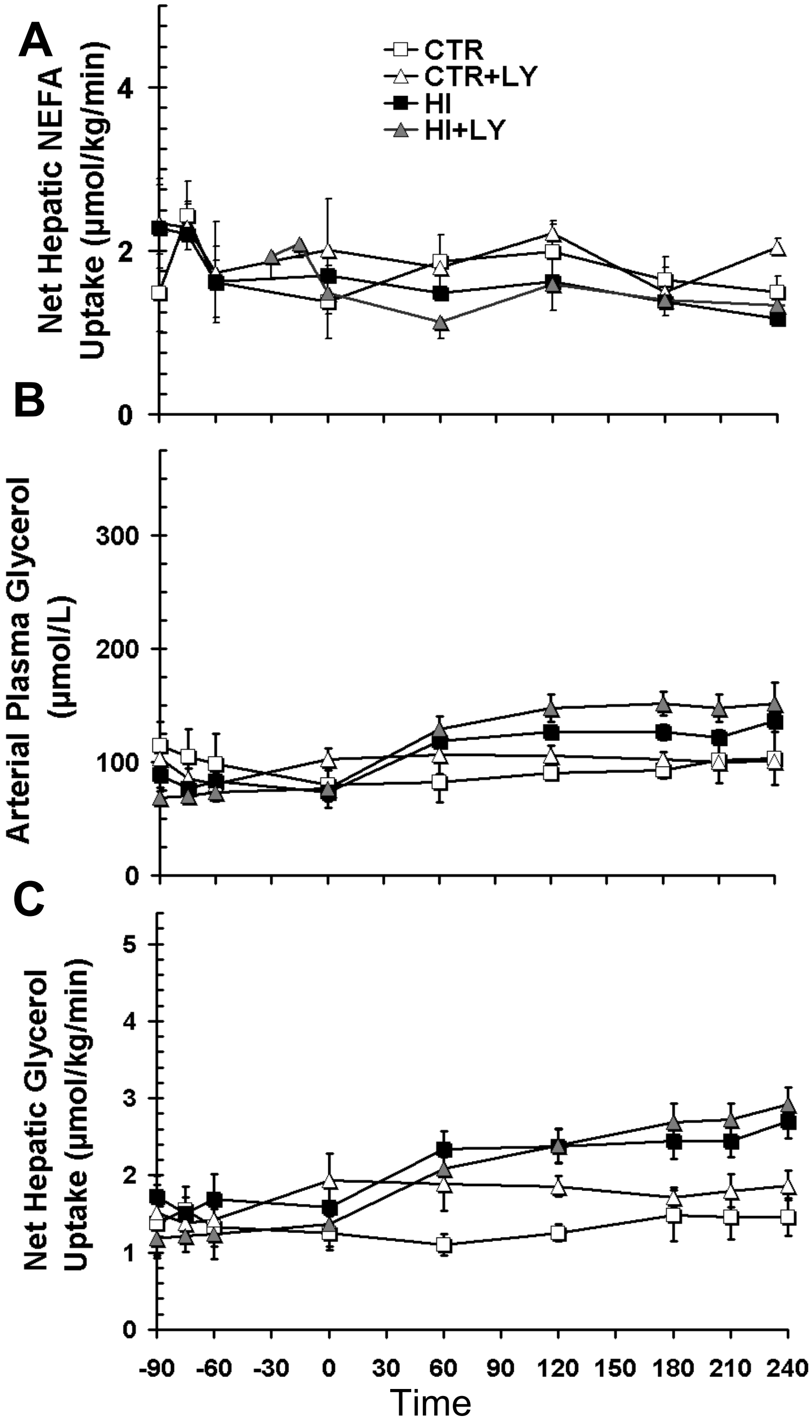
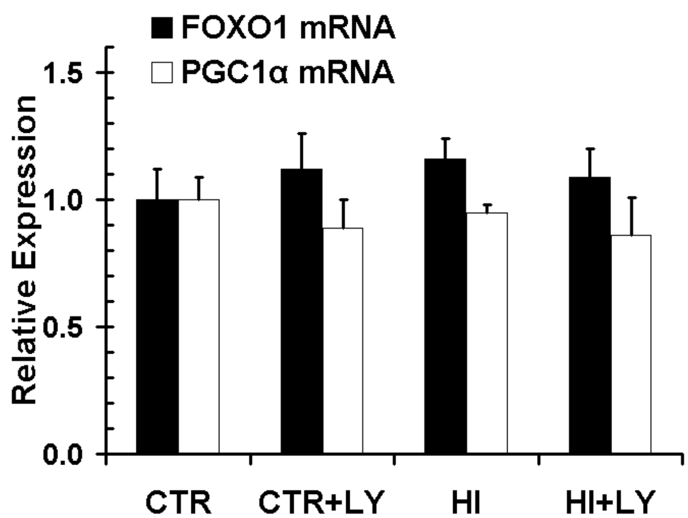
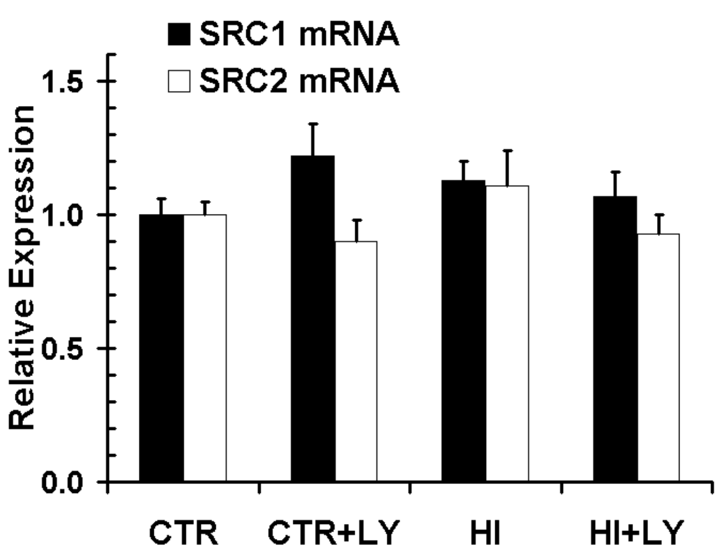
Supplemental Materials

Supplemental Figure Legend 1. (A) Net hepatic NEFA uptake, (B) arterial plasma glycerol, and (C) net hepatic glycerol uptake in 42h fasted conscious dogs. Values are means ± SEM; n values same as for Figure 4.



Supplemental Figure Legend 2. Effect of insulin administered through the head arteries in 42h fasted conscious dogs on the genetic regulation of key gluconeogenic regulators. (A) FOXO1 and PGC1α. (B) SRC1 and SRC2. Values are means ± SEM; n values same as for Figure 4.





Supplementary Table 1. Arterial plasma levels (mean ± S.E.M., n=5 in each group) of NEFA, cortisol, and catecholamines in the 18 h fasted conscious dog subjected to either artificial CSF (aCSF) or insulin (INS) infusion into the third ventricle during a basal insulin, basal glucagon pancreatic clamp.

	Basal Period	**Experimental Period (min)**				
		60	**120**	**180**	**210**	**240**
Arterial Plasma nonesterified fatty acid (NEFA) Level (µmol/L)						
aCSF	446 ± 60	484 ± 67	466 ± 76	554 ± 91	670 ± 102	644 ± 133
INS	666 ± 102	538 ± 117	563 ± 117	527 ± 109	555 ± 92	660 ± 137
Arterial Plasma Cortisol Level (ug/dl)						
aCSF	3.4 ± 0.7	2.7 ± 0.4	1.8 ± 0.5	2.2 ± 0.5	2.9 ± 0.7	2.4 ± 0.7
INS	3.4 ± 1.1	2.6 ± 0.3	2.0 ± 0.4	2.4 ± 0.7	2.0 ± 0.6	2.7 ± 1.3
Arterial Plasma Epinephrine Level (pg/mL)						
aCSF	99 ± 23	90 ± 25	72 ± 14	68 ± 7	80 ± 35	104 ± 38
INS	131 ± 30	149 ± 46	101 ± 31	89 ± 19	153 ± 53	179 ± 53
Arterial Plasma Norepinephrine (pg/mL)						
aCSF	98 ± 20	74 ± 10	74 ± 17	88 ± 19	89 ± 19	93 ± 18
INS	160 ± 22	149 ± 31	135 ± 27	126 ± 23	183 ± 50	191 ± 46

Supplementary Table 2. Arterial blood concentration and net hepatic balance (mean ± S.E.M., n=5 in each group) of gluconeogenic substrates in the 18 h fasted conscious dog subjected to either artificial CSF (aCSF) or insulin (INS) infusion into the third ventricle during a basal insulin, basal glucagon pancreatic clamp.

	Basal Period	**Experimental Period (min)**				
		60	**120**	**180**	**210**	**240**
Arterial Blood Lactate Concentration (μmol/L)						
aCSF	762 ± 249	759 ± 241	677 ± 202	575 ± 174	550 ± 147	516 ± 125
INS	805 ± 143	1018 ± 227	991 ± 142	1012 ± 153	882 ± 131	806 ± 96
Arterial Blood Glycerol Concentration (μmol/L)						
aCSF	99 ± 23	90 ± 25	72 ± 14	68 ± 7	80 ± 35	104 ± 38
INS	131 ± 30	149 ± 46	101 ± 31	89 ± 19	153 ± 53	179 ± 53
Arterial Blood Gluoneogenic Amino Acid Concentration (μmol/L)						
aCSF	1928 ± 153	1946 ± 188	2024 ± 156	1973 ± 138	2048 ± 161	1968 ± 84
INS	1743 ± 111	1871 ± 141	1937 ± 175	1966 ± 187	1989 ± 158	2020 ± 181

Supplementary Table 3. Arterial plasma levels of NEFA, cortisol, and catecholamines in the 42 h fasted conscious dog subjected to either saline (CTR, CTR+LY) or insulin infusion (HI, HI+LY) in the head arteries with and without ICV infusion of the PI3K inhibitor LY. Values are mean ± S.E.M., n=4, 4, 8 and 7 for CTR, CTR+LY, HI, and HI+LY animals, respectively.

	Basal Period	**Experimental Period (min)**				
		60	**120**	**180**	**210**	**240**
Arterial Plasma Cortisol Level (ug/dl)						
CTR	3.4 ± 0.7	4.4 ± 0.5	4.6 ± 2.5	3.3 ± 1.3	4.1 ± 2.2	3.9 ± 1.4
CTR+LY	3.7 ± 0.6	4.8 ± 0.9	4.2 ± 1.3	2.0 ± 0.5	3.3 ± 0.2	5.0 ± 1.6
HI	2.8 ± 0.4	2.4 ± 0.4	2.2 ± 0.3	3.3 ± 1.2	3.3 ± 0.5	3.6 ± 0.5
HI+LY	2.5 ± 0.3	3.0 ± 0.5	3.9 ± 0.6	2.9 ± 0.4	3.0 ± 0.4	4.4 ± 0.4
Arterial Plasma Epinephrine Level (pg/mL))						
CTR	116 ± 21	136 ± 15	100 ± 15	134 ± 15	141 ± 22	131± 23
CTR+LY	119 ± 28	214 ± 62	177 ± 55	120 ± 35	120 ± 26	146 ± 33
HI	106 ± 15	109 ± 15	99 ± 17	115 ± 17	102 ± 15	124 ± 24
HI+LY	86 ± 28	189 ± 53	196 ± 83	162 ± 49	120 ± 43	157 ± 56
Arterial Plasma Norepinephrine (pg/mL)						
CTR	137 ± 31	166 ± 23	105 ± 27	155 ± 26	175 ± 78	121 ± 24
CTR+LY	120 ± 24	128 ± 19	128 ± 23	128 ± 23	141 ± 23	141 ± 22
HI	106 ± 15	109 ± 15	99 ± 17	115 ± 17	102 ± 15	123 ± 24
HI+LY	105 ± 21	140 ± 31	155 ± 42	172 ± 40	150 ± 35	174 ± 44

Supplementary Table 4. Arterial blood levels and net hepatic balance (mean ± S.E.M., n per group same as Supplementary Table 3) for lactate and gluconeogenic amino acids in the 42 h fasted conscious dog subjected to selective head hyperinsulinemia.

	Basal Period	**Experimental Period (min)**				
		60	**120**	**180**	**210**	**240**
Arterial Blood Lactate Concentration (μmol/L)						
CTR	404 ± 41	433 ± 45	337 ± 19	355 ± 27	357 ± 36	352 ± 43
CTR+LY	560 ± 106	600 ± 67	580 ± 76	666 ± 190	567 ± 115	552 ± 106
HI	424 ± 54	561 ± 67	555 ± 61	518 ± 64	545 ± 57	542 ± 70
HI+LY	484 ± 157	685 ± 154	604 ± 187	528 ± 139	518 ± 130	563 ± 174
Net Hepatic Lactate Uptake (μmoL/kg/min)						
CTR	4.5 ± 0.6	3.8 ± 1.1	3.9 ± 0.6	4.4 ± 0.5	5.1 ± 0.6	4.7 ± 0.7
CTR+LY	3.9 ± 0.4	3.1 ± 0.7	5.7 ± 0.9	4.8 ± 0.8	4.6 ± 0.4	4.8 ± 0.4
HI	4.8 ± 0.8	3.5 ± 1.9	3.5 ± 1.4	3.8 ± 1.1	3.7 ± 1.1	4.1 ± 1.2
HI+LY	4.0 ± 0.6	2.7 ± 0.8	3.7 ± 0.8	4.4 ± 1.1	4.2 ± 0.8	3.7 ± 0.7
Arterial Blood Gluoneogenic Amino Acid Concentration (μmol/L)						
CTR	1621 ± 62	1623 ± 31	1645 ± 62	1617 ± 59	1561 ± 40	1645 ± 65
CTR+LY	1743 ± 49	1746 ± 89	1771 ± 68	1823 ± 104	1753 ± 90	1744 ± 89
HI	1850 ± 22	1799 ± 127	1728 ± 131	1653 ± 121	1631 ± 108	1539 ± 145
HI+LY	1800 ± 62	1802 ± 77	1741 ± 106	1668 ± 132	1648 ± 104	1657 ± 80
Net Hepatic Gluconeogenic Amino Acid Uptake (μmoL/kg/min)						
CTR	2.3 ± 0.7	3.7 ± 0.8	5.9 ± 0.7	2.3 ± 1.1	2.1 ± 1.4	3.0 ± 1.0
CTR+LY	3.1 ± 0.6	3.7 ± 0.7	3.1 ± 1.4	3.3 ± 1.0	4.7 ± 1.2	3.7 ± 0.9
HI	3.1 ± 0.6	3.0 ± 0.8	2.2 ± 0.5	3.0 ± 0.9	2.9 ± 0.6	3.6 ± 0.9
HI+LY	3.7 ± 0.7	3.7 ± 0.7	2.7 ± 0.4	2.0 ± 0.7	1.8 ± 0.7	1.8 ± 0.7

Supplementary Table 5. Metabolic data comparing Head insulin infused group (HI) to head insulin infused animals previously subjected to hepatic denervation (HI+DN). †P<0.05 difference versus basal period; *P<0.05 difference between HI and HI+DN animals.

	HI group (n=8)		**HI+DN group (n=4)**	
	BASAL (-90 to 0 min)	CLAMP (180-240 min)	BASAL (-90 to 0 min)	CLAMP (180-240 min)
Arterial Plasma Glucose (mg/dL)	111 ± 2	110 ± 2	104 ± 6	106 ± 5
Jugular Vein Plasma Insulin (uU/mL)	3.8 ± 0.5	26.1 ± 3.3†	2.9 ± 0.3	28.1 ± 1.9†
Hepatic Vein Plasma Insulin (uU/mL)	6.2 ± 0.7	7.0 ± 1.0	4.3 ± 1.0	4.4 ± 0.6
Portal Vein Plasma Glucagon (ng/mL)	55 ± 4	56 ± 5	43 ± 3	44 ± 7
Arterial Plasma NEFA (umol/kg/min)	842 ± 97	716 ± 56	682 ± 115	593 ± 133
Net Hepatic Glucose Output (mg/kg/min)	1.60 ± 0.13	0.97 ± 0.29†	1.35 ± 0.18	0.71 ± 0.14†

Supplementary Table 6. Molecular signaling data comparing Head insulin infused group (HI) to head insulin infused animals previously subjected to hepatic denervation (HI+DN). Data are means of averages from liver lobe 2, 3, and 7 and expressed relative to measurements in CTR group (basal insulin, basal glucagon, ICV infusion of aCSF). *P<0.05 difference CTR group; #P<0.05 difference between HI and HI+DN animals.

	CTR (n=4)	**HI (n=8)**	**HI+DN (n=4)**
Hepatic P-Akt/Total Akt	1.00 ± 0.09	1.07 ± 0.15	1.05 ± 0.16
Hypothalamic P-Akt/Total Akt	1.00 ± 0.08	2.81 ± 0.45*	2.53 ± 0.21*
Hepatic P-STAT3/Total STAT3 protein	1.00 ± 0.04	1.71 ± 0.15*	1.33 ± 0.08*#
Hepatic PEPCK mRNA expression	1.00 ± 0.09	0.46± 0.03*	0.73 ± 0.02*#
Hepatic PC mRNA expression	1.00 ± 0.08	0.52 ± 0.04*	1.26 ± 0.14#
Hepatic G6Pase mRNA expression	1.00 ± 0.13	0.44 ± 0.02*	0.51 ± 0.06*
Hepatic GK mRNA expression	1.00 ± 0.06	2.85 ± 0.20*	1.78 ± 0.11*#
Hepatic GSK3β mRNA expression	1.00 ± 0.09	0.59 ± 0.05*	0.87 ± 0.13
Hepatic GSK3β protein expression	1.00 ± 0.12	0.75 ± 0.07	0.82 ± 0.13
Hepatic P-GS/Total GS protein	1.00 ± 0.04	0.80 ± 0.07*	0.80 ± 0.15

Supplemental Table 7. Nucleotide sequences of dog-specific primers for Real-Time PCR.

Gene	Primer	Sequence (5'-3')	Temp. (^{o}C)
PEPCK	Forward Reverse	AGCTTTCAATGCCCGATTTCCAGG TCAGCTCGATGCCGATCTTTGACA	57
G6Pase	Forward Reverse	TGAAACTTTCAGCCACATCCG GCAGGTAAAATCCAAGTGCGAA	56
Pyruvate Carboxylase	Forward Reverse	AATTCTCTAACACCTATGGCTTCC GTGGCGTGGCTTCTCAATG	56
FOXO1	Forward Reverse	CTACGAGTGGATGGTCAAGAG CACGAATGAACTTGCTATGTAGG	55
PGC1α	Forward Reverse	GCTTTCTGGGTGGACTCAAGTG GCAAGTTCGCCTCGTTCTCTTC	59
SRC1	Forward Reverse	CAATGACCAAACCTGCTCCTGAAG GACCGCACCATCCATCCTCTC	60
SRC2	Forward Reverse	CACCTGTGCTGCTGCTAC GCTTGTTGTTGGCTATACTGAG	55
GK	Forward Reverse	CAGAGGGGACTTTGAAATG ATGAATCCTTACCCACAATC	57
HPRT	Forward Reverse	AGCTTGCTGGTGAAAAGGAC TTATAGTCAAGGGCATATCC	55
FOXO1	Forward Reverse	TCGCAGACTATGAAGCCTATG CCTTAATTGTTCGGTCACTAGAG	55
Glycogen Synthase	Forward Reverse	CTCAGGTGGAACAGTGTGAAC CCAAGCCGAATAGCCTATGTC	56
Glycogen phosphorylase	Forward Reverse	TCGCAGACTATGAAGCCTATG CCTTAATTGTTCGGTCACTAGAG	55
Glucokinase	Forward Reverse	CAGAGGGGACTTTGAAATG ATGAATCCTTACCCACAATC	57

GSK3β	Forward Reverse	CCACTGTAACATAGTCCGATTGC TCCAGCACCAGATTAAGATAGACC	57
HPRT	Forward Reverse	AGCTTGCTGGTGAAAAGGAC TTATAGTCAAGGGCATATCC	55