

Supplemental Information

Postprandial Hepatic Lipid Metabolism

Requires Signaling though Akt2 Independent of the Transcription Factors FoxA2, FoxO1, and SREBP1c

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Inventory for Supplemental Information

Figures S1 and S2 are controls for Akt2-FoxA2 mice on either normal chow or HFD, supporting the data in Figure 1.

Figure S3 is control data for Akt2-FoxO1 mice on normal chow, supporting the data in Figure 2.

Figure S4 showed TG secretion data to exclude one possible mechanism for hepatic TG accumulation in FoxO1 knockout livers, which was shown in Figure 2.

Figure S5 presents the gene expression data from mice we described in Figure 3

Figure S6 presents the gene expression data from mice we described in Figure 4

Figure S7 presents one potential mechanism for Akt2 to regulate postprandial lipogenesis, which we showed in Figure 5

Table S1.

Supplemental Experimental Procedures

Supplemental References

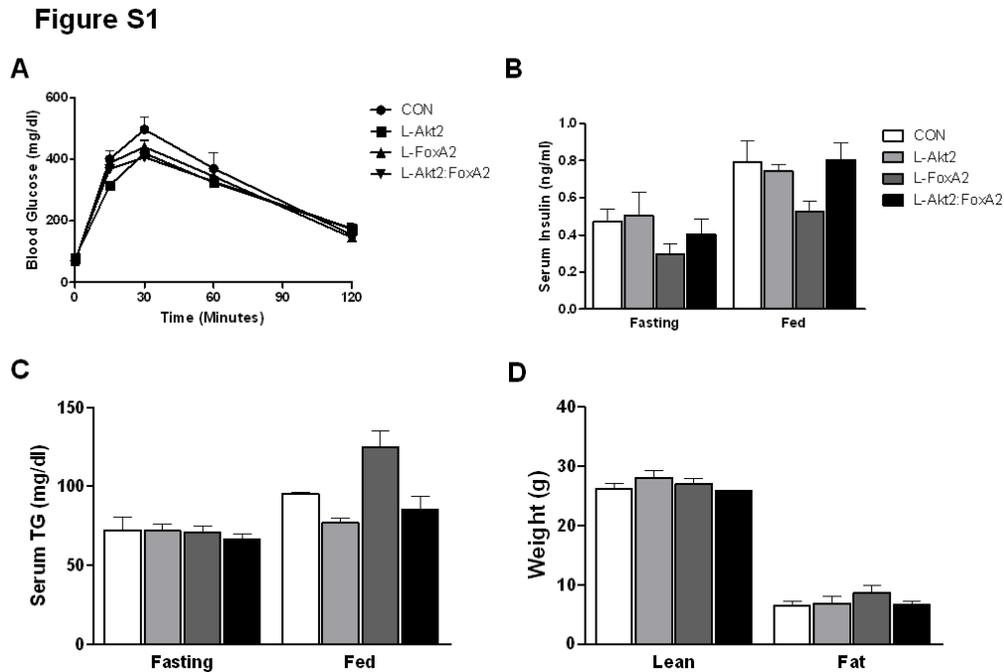


Figure S1. Hepatic *Akt2* and *FoxA2* Deletion does not Alter Glucose and Insulin Homeostasis in Mice on Normal Chow

(A) Glucose tolerance test (GTT) on wild type controls (CON), *AFP>Cre;Akt2^(loxP/loxP)* (L-Akt2), *AFP>Cre;FoxA2^(loxP/loxP)* (L-FoxA2) and *AFP>Cre;Akt2^{(loxP/loxP);FoxA2^(loxP/loxP)}* (L-Akt2:FoxA2) mice. Two-month-old male mice were fasted overnight, and injected intraperitoneally with 2g/kg bodyweight glucose, n=2-9 for each genotype, error bars represents SEM.

(B-C) Serum insulin and TG levels under fasting or fed conditions. Mice as described above were fasted, n=2-9 for each genotype, error bars represents SEM.

(D) Lean and fat mass. Mice were male and 1-year-old, n=4-6 for each genotype, error bars represents SEM.

Figure S2

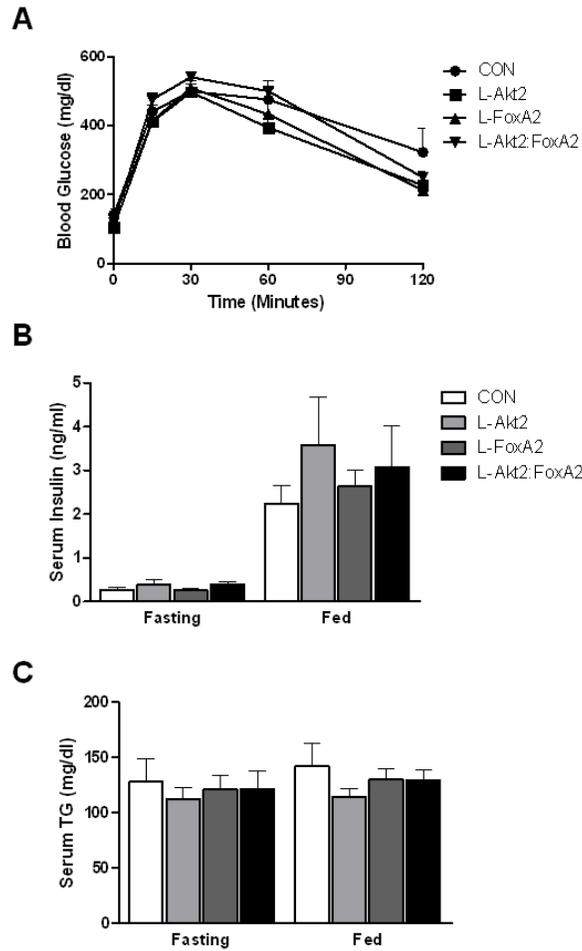


Figure S2. Hepatic *Akt2* and *FoxA2* Deletion does not Alter Glucose and Insulin Homeostasis in Mice on HFD

(A) Glucose tolerance test (GTT). At 1 month-old male mice were subject to 3-month HFD feeding. Mice were fasted overnight, and injected intraperitoneally with 2g/kg glucose, n=4-6 for each genotype, error bars represents SEM.

(B-C) Serum insulin and TG levels under fasting or fed conditions. Mice were as described above, n=6-7 for each genotype, error bars represents SEM.

Abbreviations are as indicated in Figure S1.

Figure S3

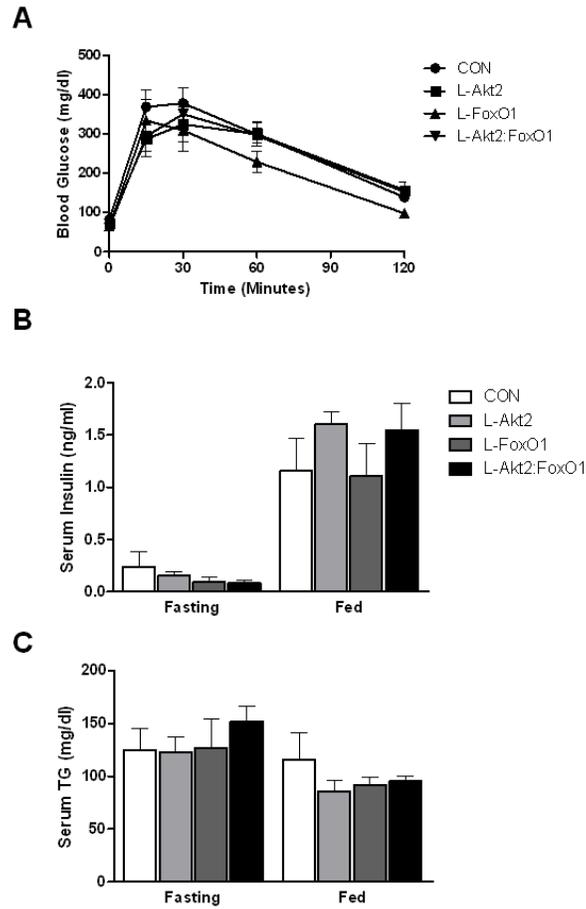


Figure S3. Hepatic *Akt2* and *FoxO1* Deletion does not Alter Glucose Homeostasis in Mice on Normal Chow

(A) Glucose tolerance test (GTT) on wild type controls (CON), $AFP>Cre;Akt2^{loxP/loxP}$ (L-Akt2), $AFP>Cre;Akt2^{loxP/loxP};FoxO1^{loxP/loxP}$ (L-FoxO1) and $AFP>Cre;Akt2^{loxP/loxP};FoxO1^{loxP/loxP}$ (L-Akt2:FoxO1) mice. Two-month-old male mice were fasted overnight, and injected intraperitoneally with 2g/kg glucose, n=4-6 for each genotype, error bars represents SEM.

(B-C) Serum insulin and TG levels under fasting or fed conditions. n=4-6 for each genotype, error bars represents SEM.

Figure S4

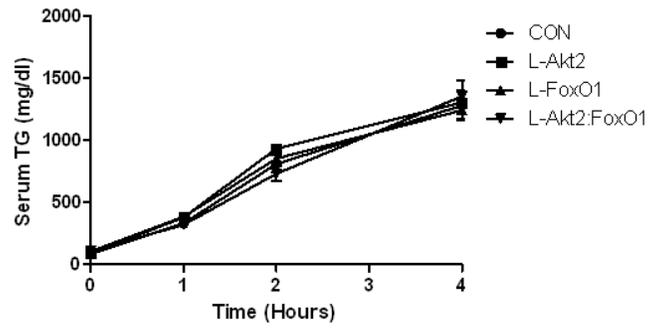


Figure S4. TG Secretion is Not Altered

VLDL-TG secretion. Mice were fasted overnight, and injected intraperitoneally with 1g/kg poloxamer 407. Blood samples were taken at designated time point, and serum TG levels were assayed, n=5-8 for each genotype, error bars represents SEM.

Abbreviations are as indicated in Figure S3.

Figure S5

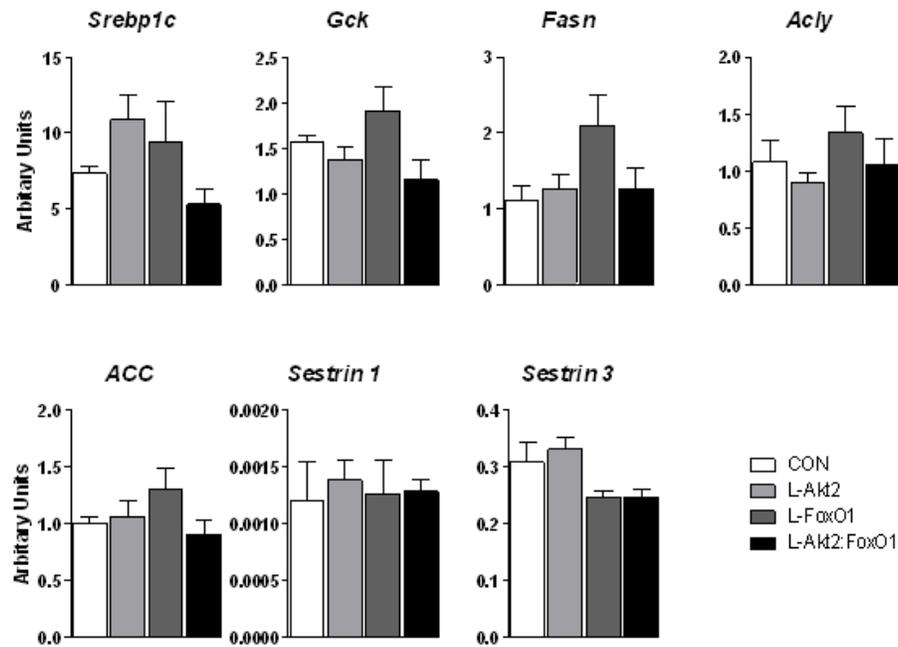


Figure S5. The Expression of Lipogenic Genes was Not Altered in Mice Refed on HCD.

Real-time PCR results for designated genes. Mice were described as in Figure 6, and lipogenic gene expression were analyzed by Realtime-PCR, n=5-7 for each group.

The variance of *Gck* and *Sestrin3* expression levels was significant among four different genotypes by one-way ANOVA, error bars represents SEM. Abbreviations are as indicated in supp. Figure 3.

Figure S6

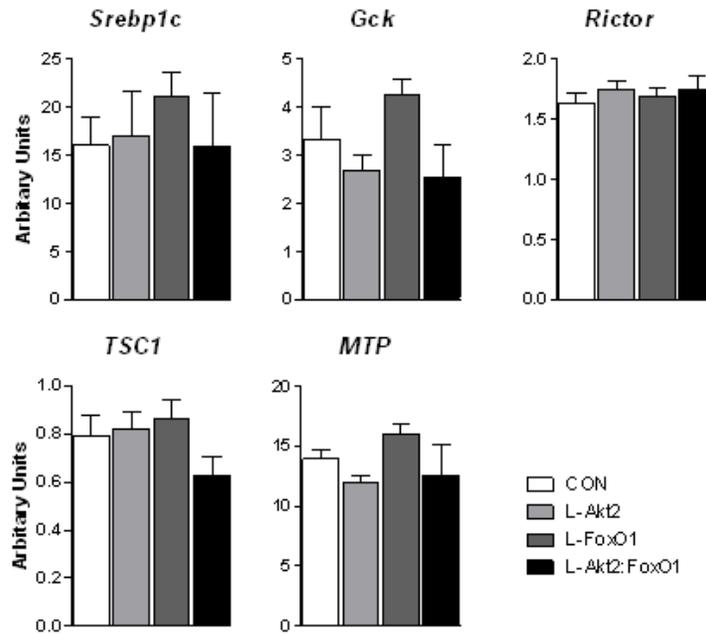


Figure S6. The Expression of Lipogenic Genes was Not Altered in Mice Injected with GTG.

Real-time PCR results for designated genes. Mice were described as in Figure 4, and lipogenic gene expression were analyzed by Realtime-PCR, n=4-8 for each group. The variance of *Gck* expression levels was significant among four different genotypes by one-way ANOVA, error bars represents SEM. Abbreviations are as indicated in Figure S3.

Figure S7

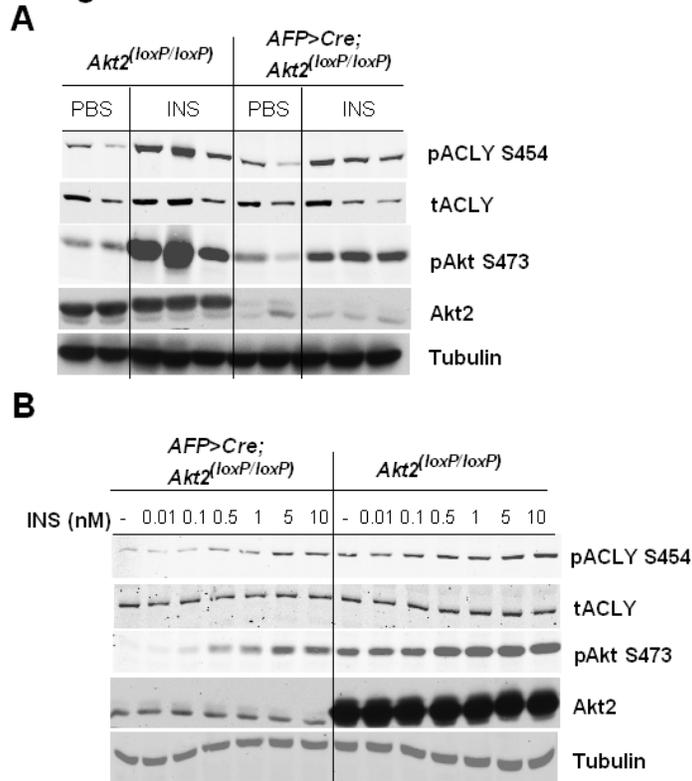


Figure S7. Insulin-Stimulated ACL Phosphorylation was Blunted in *Akt2* Deficient Hepatocytes.

(A) Western blots for the phosphorylated form of Akt and ACL in liver samples from wild type controls (*Akt2*^(loxP/loxP)) or *AFP>Cre;Akt2*^(loxP/loxP) mice after insulin injection. Mice were fasted overnight, and injected I.P. with either PBS or 1 U/kg bodyweight insulin and the liver harvested 20 minutes later.

(B) Western blots of primary hepatocytes isolated from control (*Akt2*^(loxP/loxP)) or *AFP>Cre;Akt2*^(loxP/loxP) mice. Cells were serum starved for 6 hours followed by insulin treatment at the indicated concentrations for 25 minutes.

Table S1. Real-time PCR Primers

Gene Name	Forward	Reverse
<i>TBP</i> ^a	cccctgtaccctcaccaat	gaagctgcggtacaattccag
<i>Srebp1c</i>	atgtgcgaactggacacagcg	acggagccatggattgcacattg
<i>Fasn</i>	gctgcggaaactcaggaaat	agagacgtgtcactcctggactt
<i>Gck</i>	gaagacctgaagaaggatgagc	gtctatgtcttcgtgccttacagg
<i>Acc</i>	tgacagactcatcgagagaaag	tggagagccccacacaca
<i>Acl</i>	gccagcgggagcacatc	cttgcaggtgccacttcac
<i>Gpat</i>	gccagcgggagcacatc	cttgcaggtgccacttcac
<i>FoxO1</i>	gcgtgccctactcaaggataa	tccagttcctcattctgcact
<i>TSC1</i>	gtcacgaccgtaggagaagc	gaatcaacccccacagagcat
<i>MTP</i>	tgaagaacatcctgctgtcc	tctacgtagccagtatagcc
<i>Sestrin1</i>	ggacgaggaactccaatca	atgcatctgtgcgtttcac
<i>Sestrin3</i>	catgcgtttcctcactcaga	ggcaaagtcttcgtacccaa
<i>INSIG2a</i>	ccctcaatgaatgtactgaaggatt	tgtgaagtgaagcagaccaatgt

^a, The expression level of *TATA-binding protein (TBP)* serves as the normalization control.

Supplemental Experimental Procedures

RNA Isolation and Gene Expression

Total RNA was extracted from liver, reverse-transcribed and analyzed by real-time PCR as described previously (Leavens et al., 2009). Primers are shown in Table S1.

aPKC Kinase Assay

The kinase activity of aPKC from liver samples was measured as described (Matsumoto et al., 2003; Standaert et al., 2004). Phosphatidylserine was purchased from Sigma, and the PKC zeta substrate was from EMD chemicals.

Western Blotting

Mice were sacrificed, the liver clamp-frozen in liquid nitrogen and stored at -80°C until being homogenized by TissueLyser (Qiagen) in lysis buffer containing 150 mM NaCl, 50mM Tris pH 7.4, 1% Triton-X100, 0.1% SDS, 0.5% Na-Deoxycholate, 1x Complete protease inhibitor mixture (Roche Applied Science) and 1x phosphatase inhibitor mixtures 1 and 2 (Sigma). For FoxA2 blots, primary hepatocytes were isolated as described previously (Li et al., 2007), and protein was extracted using the lysis buffer above. Blots were probed for pS6 S240/244 (2215, Cell Signaling Technology), tS6 (2217, Cell Signaling Technology), pAkt S473 (4060, Cell Signaling Technology), Akt2 (generated by our own lab)(Cho et al., 2001), FoxA2 (07-633, Upstate USA), GSK3 (9332, Cell Signaling Technology), p-pan PKC T410

(2060, Cell Signaling Technology), aPKC (sc-216, Santa Cruz), β -actin (ab6276, Abcam), p4EBP1 T37/46 (2855, Cell Signaling), 4EBP1 (9452, Cell Signaling), SREBP1 (sc-13551, Santa Cruz) or tubulin (2128, Cell Signaling Technology). Blots were then probed with either horseradish peroxidase (HRP)-conjugated secondary antibodies (Santa Cruz Biotechnology) and imaged using enzymatic chemiluminescence (ECL, Perkin Elmer life & Analytical), or fluorescently labeled secondary antibodies (Rockland) following instructions supplied by the manufacturer and imaged using the Odyssey Infrared Imager (LICOR Biosciences)

For blotting of SREBP1c, mice were sacrificed after a 24-hour fast or refed with HCD for 6 hours following a 24-h fast, then liver samples were subject to a nuclear extraction protocol modified from a previous report (Sheng et al., 1995). In brief, liver (100 mg) was homogenized in 1 ml Buffer A (20mM Tris Cl, pH 7.4; 2mM MgCl₂; 0.25M Sucrose; 10 mM EDTA; 10 mM EGTA; 5 μ M DTT and protease and phosphatase inhibitors), and then centrifuged at 3000g for 5 min at 4 °C. 0.8 ml supernatant (S1) was removed and centrifuged at 100,000g for 30 min at 4 °C. The supernatant (S2, cytoplasmic fraction) was collected and pellet (P1, membrane fraction) resuspended. The pellet from the first spin was washed with 1 ml Buffer A and then pellet from the wash (P2) resuspended in Buffer B (20 mM HEPES, pH 7.5; 2.5% Glycerol; 0.42 M NaCl; 1.5 mM MgCl₂; 1 mM EDTA; 1mM EGTA and protease and phosphatase inhibitors). P2 was incubated in Buffer B for 1 hour at 4 °C, spin at 100,000g for 30 min at 4 °C, and supernatant collected as the nuclear fraction.

Supplemental References

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