Supplemental Information

Supplementary Figure 1

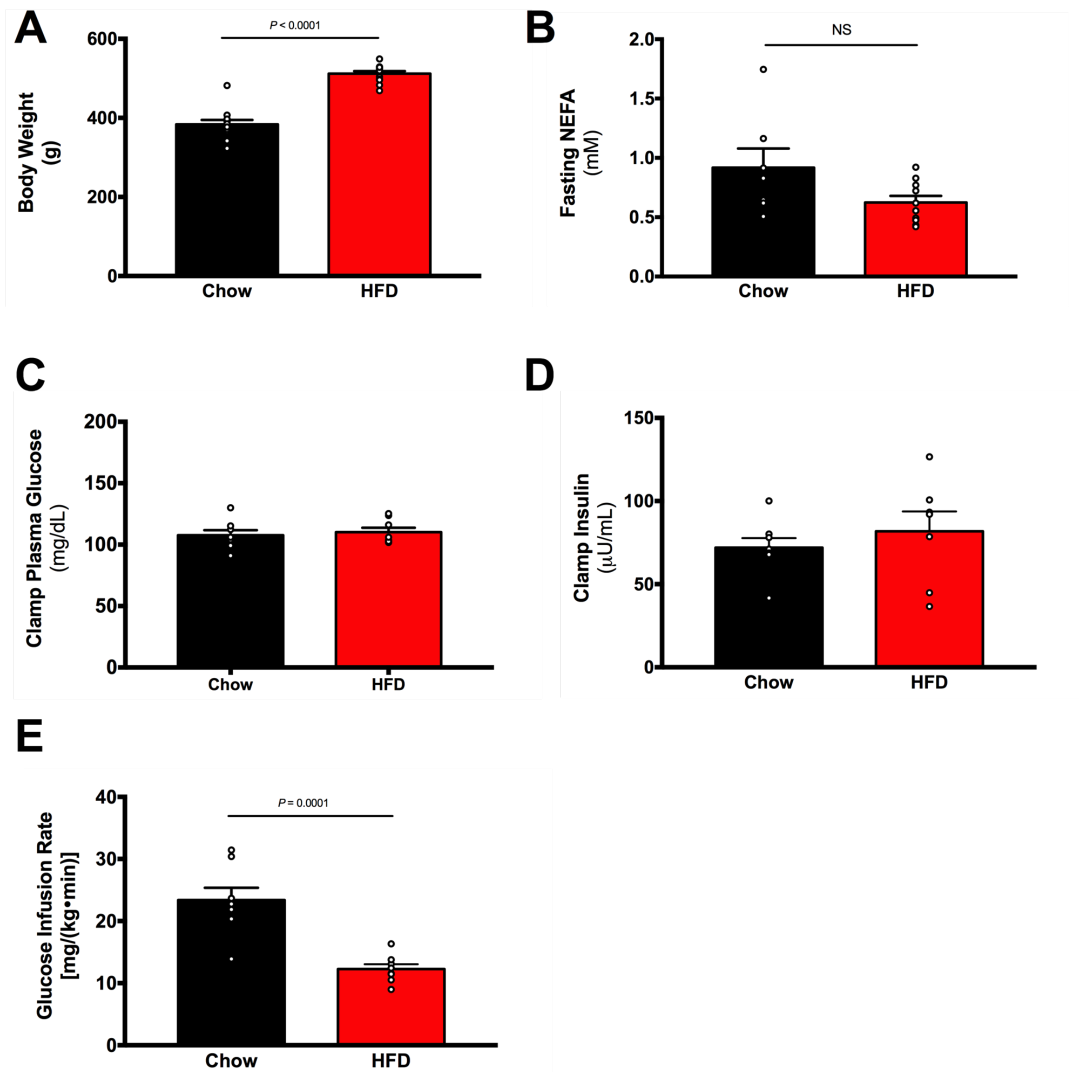


Figure S1. Related to Figure 1

(A) HFD increases body weight in rats compared to regular chow

(B) Fasting NEFA levels are unchanged with HFD

(C) Plasma glucose at the end of the hyperinsulinemic euglycemic clamp is similar between chow and HFD rats

(D) Insulin levels at the end of the clamp is similar between chow and HFD rats

(E) Glucose infusion rate needed to maintain euglycemia during the hyperinsulinemic clamp is lower in HFD rats

In all panels, data are the mean±SEM of *n* = 7-9 per group

Supplementary Figure 2

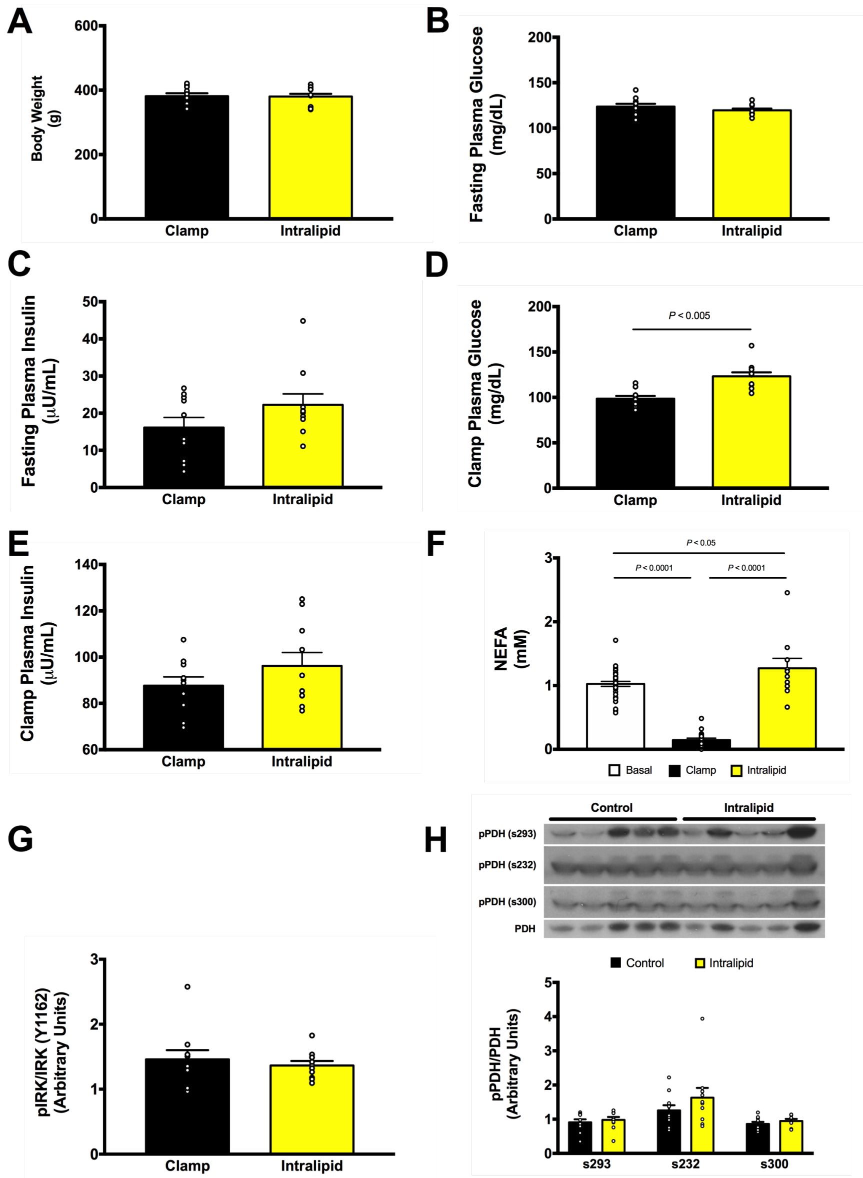


Figure S2. Related to Figure 3

(A) Rats in both the control and acute lipid infusion groups were fed regular chow and similar in body weight

(B) Fasting plasma glucose levels were similar between control and lipid infusion groups

(C) Fasting plasma insulin levels were similar between control and lipid infusion groups

(D) Plasma glucose levels at the end of the hyperinsulinemic euglycemic clamp was slightly but significantly higher in the lipid infusion, but still within the range of euglycemia

(E) Plasma insulin levels at the end of the clamp were comparable between the groups

(F) Insulin suppresses plasma NEFA levels, but co-infusion of lipid during the clamp increases NEFA back to approximately fasting levels.

(G) Phosphorylation of IRK is unchanged between the groups after the clamp

(H) Phosphorylation of PDH (3 subtypes) are unchanged after the clamp

In all panels, data are the mean±SEM of *n* = 7-9 per group

Supplementary Figure 3

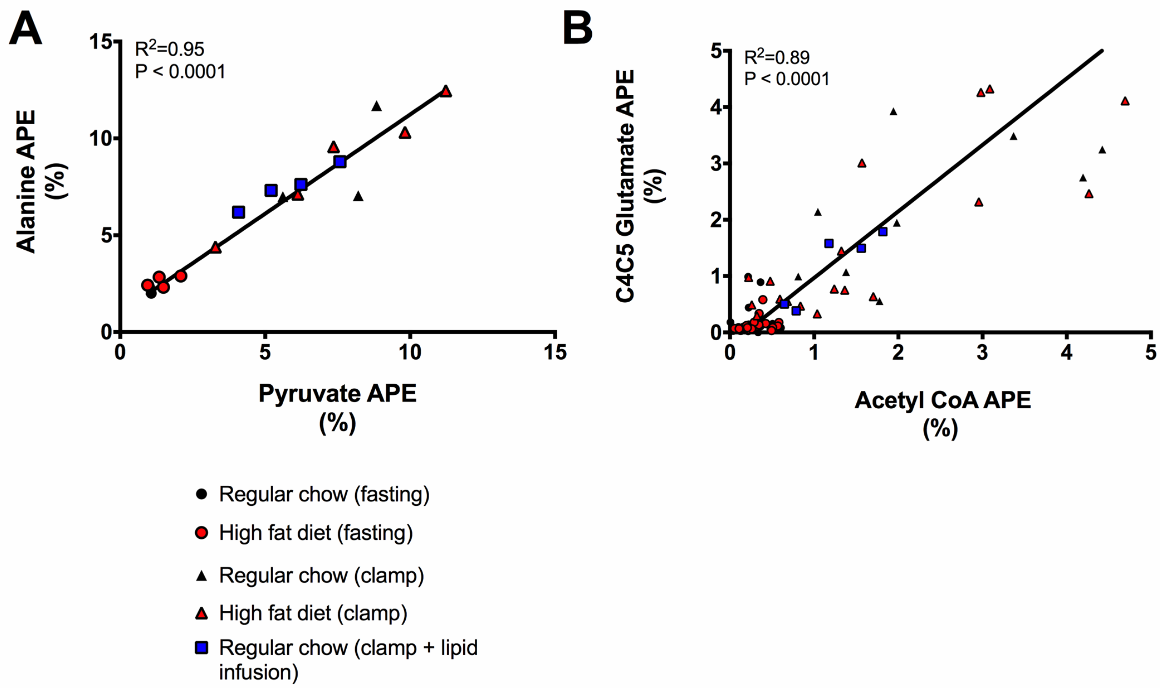


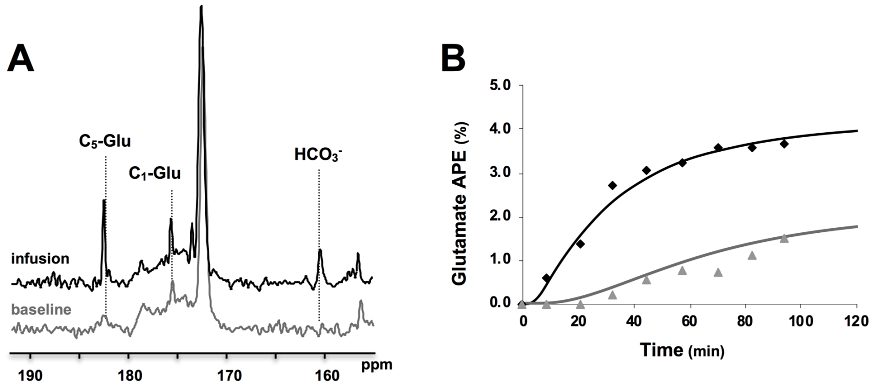
Figure S3. Related to Figure 1 and 3

(A) Correlation between pyruvate [m+3] and alanine [m+3] ^{13}C enrichments in muscle after steady state infusion of [$^{13}C_6$] glucose [m+6] in a variety of experimental conditions

(B) Correlation between C4C5 glutamate [m+2] and acetyl CoA [m+2] ^{13}C enrichments in muscle after [$^{13}C_6$] glucose [m+6] infusion

In each panel, the best fit line is shown with each group containing 3-8 data points

Supplementary Figure 4



C

Isotopic and mass balance differential equations for metabolic modeling analysis of the TCA cycle in muscle

$$\frac{dC_1AcCoA}{dt} = V_{DIL}\left(\frac{\text{unlabeled FA + Pyr}}{\text{FA + Pyr}}\right) + V_{AC}\left(\frac{C_1Acetate}{Acetate}\right) - V_{CS}\left(\frac{C_1AcCoA}{AcCoA}\right)$$

$$\frac{dC_5Citate}{dt} = V_{CS}\left(\frac{C_1AcCoA}{AcCoA}\right) - V_{CS}\left(\frac{C_5Citate}{Citrate}\right)$$

$$\frac{dC_5\alpha KG}{dt} = V_{CS}\left(\frac{C_5Citate}{Citrate}\right) + V_X\left(\frac{C_5Glutamate}{Glutamate}\right) - [V_{CS} + V_X]\left(\frac{C_5\alpha KG}{\alpha KG}\right)$$

$$\frac{dC_5Glutamate}{dt} = V_X\left(\frac{C_5\alpha KG}{\alpha KG}\right) - V_X\left(\frac{C_5Glutamate}{Glutamate}\right)$$

$$\frac{dC_4Malate}{dt} = \frac{V_{CS}}{2}\left(\frac{C_5\alpha KG}{\alpha KG}\right) + \frac{V_{CS}}{2}\left(\frac{C_2\alpha KG}{\alpha KG}\right) - V_{CS}\left(\frac{C_4Malate}{Malate}\right)$$

$$\frac{dC_1Malate}{dt} = \frac{V_{CS}}{2}\left(\frac{C_5\alpha KG}{\alpha KG}\right) + \frac{V_{CS}}{2}\left(\frac{C_2\alpha KG}{\alpha KG}\right) - V_{CS}\left(\frac{C_1Malate}{Malate}\right)$$

$$\frac{dC_4OAA}{dt} = V_{CS}\left(\frac{C_4Malate}{Malate}\right) - V_{CS}\left(\frac{C_4OAA}{OAA}\right)$$

$$\frac{dC_1Citate}{dt} = V_{CS}\left(\frac{C_4OAA}{OAA}\right) - V_{CS}\left(\frac{C_1Citate}{Citrate}\right)$$

$$\frac{dC_1\alpha KG}{dt} = V_{CS}\left(\frac{C_1Citate}{Citrate}\right) + V_X\left(\frac{C_1Glutamate}{Glutamate}\right) - [V_{CS} + V_X]\left(\frac{C_1\alpha KG}{\alpha KG}\right)$$

$$\frac{dC_1Glutamate}{dt} = V_X\left(\frac{C_1\alpha KG}{\alpha KG}\right) - V_X\left(\frac{C_1Glutamate}{Glutamate}\right)$$

$$V_{CS} = V_{DIL} + V_{AC}$$

Figure S4. Related to Figure 4

(A) Representative ^{13}C-MRS peak for positional glutamate enrichment in human muscle with infusion of [1-^{13}C]glucose

(B) Time course of glutamate enrichment with infusion of tracer

(C) Isotopic and mass balance differential equations for metabolic modeling analysis of the TCA cycle in muscle

Supplemental Table S1

	Basal	**Clamp**
Normal chow-fed	83±6 (nmol/g)	81±4 (nmol/g)
HFD-fed	79±6 (nmol/g)	72±8 (nmol/g)

Table S1. Related to Figure 2
Muscle acylcarnitines in normal chow fed rats and insulin resistant high fat diet (HFD)-fed rats. Data are mean±SEM with n = 8 per group.

Supplemental Table 2

	Insulin Sensitive (n=9)	Insulin Resistant (n=9)	*p-value*
Gender	(3f/6m)	(6f/3m)	
Age (Years)	27±2	22±1	*NS*
Height (m)	1.73±0.04	1.71±0.04	*NS*
Body Weight (Kg)	70.8±3.9	71.3±3.8	*NS*
BMI (Kg/m^2)	23.5±0.8	24.4±0.7	*NS*
Insulin Sensitivity Index (dL/min per microunit/mL)	7.71±0.80	1.17±0.16	*0.0001*

7T MRS Study	Insulin Sensitive (n=6)	Insulin Resistant (n=8)	*p-value*
Gender	(2f/4m)	(4f/4m)	
Age (Years)	36±3	27±2	*<0.05*
Height (m)	1.78±0.05	1.71±0.04	*NS*
Body Weight (Kg)	71.7±5.7	68.0±5.2	*NS*
BMI (Kg/m^2)	22.5±1.2	22.7±0.7	*NS*
Insulin Sensitivity Index (dL/min per microunit/mL)	6.24±0.67	3.90±0.50	*0.016*

Table S2. Related to Figure 4
Baseline characteristics of human subjects