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Supplemental Data

Article

Fast/Glycolytic Muscle Fiber Growth

Reduces Fat Mass and Improves

Metabolic Parameters in Obese Mice

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Supplemental Experimental Procedures

Measurement of Fatty Acid β-Oxidation in Skeletal Muscle

The rate of fatty acid β -oxidation in muscle was examined by the method of Muoio et al. (2002) and Watanabe et al (2000) with some modifications. Unfrozen skeletal muscle was homogenized in four volumes of 0.25 M sucrose containing 1 mM EDTA in a in a dounce tissue grinder (Wheaton). Approximately 500 µg of homogenate was incubated with the assay medium in 0.2 ml of 150 mM potassium chloride, 10 mM HEPES, pH 7.2, 0.1 mM EDTA, 1 mM potassium phosphate buffer, pH 7.2, 5 mM Tris malonate, 10 mM magnesium chloride, 1 mM carnitine, 0.15% bovine serum albumin, 5 mM ATP, and 50 µM palmitic acid containing 1 µCi of [9, 10 (n)-H3] palmitic acid. The reaction was run for 30 min at 25 °C and stopped by the addition of 0.2 ml of 0.6 N perchloric acid. The mixture was centrifuged at 2,000 × g for 10 min, and the unreacted fatty acid in the supernatant was removed with three extractions of 2 ml of *n*-hexane. Radioactive degradation products in the water phase were counted.

Supplemental References

Muoio, D. M., MacLean, P. S., Lang, D. B., Li, S., Houmard, J. A., Way, J. M., Winegar, D. A., Corton, J. C., Dohm, G. L., and Kraus, W. E. (2002). Fatty acid homeostasis and induction of lipid regulatory genes in skeletal muscles of peroxisome proliferator-activated receptor (PPAR) alpha knock-out mice. Evidence for compensatory regulation by PPAR delta. J Biol Chem 277, 26089-26097.

Watanabe, K., Fujii, H., Takahashi, T., Kodama, M., Aizawa, Y., Ohta, Y., Ono, T., Hasegawa, G., Naito, M., Nakajima, T., *et al.* (2000). Constitutive regulation of cardiac fatty acid metabolism through peroxisome proliferator-activated receptor alpha associated with age-dependent cardiac toxicity. J Biol Chem *275*, 22293-22299.

	Primer Sequence	
Gene	Forward	Reverse
PGC-1a	5'-AAAACAGGAACAGCAGCAGAGAC-3'	5'-GGGGTCAGAGGAAGAGATAAAGTTG-3'
PPARa	5'-TTGCTGTGGAGATCGGCCT-3'	5'-GGATGGTTGCTCTGCAGGTG-3'
PPARδ	5'-TCACCGGCAAGTCCAGCCA-3'	5'-ACACCAGGCCCTTCTCTGCCT-3'
Hk2	5'-TGTCCCTACCTTTGGGTTTC-3'	5'-ATGCCTTGAATCCCTTTGTC-3'
Pfk	5'-GGCCAATCCTCAAAATCCTA-3'	5'-CCAGACCGTTTCCTTGAAAT-3'
LDHA	5'-TGTGTGGAGTGGTGTGAATG-3'	5'-ACCTGCTTGTGAACCTCCTT-3'
PEPCK	5'-GCATAACTAACCCCGAAGGCAAG-3'	5'-CATCCAGGCAATGTCATCGC-3'
G6Pase	5'-CAGAATGGGTCCACCTTGACAC-3'	5'-AGCGGAATGGGAGCAACTTG-3'
HNF4a	5'-CCTGCAGGTTTAGCCGACAAT-3'	5'-ATCCGGTCCCGCTCATTTT-3'
CPT1	5'-ATGGCAGAGGCTCACCAAGC-3'	5'-GATGAACTTCTTCTTCCAGGAGTGC-3'
SCD1	5'-CAAGCTGGAGTACGTCTGGA-3'	5'-CAGAGCGCTGGTCATGTAGT-3'
36B4	5'-GCTCCAAGCAGATGCAGCA-3'	5'-CCGGATGTGAGGCAGCAG-3'

Table S1. Primer Sequences Used for Quantitative Real-Time PCR

Figure Legends

Figure S1. Generation of Skeletal Muscle-Specific Conditional Akt1 Transgenic Mice

(A) Schematic illustration of binary TG system. The rtTA protein is expressed from the 1256[3Emut] promoter fragment of the MCK promoter. Constitutively-active Akt1 (myrAkt1) is under the control of multiple copies of the tetracycline regulatory element (TRE). When DOX is administered via the drinking water, the *myrAkt1* transgene is expressed.

(B) DOX-dependent expression of *Akt1* transgene. Top: Schematic of DOX-treatment time course. The transgene is induced between 8 and 10 weeks of age by administering DOX. Bottom: Western blot analysis of transgene expression in gastrocnemius muscle. An increase in Akt phosphorylation is only seen in DTG mice following DOX administration. Induction of the transgene is also indicated by the inducible expression of the HA tag on the *myrAkt1* transgene.

(C) Western blot analysis of transgene expression in different tissues after 2 weeks of induction by DOX.

(D) Western blot analysis of transgene expression in different muscle groups after 2 weeks of induction. MCK-rtTA single transgenic mice were used as a control. Representative blots of 3 control and 3 DTG animals are shown. HA, hemagglutinin epitope on transgene, EDL, extensor digitorum longus; DOX, doxycycline; Cont, control; DTG, double transgenic.

Figure S2. Akt2 Is Not Regulated in Akt1 Transgenic Mice

(A) Western blot analysis of Akt2 protein expression in control or DTG mice 2 weeks after DOX treatment.

(B) The activating phosphorylation of Akt2 was examined by immunoprecipitation (by Akt2 antibody) and Western blot analysis (by phosphor-Akt antibody). MCK-rtTA single transgenic mice were used as a control.

Figure S3. Body Composition Changes Caused by Akt1-Mediated Muscle Growth Are Reversible and Blocked by Rapamycin

(A) Body weight of normal or HF/HS diet-fed control and DTG mice. At 8 weeks of age mice were treated with DOX in their drinking water for 5 weeks followed by 5 weeks of regular water (n = 6-9 in each group). *p < 0.05 vs. HF/HS diet-fed control mice.

(B) Gastrocnemius muscle, inguinal and subcutaneous fat pad weight in control and DTG mice 5 weeks after the withdrawal of DOX (n = 6-9 in each group).

(C) Western blot analysis of gastrocnemius muscle lysates from control or DTG mice administered vehicle or rapamycin during the 6 week time course of DOX-treatment. Tissue was analyzed for transgene expression, and phosphorylation of Akt and S6K.

(D) Gastrocnemius muscle, inguinal and subcutaneous fat pad weight in control and DTG mice after 6 weeks of DOX and rapamycin treatment (n = 6 in each group). MCK-rtTA single transgenic mice were used as a control. Results are presented as mean \pm SEM. Cont, control; DTG, double transgenic; Normal, normal diet; HF/HS, high fat/sucrose diet; DOX, doxycycline; HA, hemagglutinin tag on transgene.

Figure S4. Serum Free Fatty Acid, Triglyceride, and Glycerol Levels

Serum free fatty acid, triglyceride and glycerol levels in normal or HF/HS diet-fed control and DTG mice at 6 weeks after DOX-treatment (n = 7-12 in each group). MCK-rtTA single transgenic mice were used as a control. Results are presented as mean \pm SEM. Cont, control; DTG, double transgenic; Normal, normal diet; HF/HS, high fat/sucrose diet.

Figure S5. Glucose Uptake into Nontarget Tissues

Control and DTG mice fed HF/HS diet were injected intraperitoneally with D-glucose (2 g/kg of body weight) containing ³H-2-deoxyglucose. After 2 hours, tissues were processed and assayed for radioactive tracer uptake (n = 6 in each group). MCK-rtTA single transgenic mice were used as a control. The transgene is not expressed in soleus or EDL muscle. Results are presented as mean \pm SEM. *p < 0.05. Cont, control; DTG, double transgenic.

Figure S6.

(A) Relative mRNA expression level of interleukin-6 (IL-6) as measured by qRT-PCR in gastrocnemius muscle of HF/HS diet-fed control and DTG mice at the 3 week time point after DOX treatment.

(B) Serum IL-6 level in normal or HF/HS diet-fed control and DTG mice at 6 weeks after DOXtreatment (n = 5-7 in each group). MCK-rtTA single transgenic mice were used as a control. Results are presented as mean \pm SEM. *p < 0.05. Cont, control; DTG, double transgenic; Normal, normal diet; HF/HS, high fat/sucrose diet.

Figure S7.

Representative western blot analysis of PGC-1 α , PPAR α and PPAR δ protein expression in gastrocnemius muscle of HF/HS diet-fed control and DTG mice at the 3 week time point after DOX treatment. MCK-rtTA single transgenic mice were used as a control. Cont, control; DTG, double transgenic.



В

Α



С



D





Α

Cont DTG





В

Α



С



D















<u>Heart</u>

Figure S7

