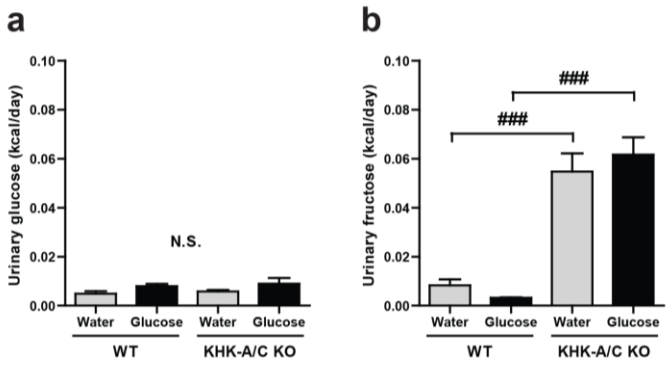
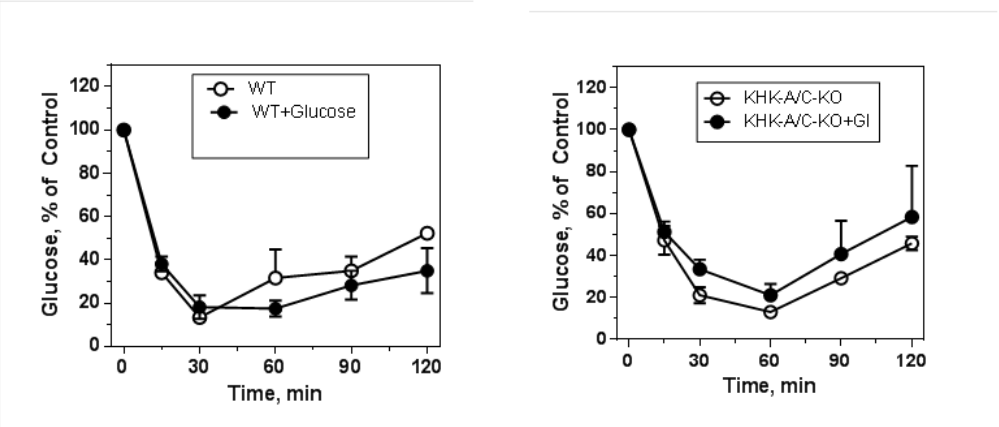
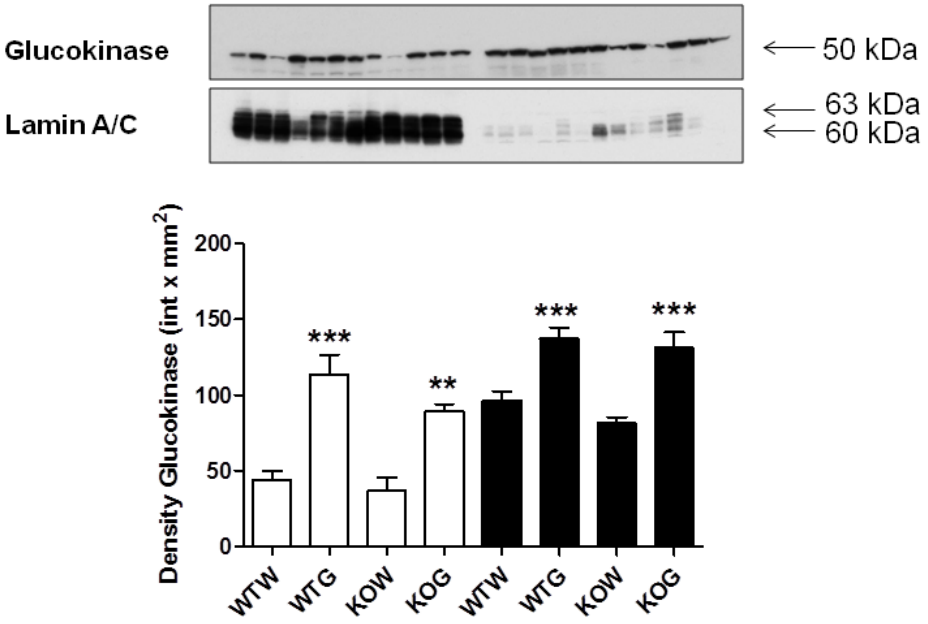


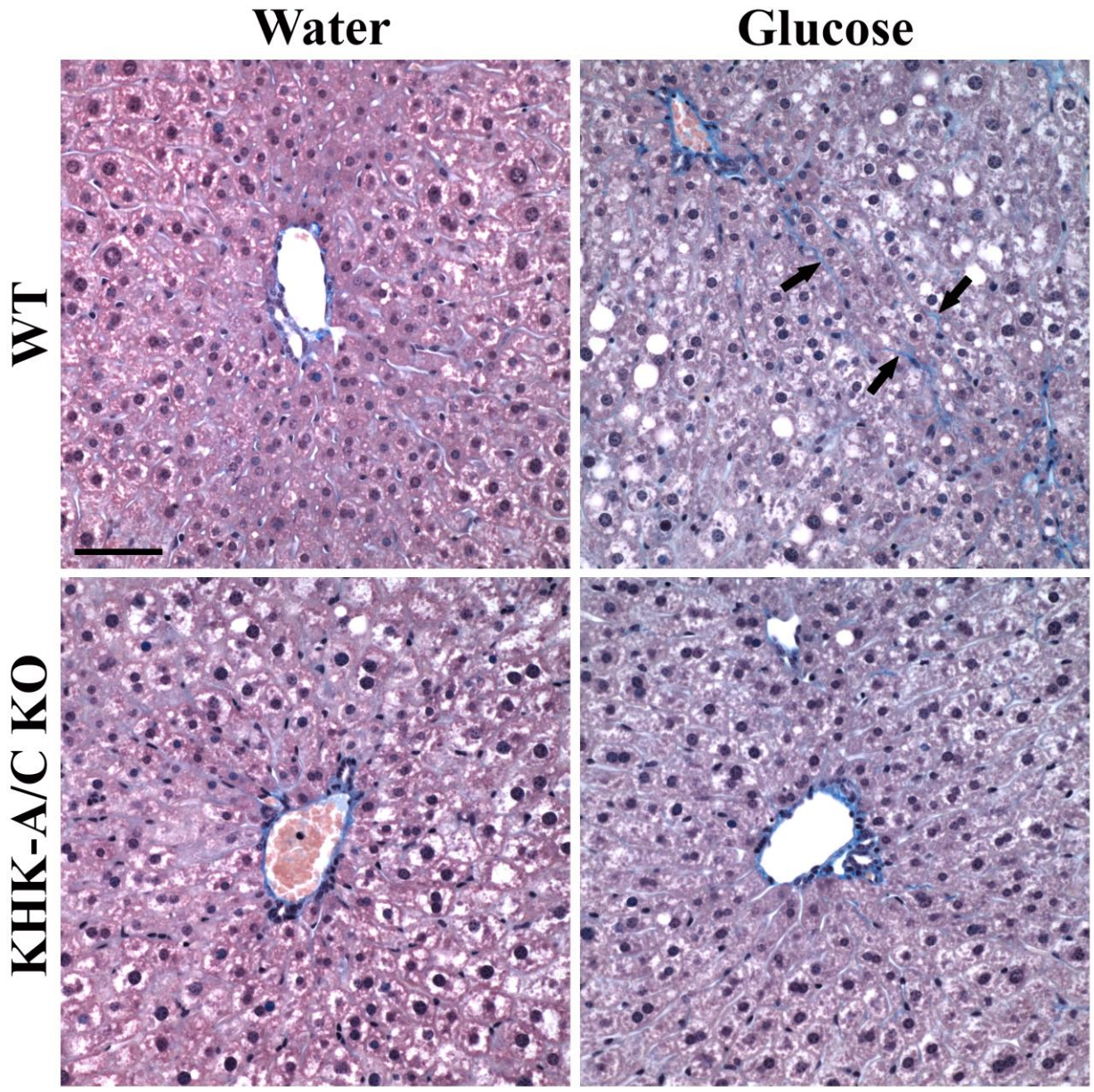
Supplementary Figure S1. Effect of Glucose on Energy Balance in WT and KHK A/C KO Mice. WT mice and KHK-A/C KO mice were provided drinking water containing 10% glucose or tap water with normal chow *ad libitum* for 14 weeks (n = 6 per group). Urinary glucose excretion (a) and urinary fructose excretion (b). Data represent means ± S.E.M. ***$P < 0.001$ vs. respective water control. #$P < 0.05$. ### $P < 0.001$. N.S., not significant. (ANOVA, Tukey post hoc analysis)



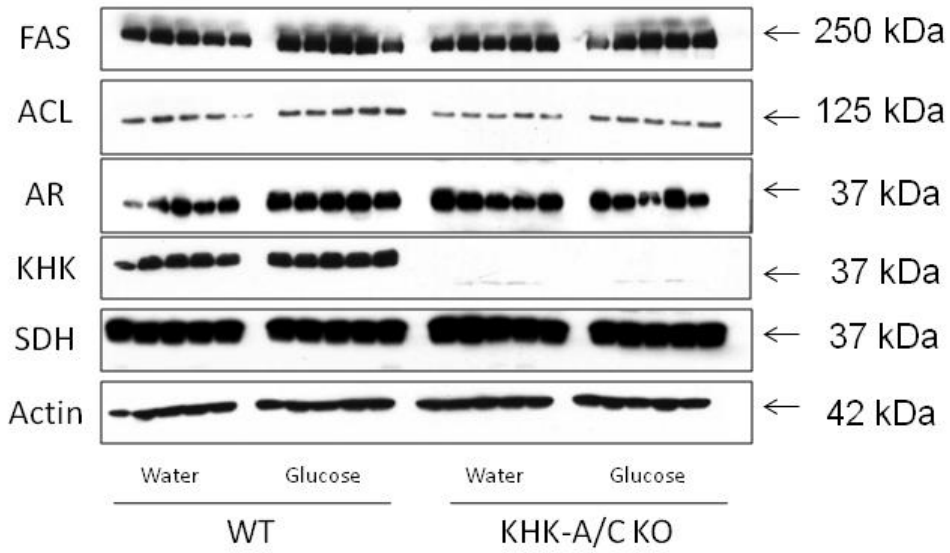
Supplementary Figure S2. Effect of Glucose Diet on Insulin resistance Assessed by Insulin Tolerance Test (ITT) in WT and KHK A/C KO Mice. ITT was performed one week prior to the end of experiment. Animals(n=6) were fasted for 4 hours and then injected intraperitoneally with insulin (Humulin: R; Eli Lilly, Indianapolis, IN) at 1 U/kg body weight. Glucose in tail vein blood was measured immediately before injection (time 0) and 15, 30, 60 90, and 120 minutes after injection using One Touch Ultra glucometer (Lifescan, Milpitas, CA). Data represent means ± S.E.M.



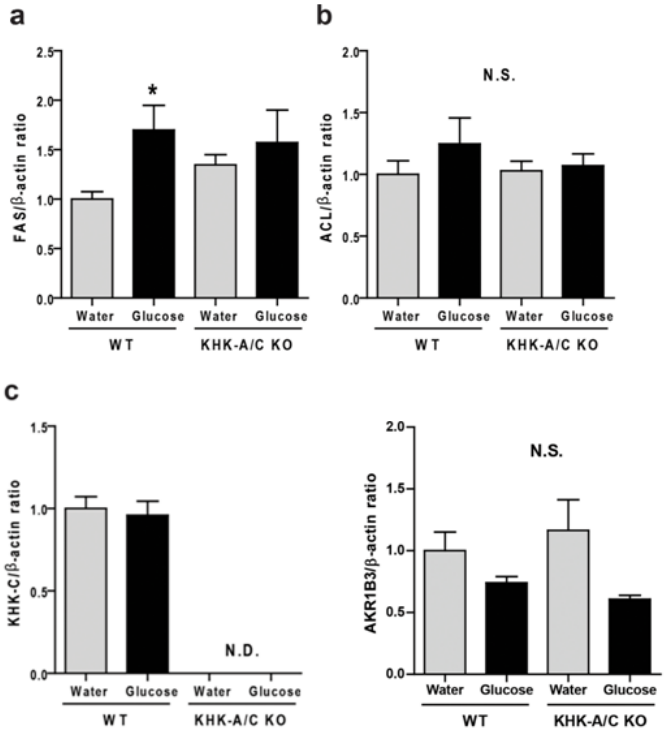
Supplementary Figure S3. Effect of Glucose Diet on Glucokinase expression and cellular location in WT and KHK A/C KO Mice. Glucokinase expression in nuclear (white bars) and cytosolic (black bars) fractions from WT and KHK-A/C KO mice drinking water (WTW or KOW). Nuclear fractions were obtained using the nuclear/cytosol fractionation kit (Biovision). Western blot was performed as described in the methods section employing the following antibodies: glucokinase, Santa Cruz Biotechnologies sc-130765. Lamin A/C, nuclear amrker, sc-20681. ***P*<0.01 and ****P*<0.001 versus respective water controls (ttest, n=3).



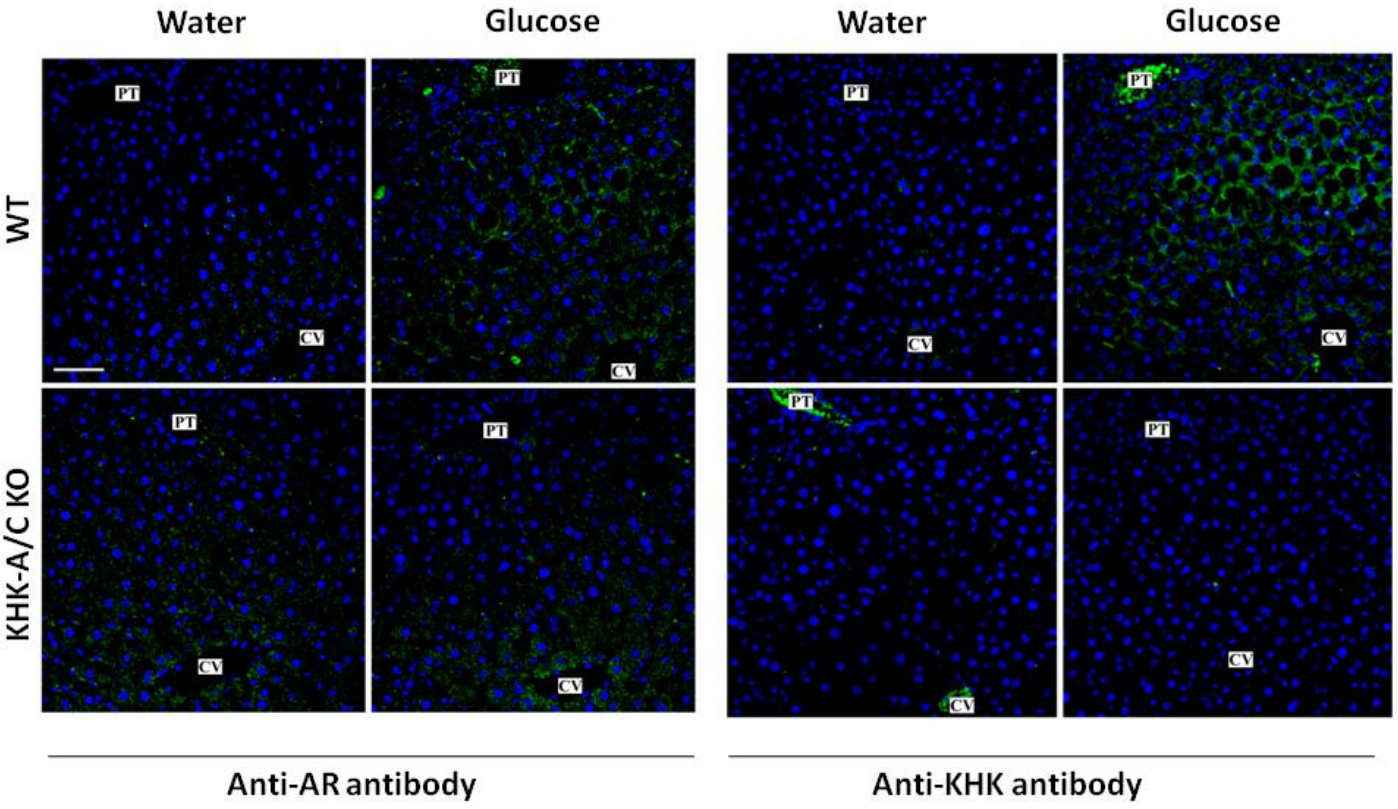
Supplementary Figure S4. Effect of glucose on liver fibrosis in WT and KHK-A/C KO mice. WT and KHK-A/C KO mice were provided drinking water containing 10% glucose or tap water with normal chow *ad libitum* for 14 weeks (n=6 per group). Fibrosis was assessed by Mallory trichrome staining. Trichrome stain analysis demonstrated a small amount of collagen in the periportal sinusoids of the glucose fed WT mice (black arrows). Staining of tissue from KHK-A/C KO mice revealed an attenuated quantity of collagen. Size bar: 50 μM.



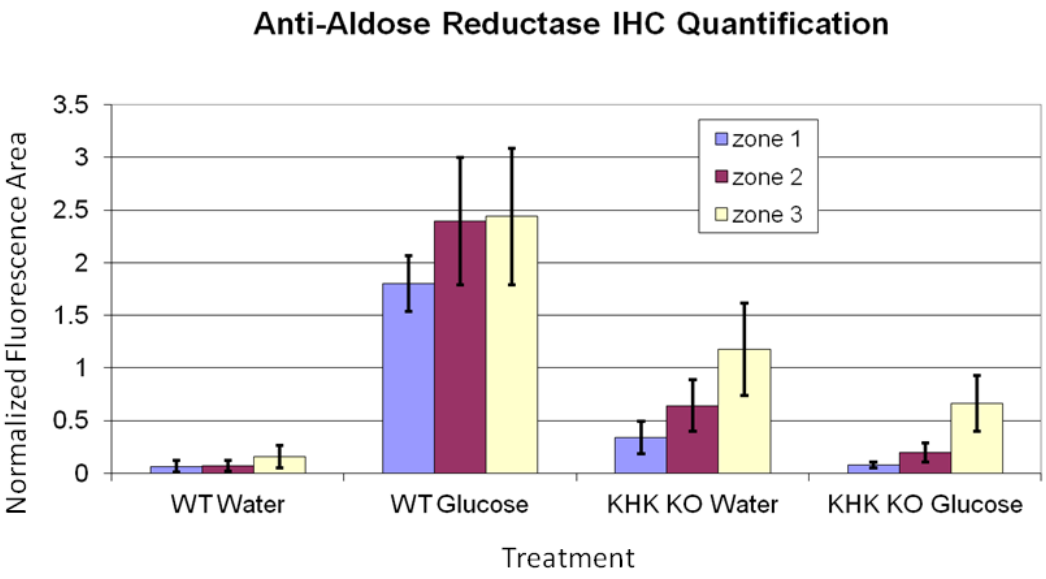
Supplementary Figure S5. Effect of Glucose on Hepatic expression of enzymes involved in fat synthesis and the polyol pathway. WT mice and KHK-A/C KO mice were provided drinking water containing 10% glucose or tap water with normal mouse chow *ad libitum* for 14 weeks (*n* = 6 per group). Representative western blot from the livers of these mice (n=5 per group). FAS (fatty acid synthase), ACL (ATP citrate lyase), AR (aldose reductase), KHK (fructokinase), SDH (sorbitol dehydrogenase).

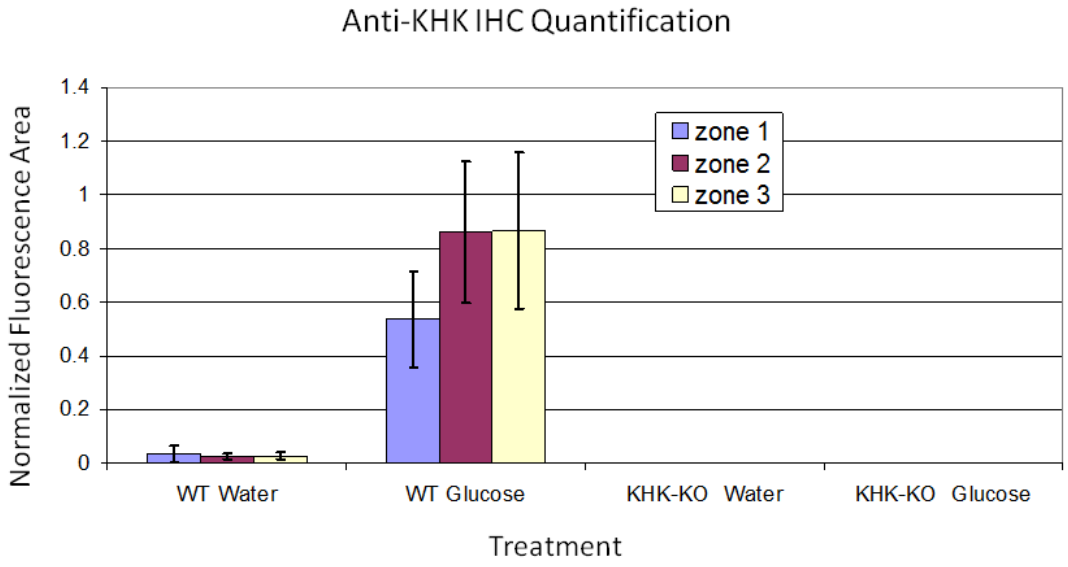


Supplementary Figure S6. Effects of 10% drinking glucose solution on mRNA expression of lipogenic and polyol pathway-related enzymes in the liver of WT and KHK-A/C KO mice. Data depicts fatty acid synthase (FAS,a), ATP citrate lyase (ACL, b), fructokinase (KHK, c left) and aldose reductase (akr1b1, c right) mRNA levels Data represents means ± S.E.M..(n=6 per group) **P*<0.05, N.S. non significant vs rest of conditions (ANOVA, Tukey post hoc analysis).

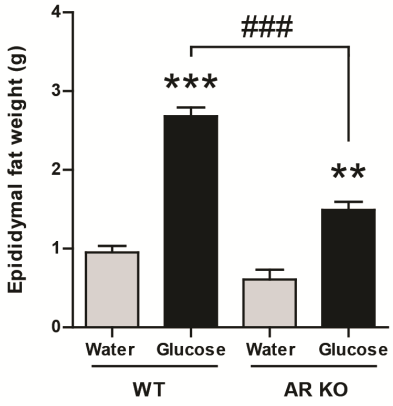


Supplementary Figure S7. Hepatic distribution of AR and KHK in control and glucose-fed mice. WT mice and KHK-A/C KO mice were provided drinking water containing 10% glucose or tap water with normal mouse chow *ad libitum* for 14 weeks (n = 6 per group). Representative immunofluorescence images showing AR (green, left panel) and KHK (green, right panel) distribution in WT (top) and KHK-A/C KO mice (bottom). As shown in the representative images, both AR and KHK are predominantly found in zones 1 and 2 of the liver with no or minimal expression in zone 3. PT (portal triad), CV (cental vein). Scale bar: 50 μM.

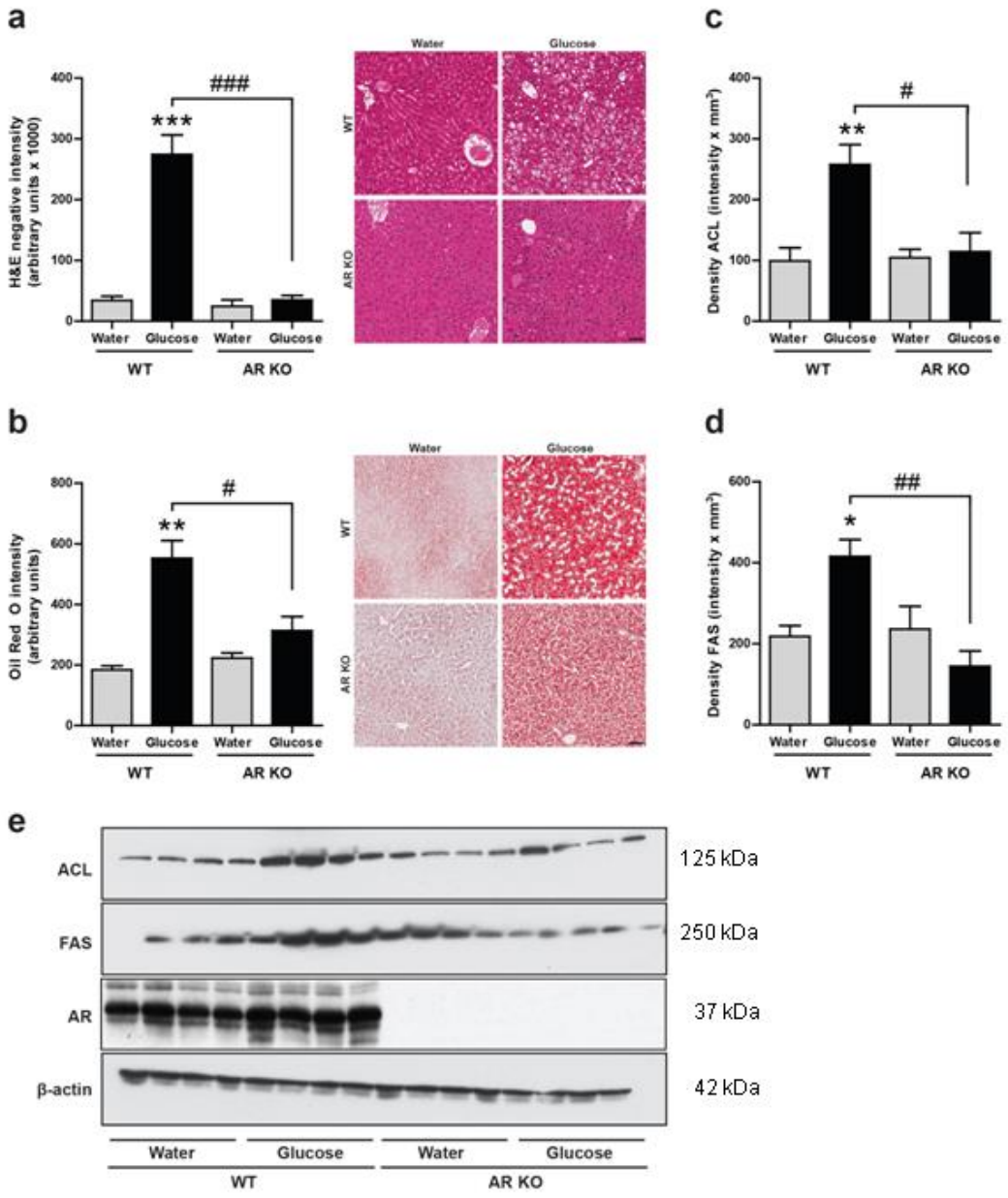




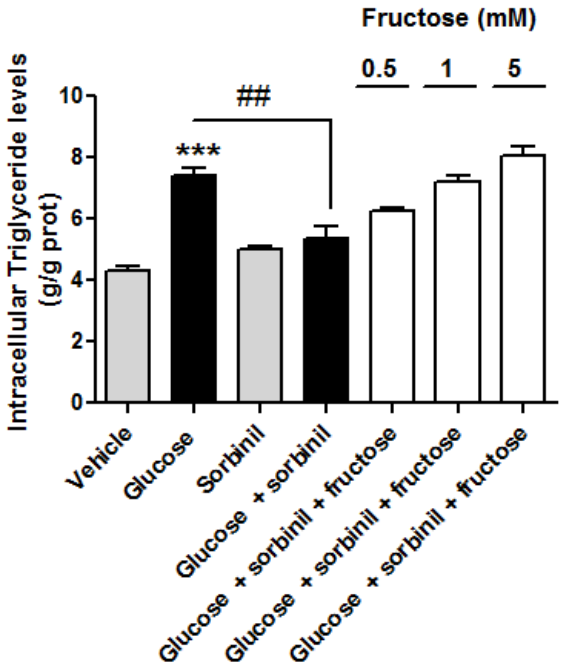
Supplementary Figure S8. Quantification of AR (top) and KHK (bottom) expression in different liver zones of WT and KHK-AC KO mice. Data represent means ± SEM, n=8. Statistics: No Statistically significant differences (P<0.01) between zones within any group. WT glucose is significantly different (P<0.01) from WT water and KHK-A/C KO for both AR and KHK expression. (ANOVA, Tukey post hoc)



Supplementary Figure S9: Effect of glucose on epidimal fat of WT and AR KO mice Reduced white adipose tissue in aldose reductase knockout mice drinking glucose compared to WT mice. Epididymal fat weight of WT mice and aldose reductase knockout mice (AR KO) given *ad libitum* normal chow diet with 10% glucose water, or tap water for 14 weeks (n=3-4). Data represents means ± S.E.M. **P<0.01, ***P<0.001 vs. respective water control. ### P<0.001.(ANOVA, Tukey post hoc analysis)



Supplementary Figure S10. Aldose reductase knockout mice do not develop fatty liver after glucose load. WT mice and aldose reductase knockout mice (AR KO) given *ad libitum* normal chow diet with 10% glucose water, or tap water for 14 weeks (n=3-4). (a) Representative images of H and E staining. Bar, 50 µm. (b) Representative images of oil red O staining. Bar, 50 µm. (c-e) Western blot of ATP citrate lyase (ACL, c, e), fatty acid synthase (FAS, d,e) and aldose reductase (AR, e). Relative intensity to β-actin in liver (e). Data represents means ± S.E.M. *P<0.05,**P<0.01, ***P<0.001 vs. respective water control. ### P<0.001.(ANOVA, Tukey post hoc analysis).



Supplementary Figure S11. Aldose reductase inhibition blocks glucose-induced triglyceride accumulation in human HepG2 cells which is restored by adding back fructose in a dose dependent manner. Data represents means ± S.E.M (ANOVA, Tukey post hoc analysis). , ***P<0.001 vs. respective water control. ## P<0.01. n=3 per group

Supplementary Table S1 Characteristics of the mice given ad libitum normal chow diet with 10% glucose water or tap water for 14 weeks (*n* = 6).

	WT		KHK-A/C KO		WT glucose vs. KHK-A/C glucose
	Water (*n*=6)	Glucose (*n*=6)	Water (*n*=6)	Glucose (*n*=6)	
Body weight (0W, g)	28.0 ± 0.7	28.2 ± 0.6	27.5 ± 0.7	27.4 ± 0.6	N.S.
Body weight (14W, g)	34.9 ± 1.1	46.7 ± 1.7***	34.9 ± 1.3	40.6 ± 2.2***	N.S.
Kidney weight (14W, g)	0.21 ± 0.01	0.19 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	N.S.
BUN (mg/dl)	24.2 ± 0.8	19.2 ± 1.6	25.3 ± 0.6	19.2 ± 0.5	N.S.
Creatinine (mg/dl)	0.22 ± 0.04	0.26 ± 0.05	0.23 ± 0.02	0.28 ± 0.06	N.S.
Total cholesterol (mg/dl)	137.0 ± 2.6	185.6 ± 7.5***	134.2 ± 9.3	163.8 ± 6.2*	*P* < 0.05 (ttest)
LDL cholesterol (mg/dl)	6.5 ± 0.7	13.4 ± 0.7***	7.8 ± 1.0	12.3 ± 1.4*	N.S.
HDL cholesterol (mg/dl)	78.7 ± 1.6	89.6 ± 4.0	77.5 ± 5.7	89.5 ± 3.6	N.S.
Triglyceride (mg/dl)	68.8 ± 13.9	76.2 ± 23.5	57.3 ± 5.3	69.8 ± 10.3	N.S.
Glucose (mg/dl)	265.5 ± 20.8	346.0 ± 45.4	266.8 ± 11.1	345.3 ± 27.2	N.S.
HbA1c (%)	3.2 ± 0.10	3.8 ± 0.14***	3.3 ± 0.05	3.7 ± 0.06*	N.S.
Uric acid (mg/dl)	2.9 ± 0.5	2.9 ± 0.7	3.1 ± 0.3	2.7 ± 0.4	N.S.

BUN, blood urea nitrogen

***P* < 0.05, ****P* < 0.01, *****P* < 0.001 vs. respective water control. N.S., not significant. (ANOVA, Tukey post hoc) Data represents means ± S.E.M**

SupplementaryTable S2 Liver pathology of the mice given ad libitum normal chow diet with 10% glucose water or tap water for 14 weeks (*n* = 5).

			WT		KHK-A/C KO	
			Water (*n*=5)	**Glucose (*n*=5)**	**Water (*n*=5)**	**Glucose (*n*=5)**
Steatosis		points				
grade (% cells with steatosis)	<10%	0	100%		100%	100%
	10-33%	1		100%		
	33-66%	2				
	>66%	3				
		points				
location (zone)	not relevant	0	100%		100%	100%
	zone 3	1				
	zone 2	1		20%		
	zone 1 and 2	2		80%		
	zone 1	1				
	azonal					
	panacinar	3				
microvesicular steatosis	not present		100%	20%	60%	80%
	present (which zone)			80%	40%	20%
Fibrosis						
stage	none		100%	40%	60%	80%
	perisinusoidal or periportal			60%	40%	20%
	mild, zone 3, periportal					
	moderate, zone 3, perisinusoidal					
	portal/periportal					
	perisinusoidal and portal/periportal					
	bridging fibrosis					
	cirrhosis					
Inflammation						
lobular inflammation	<2 per 200x field		100%	80%	100%	100%
	2 per 200x field			20%		
	2-4 per 200x field					
	>4 per 200x field					
Small foci of inflammatory cells	absent (less then 1 per 200x field)		100%	80%	100%	100%

	present (1 or more per 200x field)		20%		
large lipogranulomas	absent	100%	80%	100%	100%
	present		20%		
portal inflammation	none to minimal	100%	100%	100%	100%
	more than minimal				
Liver cell injury					
ballooning	none	100%	100%	100%	100%
	very few				
	few				
	many				
acidophil bodies	none	100%	100%	100%	100%
	few				
	many				
pigmented macrophages	none	100%	100%	100%	100%
	many				
megamitochondria	none	100%	100%	100%	100%
	many				
Other					
Mallory's hyaline	none	100%	100%	100%	100%
	many				
Glycogenated nuclei	none (less then 1 per 200x field)	100%	100%	100%	100%
	few (1-3 per 200x field)				
	many (more then 3 per 200x field)				

Supplementary Table S3 Characteristics of the mice given ad libitum normal chow diet with 10% glucose water or tap water for 14 weeks (*n* = 4).

	WT Glucose (*n*=4)	KHK-AC KO Glucose (*n*=4)
Cumulative total energy intake (kcal)	1380 ± 46	1307 ± 22
Cumulative glucose intake (kcal)	574 ± 35	585 ± 17
Body weight (0W, g)	28.3 ± 0.7	27.6 ± 0.6
Body weight (14W, g)	45.6 ± 1.7	41.7 ± 2.6
ΔBody weight (g)	17.3 ± 1.5	14.2 ± 2.0
Liver weight (g)	2.00 ± 0.13	1.66 ± 0.23
Epididymal fat weight (g)	2.61 ± 0.10	2.25 ± 0.26
ALT (IU/l)	87.5 ± 16.2	46.5 ± 13.3
Total cholesterol (mg/dl)	183.8 ± 9.4	171.8 ± 5.3
LDL cholesterol (mg/dl)	13.5 ± 0.9	13.5 ± 1.8
HDL cholesterol (mg/dl)	92.5 ± 3.6	91.5 ± 5.2
Triglyceride (mg/dl)	85.8 ± 27.7	80.3 ± 12.4
Glucose (mg/dl)	339.5 ± 58.0	378.3 ± 27.6
HbA1c (%)	3.8 ± 0.18	3.7 ± 0.10
Fructose (μmol/l)	723.3 ± 78.6	763.6 ± 30.3
Uric acid (mg/dl)	3.3 ± 0.7	3.2 ± 0.3
Leptin (ng/ml)	58.5 ± 2.4	41.2 ± 5.3

ALT, alanine aminotransferase. Data represents means ± S.E.M