	**37.3 ± 1.2****	**44.9 ± 3.4****	*NS*
ΔBody Weight; 30W (g)	10.5 ± 1.4	**22.9 ± 2.1****	**28.8 ± 1.6****	9.8 ± 0.8	**14.4 ± 0.7****	**22.1± 1.9****	*NS*
Average Food Intake (g/day)	3.16 ± 0.4	**2.75 ± 0.4****	**2.88 ± 0.2****	3.06 ± 0.2	**2.65 ± 0.5****	**2.70 ± 0.1****	*NS*
Water/FG Intake (ml/day)	3.1 ± 0.2	**14.1 ± 1.1****	**7.8 ± 1.1****	3.1 ± 0.1	3.1± 0.2	3.7 ± 04	*P*<0.01
Cumulative Caloric Intake 30W	2104±97	**3016±118****	**3694 ± 172****	2050 ± 111	2027 ± 58	**2835±111****	*NS*
Cumulative Caloric Intake/BW 30W	63.7 ± 8	65.1 ± 22	72.0 ± 36	61.3 ± 8	54.3 ± 10	63.1 ± 12	*NS*
Cumulative Calories from Fructose; 30W	0	**709 ± 45****	**1181 ± 73****	0	**155 ± 19****	**661 ±28****	*NS*
Cumulative Calories from Fructose/BW 30W	0	**15.3 ± 5****	**23.1 ± 3****	0	**4.1 ± 1****	**14.7 ± 5****	*NS*
Liver Weight; 30 W (g)	1.49 ± 0.10	**2.45±0.08****	**3.01± 0.25****	1.49± 0.12	**1.96±0.20****	**2.63±0.18****	*NS*
Epididymal Fat Weight; 30W (g)	0.94 ± 0.23	**2.06±0.12****	**2.75 ± 0.33***	0.89 ± 0.25	1.16 ± 0.20	**1.91± 0.35****	*NS*
Biochemical Blood Analysis							
AST (IU/L)	42 ± 7	**104 ± 12****	**145 ± 13****	41 ± 4	50 ± 11	**80 ± 11****	*P*<0.01
ALT (IU/L)	23 ± 6	**56 ± 9****	**94 ± 8****	20 ± 7	25 ± 5	**61 ± 10****	*NS*

Serum Triglycerides (mg/dl)	44.6 ± 5.8	64.2 ± 10.1*	**68.2 ± 8.6***	39.3 ± 4.6	42.7 ± 4.6	**63.3 ± 5.2****	*NS*
Fasting Serum Glucose (mg/dl)	92 ± 8.0	101 ± 4.5	129 ± 8.5**	93 ± 3.5	94 ± 3.6	**114.5 ± 4.4****	*P*<0.01
Fasting Insulin (ng/ml)	2.06 ± 0.3	**4.97 ± 0.9****	**7.89 ± 1.6****	2.13 ± 0.2	2.55 ± 0.3	**4.16 ± 0.5****	*NS*
HOMA-IR	1.2 ± 0.2	**3.2 ± 0.4****	**6.2 ± 1.2****	1.2 ± 0.2	1.4 ± 0.2	**3.4 ± 0.2****	*NS*
OGTT (AUC/10)	433 ± 35	**612 ± 23****	**812 ± 36****	404 ± 40	416 ± 33	**585 ± 55****	*NS*
Fasting Leptin (ng/ml)	4.33± 0.3	**7.72 ± 1.4****	**15.55 ± 2.3****	4.22 ± 0.3	4.46 ± 0.3	8.75 ± 1.7**	*NS*
Serum Creatinine (mg/dl)	0.12± 0.02	**0.07±0.02****	0.15 ± 0.03	0.12 ± 0.02	0.14 ± 0.02	0.13± 0.02	*P*<0.01
Serum BUN (mg/dl)	25.6 ± 5.8	**16.4 ± 4.5***	26.7 ± 3.3	22.6 ± 3.4	24.1 ± 3.1	21.6 ± 6.7	*NS*
FGF21 (pg/ml)	544 ± 83	**5580 ± 428****	**5956 ± 363****	428 ± 45	**3680 ± 125***	**5152 ± 556****	*NS*
Liver Analysis							
Triglycerides (mg/g)	46 ± 8	**150 ± 26****	**180 ± 14****	45 ± 10	**84 ± 15****	**140 ± 12****	*NS*
Fgf21 mRNA (AU)	0.96 ± 0.4	**6.62 ± 0.7****	**7.97 ± 0.6****	1.22 ± 0.2	3.34 ± 0.8	6.16 ± 0.9	*NS*

Supplemental Table 3: General Parameters in *KHK$^{Fl/Fl}$* and liver-specific fructokinase knockout (*KHK$^{Fl/Fl}$XCre-Alb*) exposed to water control or 10% FG (related to Figure 4). * *P*<0.05 and ** *P*<0.01 versus respective water controls. One way ANOVA Tukey post hoc analysis, n=6 mice per group.

	KHK$^{Fl/Fl}$ **Water (n=6)**	***KHK$^{Fl/Fl}$*** **FG 10% (n=6)**	***KHK$^{Fl/Fl}$XCre-Alb*** **Water (n=6)**	***KHK$^{Fl/Fl}$XCre-Alb FG 10%*** **(n=6)**	***KHK$^{Fl/Fl}$*** **FG 10% vs *KHK$^{Fl/Fl}$XCre-Alb* FG 10% ANOVA**
Body Weight and composition					
Body Weight; 0W (g)	22.7 ± 0.3	22.8 ± 0.4	23.1 ± 0.5	23.4 ± 0.5	*NS*
Body Weight; 30W (g)	33.0 ± 0.5	**43.1 ± 0.8****	32.8 ± 0.9	32.6 ± 1.5	*P*<0.01
ΔBody Weight; 30W (g)	10.3 ± 0.8	**20.3 ± 1.2****	9.7 ± 1.5	9.2 ± 2.3	*P*<0.01
Average Food Intake (g/day)	3.20 ± 0.2	**2.71 ± 0.1****	3.22 ± 0.1	2.66 ± 0.2	*NS*
Water/FG Intake (ml/day)	3.3 ± 0.2	**13.7 ± 1.4****	3.1 ± 0.3	**14.6 ± 1.6****	*NS*
Cumulative Caloric Intake 30W	2083±86	**2915±95****	2083 ± 103	**2963 ± 90****	*NS*
Cumulative Caloric Intake/BW 30W	63.1 ± 9	67.6 ± 10	63.5 ± 12	**90.8 ± 12****	*P*<0.01
Cumulative Calories from Fructose; 30W	0	**687 ± 38****	0	**736 ± 53****	*NS*
Cumulative Calories from Fructose/BW 30W	0	**15.9 ± 5****	**0**	**22.5 ± 4****	*P*<0.05
Liver Weight; 30 W (g)	1.52 ± 0.13	**2.43±0.15****	1.42± 0.11	1.49± 0.08	*P*<0.01
Epididymal Fat Weight; 30W (g)	0.89 ± 0.34	**2.25±0.32****	1.05 ± 0.20	0.96 ± 0.4	*P*<0.01
Biochemical Blood Analysis					
AST (IU/L)	42 ± 8	**107 ± 26****	36 ± 8	33 ± 4	*P*<0.01
ALT (IU/L)	28 ± 4	**59 ± 5****	25 ± 4	24 ± 6	*P*<0.01

Serum Triglycerides (mg/dl)	55.5 ± 7.7	69.2 ± 6.8 *	53.3 ± 4.5	44.8 ± 9.6	*P*<0.01
Fasting Serum Glucose (mg/dl)	93 ± 7.0	105 ± 10	91 ± 6.5**	99 ± 4.0	*NS*
Fasting Insulin (ng/ml)	2.40 ± 0.3	**4.84 ± 1.1****	2.08 ± 0.3	1.98 ± 0.5	*P*<0.01
HOMA-IR	1.2 ± 0.2	**3.3 ± 0.3****	1.0 ± 0.4	1.2 ± 0.2	*P*<0.01
OGTT (AUC/10)	466 ± 56	**735 ± 35****	385 ± 55	426 ± 60	*P*<0.01
Fasting Leptin (ng/ml)	4.01± 0.4	**9.91 ± 1.7****	4.57 ± 0.7	4.55 ± 0.3	*P*<0.01
Serum Creatinine (mg/dl)	0.15± 0.04	**0.08±0.02****	0.14± 0.02	**0.06 ± 0.02****	*NS*
Serum BUN (mg/dl)	23.7 ± 3.3	**16.4 ± 3.3***	25.0 ± 2.6	**13.8 ± 3.4****	*NS*
FGF21 (pg/ml)	416 ± 56	**5336 ± 126****	505 ± 45	**1089 ± 154***	*P*<0.01
Urine Analysis					
Fructose (nmol/mgCre)	74.5 ± 35.2	61.6 ± 40.2	249.3 ± 83.3	**617.5± 250****	*P*<0.01
Liver Analysis					
Triglycerides (mg/g)	55 ± 6	**156 ± 23****	**38 ± 4**	46 ± 7	*P*<0.01
Fgf21 mRNA (AU)	0.99 ± 0.2	**6.76 ± 0.5****	0.87 ± 0.1	1.06 ± 0.2	*P*<0.01