

SUPPLEMENTARY DATA

Supplementary Table 1. Characteristics of Male C57BL/6J Mice Fed a High Fat Diet. Mice were fed either a low fat (control diet) or a high (60%kcal) fat diet for 12 weeks starting at 6 weeks of age. Following an overnight fast, mice were weighed and blood samples taken to assess glucose and insulin levels, and calculate the homeostatic model assessment of insulin resistance (HOMA-IR). Mice were randomly assigned to one of three experimental groups as follows: 1) Ex vivo muscle glucose uptake; 2) In vivo muscle glucose uptake; or 3) Skeletal muscle intracellular signaling. Statistical significance was defined as $P < 0.05$ and denoted by ‘*’ vs Control Diet within an experimental group.

Experimental Group:	Ex Vivo Muscle Glucose Uptake		In Vivo Muscle Glucose Uptake			Skeletal Muscle Intracellular Signaling	
	Control Diet (N=20)	High Fat Diet (N=21)	Control Diet (N=8)	High Diet (N=8)	Fat	Control Diet (N=10)	High Fat Diet (N=9)
Diet (Number of Mice)							
Age (weeks)	18 ± 0	18 ± 0	18 ± 0	18 ± 0		18 ± 0	18 ± 0
Body Weight (g)	26.1 ± 0.3	34.1 ± 0.6*	25.6 ± 0.4	36.5 ± 1.9*		27.0 ± 0.6	35.0 ± 1.9*
Fasted Blood Glucose (mM)	6.7 ± 0.4	9.4 ± 0.6*	5.6 ± 0.3	9.4 ± 1.0*		7.7 ± 0.5	10.3 ± 0.6*
Fasted Serum Insulin (pM)	61.1 ± 3.8	116.7 ± 13.0*	56.5 ± 6.9	106.3 ± 28.2		35.2 ± 5.0	148.0 ± 52.7*
HOMA-IR (mM x mU/L)	2.5 ± 0.1	6.6 ± 0.7*	2.0 ± 0.2	6.5 ± 1.9*		1.7 ± 0.2	9.8 ± 3.6*

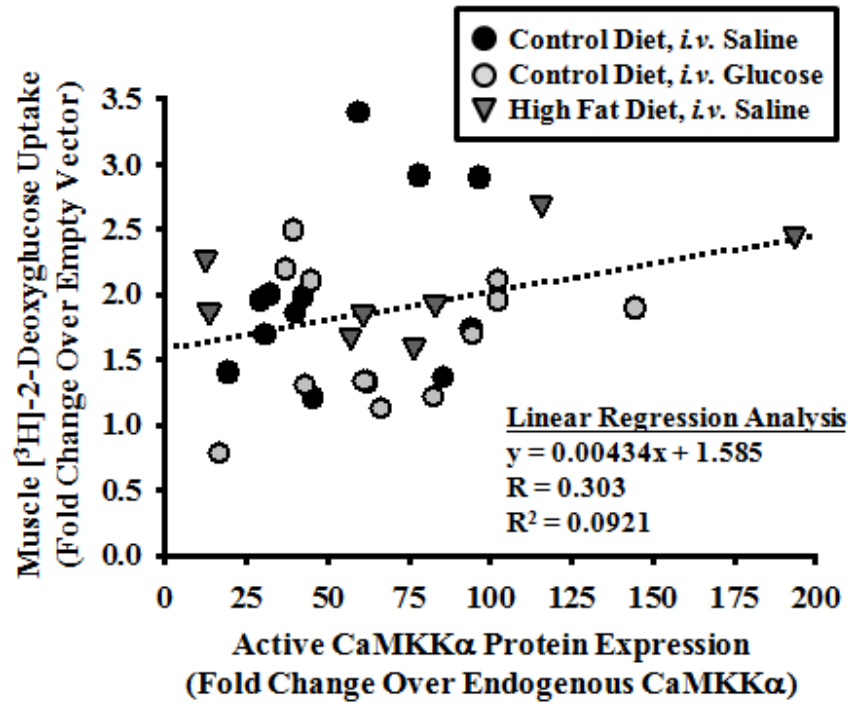
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Supplementary Table 2. Characteristics of Mice Pre- and 2 Weeks Post-Muscle Transfection. Mice were fed either a low fat (14%kcal, control diet) or a high (60%kcal) fat diet for 12 weeks starting at 6 weeks of age. Following an overnight fast, mice were weighed and blood samples taken to assess glucose and insulin levels, and calculate the homeostatic model assessment of insulin resistance (HOMA-IR). Mice were anesthetized and the tibialis anterior muscle of one leg was transfected with plasmid DNA containing the sequence for constitutively active CaMKK α while the contralateral muscle was transfected with empty vector DNA. Two weeks post-transfection, mice were fasted overnight, weighed and blood samples taken to assess glucose and insulin levels, and HOMA-IR. Statistical significance was defined as P<0.05 and denoted by ‘*’ vs Control Diet, and ‘#’ vs Pre-Transfection.

Treatment Group (Number of Mice)	Pre-Transfection		2 Weeks Post-Transfection	
	Control Diet (N=18)	High Fat Diet (N=17)	Control Diet (N=18)	High Fat Diet (N=17)
Age (weeks)	18 \pm 0	18 \pm 0	20 \pm 0	20 \pm 0
Body Weight (g)	26.3 \pm 0.4	35.7 \pm 1.3 *	27.0 \pm 0.4	40.4 \pm 1.5 *#
Fasted Blood Glucose (mM)	6.8 \pm 0.4	9.9 \pm 0.5*	7.9 \pm 0.4 #	9.8 \pm 0.3 *
Fasted Serum Insulin (pM)	43.1 \pm 5.1	128.4 \pm 30.4 *	91.6 \pm 8.0	336.8 \pm 50.6 *#
HOMA-IR (mM x mU/L)	1.7 \pm 0.2	8.3 \pm 2.1 *	4.7 \pm 0.4	22.1 \pm 3.7 *#

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Supplementary Figure 1. Relationship Between Expression of Constitutively Active CaMKK α and Muscle Glucose Uptake. Linear correlation analysis between muscles expressing active CaMKK α and muscle glucose uptake. Data taken from new Figures 1 and 3.



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Supplementary Figure 2. Representative Immunoblots for CaMKK α . (*Top*): Immunoblot with endogenous and constitutively active CaMKK α proteins shown at different exposure times. (*Bottom*): Immunoblot with endogenous and constitutively active CaMKK α proteins shown at the same exposure time. (Image taken from new Figures 2D.)

