TRPV1 activation improves exercise endurance and energy metabolism through PGC-1 upregulation in mice

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Impaired aerobic exercise capacity and skeletal muscle dysfunction are associated with cardiometabolic diseases. Acute administration of capsaicin enhances exercise endurance in rodents, but the long-term effect of dietary capsaicin is unknown. The capsaicin receptor, the transient receptor potential vanilloid 1 (TRPV1) cation channel has been detected in skeletal muscle, the role of which remains unclear. Here we report the function of TRPV1 in cultured C2C12 myocytes and the effect of TRPV1 activation by dietary capsaicin on energy metabolism and exercise endurance of skeletal muscles in mice. *In vitro*, capsaicin increased cytosolic free calcium and peroxisome proliferator-acti- - P - a d- e e c t o d a p t 2 1 t 2 r) (i f G d P o p s s m P r n C 6 e h t g h b - a i t a *v i r o,* i V 1 i k l e m a t c s P l e a s g l s a d c p i a c i a r b c d s p d R n P i T i V t o i g c e n œ i r e 1 o s r in mice. TRPV1 activation increased the expression of genes involved in fatty acid oxidation and mitochondrial res- i, i a t o m p o i m h c p , t h o d i a l i g u e c b r s i s d i n d a s t e r e d h a b s d i c e n e m e d c a s a n d n e e - i h g h i a , d e t d f m l i n a t i l o d d b o m s s t l P h t e n d c r c s i - i c f f a f s p e a i n l e t R s b r i d h e V mice. We conclude that TRPV1 activation by dietary capsaicin improves energy metabolism and exercise endurance - i g l a t g p i n k G e a t c s l e h s e s T e t i e p l s t n d s a t e a n d e h t c i a n c e t t a p t g n r o i a a g metabolic diseases and improving exercise endurance.

***Keywords*:** TRPV1; exercise endurance; PGC-1α; skeletal muscle; energy metabolism

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Introduction

influx

into different fiber types in response to exercise training. The conversion of muscle fiber from glycolytic type II

vated receptor-γ coactivator-1α (PGC-1α)

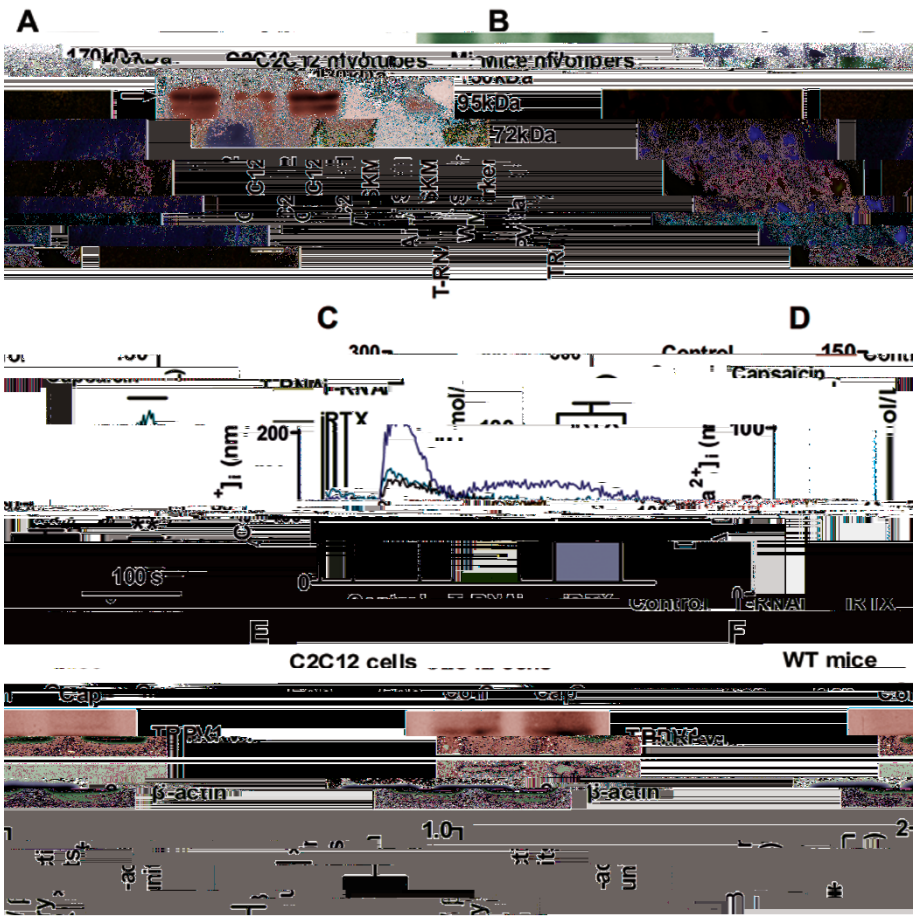

Figure 1 TRPV1 characterization in skeletal muscles. **(A)** Immunoblot of TRPV1 in skeletal muscle (SKM) from wild-type (WT) and TRPV1 knockout (TRPV1$^{-/-}$) mice and in C2C12 myotubes with or without TRPV1 RNAi (T-RNAi). The arrowhead indicates the band corresponding to TRPV1 protein. **(B)** TRPV1 localization in C2C12 myotubes and mice myofibers was shown with immunofluorescence. Bar = 50 m. **(C**, **D)** Representative curves **(C)** and summary data **(D)** showing capsaicin (100 nM)-induced $[Ca^{2+}]_i$ changes in cells with or without T-RNAi and cells pretreated with the specific TRPV1 inhibitor iRTX (1 M) for 5 min. ***P* < 0.01 vs control. **(E)** Immunoblot of TRPV1 in C2C12 myotubes with (Cap) or without (Con) capsaicin (100 nM) treatment for 24 h. **P* < 0.05 vs Con. **(F)** Immunoblot of TRPV1 in skeletal muscles of WT mice with (Cap) or without (Con) 4 months of capsaicin administration. **P* < 0.05 vs Con. Summary data are means ± S.E.M. for three to four independent experiments.

1α is a principal regulator of the expression of genes

showed that TRPV1 activation by capsaicin significantly

-dependent PGC-1α upregulation.

inhibited by PGC-1α activation

Results

, was significantly decreased by cap

TRPV1 characterization in skeletal muscles

%

(Figure 1A). Immunofluorescence staining showed that

blockade of TRPV1 by 5′-iodo-resiniferatoxin (iRTX)

% %

In

vitro

In vivo

in vitro

TRPV1 activation by capsaicin increases the PGC-1α ex

Activation of TRPV1 by dietary capsaicin upregulates PGC-1α and improves mitochondrial function and muscle remodeling

in vitro

of PGC-1α, CD36 and carnitine palmityl transferase 1,

TRPV1 activation increases PGC-1α expression and mitochondrial biogenesis in a Ca^{2+}-dependent manner

the characteristic protein for oxidative slow fibers, were

1α and its target genes . PGC-1α is a master

PGC-1α through TRPV1-mediated Ca

PGC-1α expression was elevated after 24-h capsaicin

muscle fiber types are partly determined by mitochondrial

showed that there were more Type I (oxidative) fibers and fewer Type II (glycolytic) fibers in capsaicin-treated mice

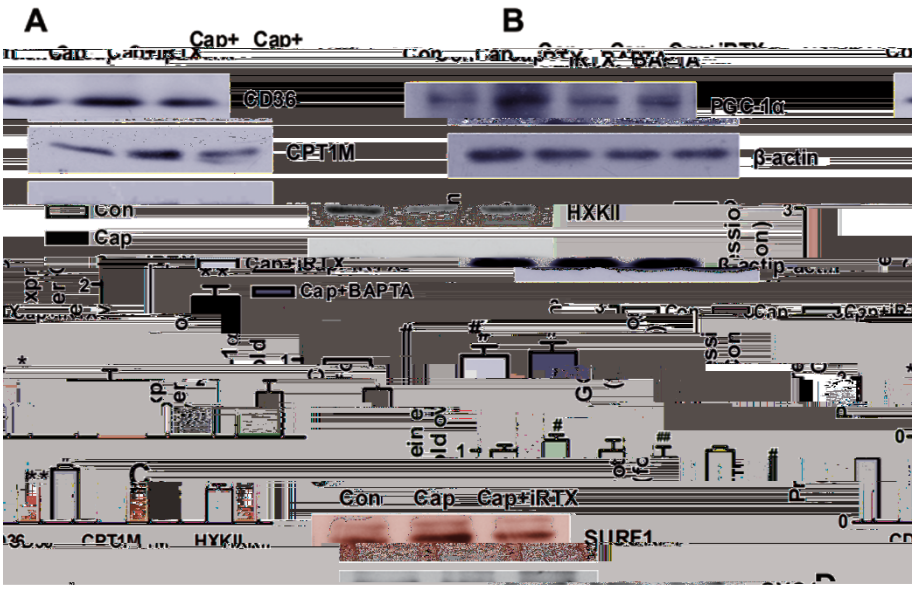

Figure 2 TRPV1 activation increases PGC-1α expression and mitochondrial biogenesis in a Ca^{2+}-dependent manner. **(A)** Immunoblot of PGC-1α in C2C12 myotubes treated with capsaicin (100 nM) in the presence or absence of the TRPV1 inhibitor iRTX (1 M) or the intracellular Ca^{2+} chelator BAPTA (10 M). **(B**, **C)** Protein expression of genes involved in fatty acid oxidation, glycolysis **(B)** and mitochondrial respiration **(C)** in myotubes treated with capsaicin (100 nM) in the presence or absence of iRTX (1 M). **(D**, **E)** Mitochondrial content **(D)** and ATP production **(E)** in myotubes. C2C12 cells were treated with capsaicin (100 nM) in the presence or absence of iRTX (1 M) or BAPTA (10 M) for 24 h. Data are means ± S.E.M. for three independent experiments. Con, control; Cap, capsaicin. **P* < 0.05, ***P* < 0.01 vs Con. $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01 vs Cap.

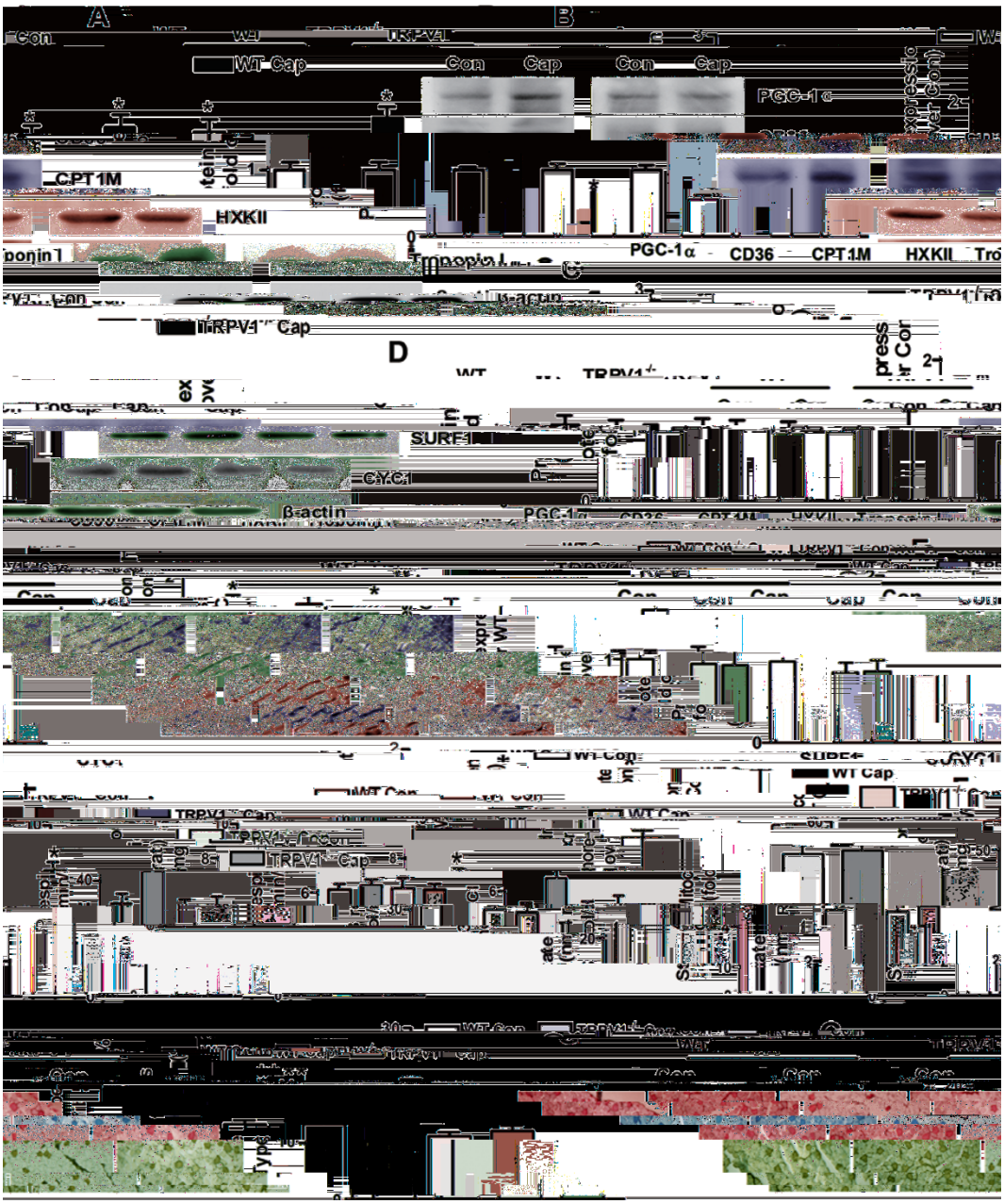

Figure 3 Activation of TRPV1 by dietary capsaicin up-regulates PGC-1α and improves mitochondrial function and muscle remodeling. **(A-D)** Protein expression of genes involved in fatty acid oxidation, glycolysis, fiber specification **(A-C)** and mitochondrial respiration **(D)** in skeletal muscles from WT and TRPV1$^{-/-}$ mice fed a regular diet (Con) or a capsaicin diet (Cap). **(E)** Mitochondrial mass in gastrocnemius muscle shown by transmission electron microscopy. **(F)** State 3 and state 4 respiration and respiratory control index (RCI) in mitochondria isolated from fresh quadriceps femoris muscles. **(G)** Percentage of type I fibers in gastrocnemius muscle shown by the metachromatic staining. Oxidative (Type I) fibers were stained dark blue. Data are means ± S.E.M. *n* = 3-8. **P* < 0.05, ***P* < 0.01 vs WT.

ference in fiber type percentages between TRPV1

TRPV1 with dietary capsaicin upregulates PGC-1α, pro

capacity, and consequently improves skeletal muscle fi

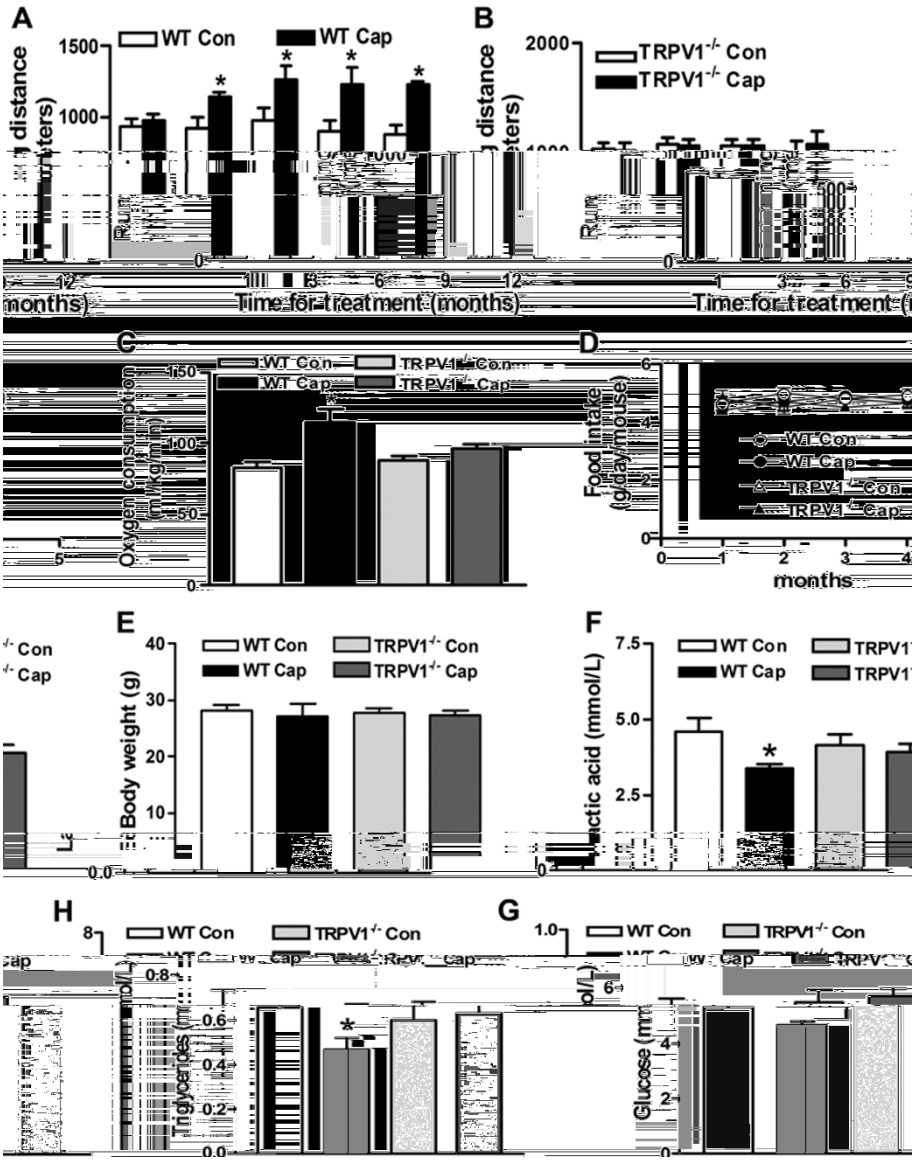

Figure 4 Dietary capsaicin enhances exercise endurance and reduces blood lactic acid and triglycerides through TRPV1 activation. **(A**, **B)** Exercise endurance test with treadmill exercise. WT **(A)** and TRPV1$^{-/-}$ mice **(B)** on a regular diet (Con) and a capsaicin diet (Cap) for indicated months were tested. Running distance before reaching exhaustion was recorded. Data are presented as the means ± S.E.M. for 6-10 mice. **P* < 0.05 vs Con. **(C)** Oxygen consumption (ml/kg/min) examined when mice ran for 30 min at a speed of 10 m/min. **(D)** Average daily food intake (g/d) per mouse determined during the last week of each month. **(E**-**H)** Body weight and blood levels of lactic acid, triglycerides and glucose. Mice were treated with control or capsaicin diet for 4 months. Data are means ± S.E.M. for 5-9 mice. **P* < 0.05 vs Con.

Dietary capsaicin enhances exercise endurance and reduces blood lactic acid and triglycerides through TRPV1 activation

. Capsaicin treatment significantly

Transgenic TRPV1 gene increases PGC-1α expression, oxidative fibers and exercise endurance

TRPV1

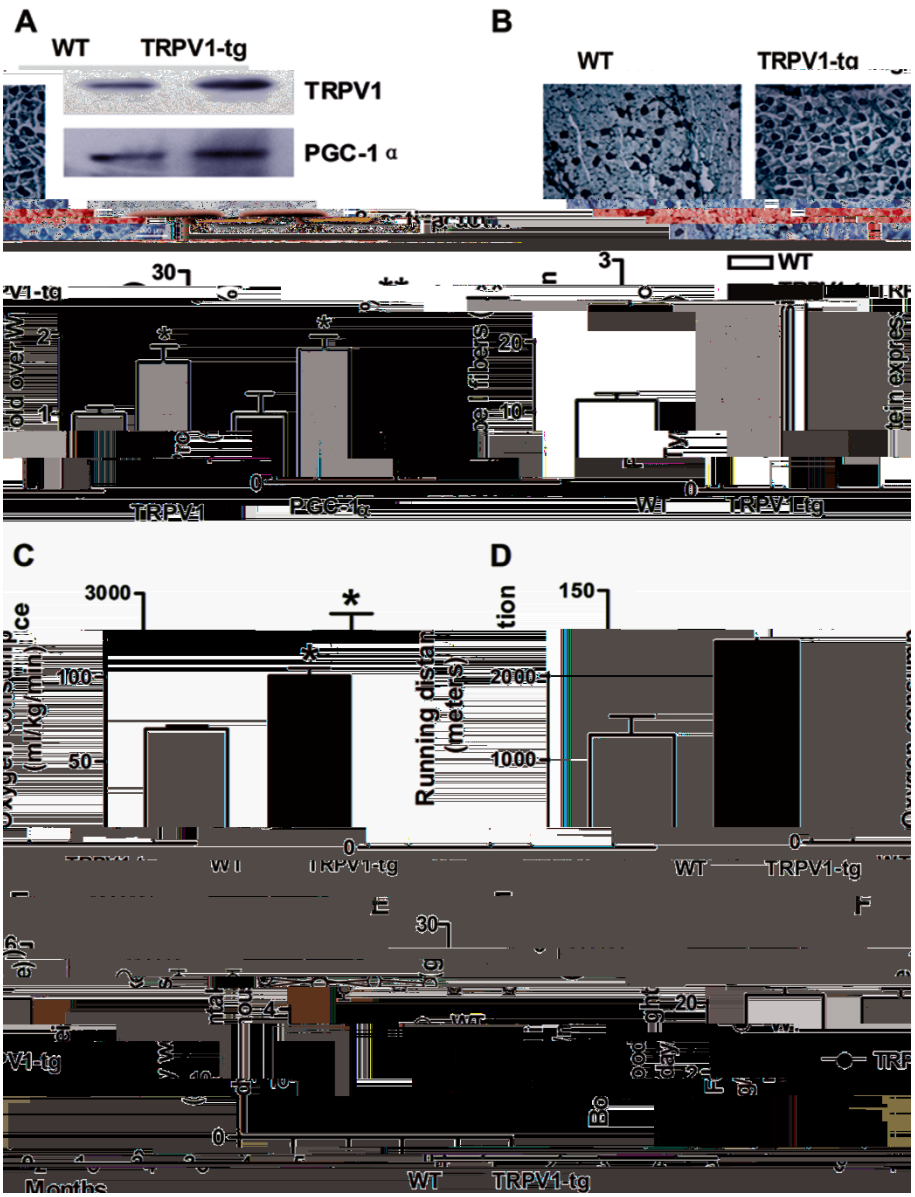

Figure 5 Transgenic *TRPV1* gene increases PGC-1α expression, oxidative fibers and exercise endurance. **(A)** Immunoblot of TRPV1 and PGC-1α in skeletal muscle from TRPV1-tg and their wild-type littermates (WT). **P* < 0.05, ***P* < 0.01 vs WT. **(B)** Metachromatic staining of gastrocnemius muscle. Type I fibers were stained dark blue. **(C)** Exercise endurance, **(D)** oxygen consumption, **(E)** body weight and **(F)** average daily food intake in TRPV1 transgenic mice and their WT littermates. **P* < 0.05, ***P* < 0.01 vs WT. Mice were fed a regular diet until they reached 6 months of age. Data are means ± S.E.M. for 3 mice.

α

TRPV1 and PGC-1α in skeletal muscles (Figure 5A).

Figure S2) and the percentage of oxidative type I fibers

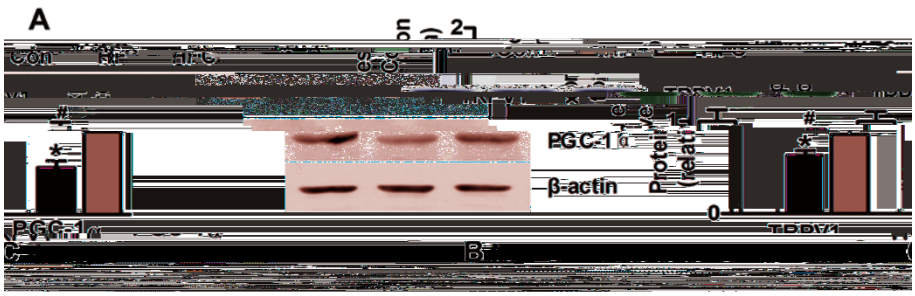

Figure 6 TRPV1 activation restores the HFD-induced PGC-1α downregulation, endurance impairment and metabolic disorders. **(A)** Immunoblot of TRPV1 and PGC-1α in skeletal muscle from WT mice on normal diet (Con) and HFD without (HF) or with capsaicin supplementation (HFC). **(B**-**E)** Running endurance **(B)**, body weight **(C)** and blood levels of triglycerides **(D)** and insulin **(E)** in mice. **(F)** Lipid contents of quadriceps femoris muscles shown by Oil-red O staining. **P* < 0.05, ***P* < 0.01 vs Con; #*P* < 0.05, ##*P* < 0.01 vs HF. **(G**, **H)** Intraperitoneal glucose tolerance test (IPGTT) and intraperitoneal insulin tolerance test (IPITT). **P* < 0.05, ***P* < 0.01 vs HF; #*P* < 0.05, ##*P* < 0.01 vs HFC. Data are means ± S.E.M. for three to six mice.

functional roles in the tissue-specific regulation of micro

first time that chronic TRPV1 activation induces PGC-1α expression and improves mitochondrial function in a

TRPV1 activation restores the high-fat diet (HFD)-induced PGC-1α downregulation, endurance impairment and metabolic disturbance

PGC-1α (Figure 6A), mitochondrial content (Supple

duced a significant increase of [Ca

fibers (Supplementary information, Figure S4) in skeletal

tivate the PGC-1α gene promoter and induce PGC-1α

sure resulted in a significant increase in PGC-1α expres

capsaicin-induced PGC-1α expression in myotubes.

capsaicin treatment significantly attenuated HFD-induced

TRPV1-mediated PGC-1α upregulation.

Discussion

in vitro

PGC-1α expression, mitochondrial biogenesis and ATP

tion of cardiometabolic diseases. Major findings of the

pression of TRPV1 and PGC-1α in skeletal muscles, and consequently upregulated PGC-1α target genes involved

through TRPV1-mediated PGC-1α upregulation in skel

tion from glycolytic type II fibers to more oxidative type I fibers. It is known that type I fibers contain more mi

but less from glycolysis compared to type II fibers, thus

oxidative fibers can lead to improved insulin action and

downregulation of PGC-1α and reduced percentage of type 1 fibers, which is consistent with previous studies . PGC-1α downregulation was supposed

PGC-1α results in increased mitochondrial function and oxidative muscle fibers and protects from metabolic dis

and glucose and lipid disorders. Both of the benefits of

ed PGC-1α upregulation in skeletal muscles.

in vitro *in vivo*

significantly increased TRPV1 expression (Supplementa

pendent upregulation of PGC-1α and its target genes in

novel findings also suggest that dietary capsaicin supple

Materials and Methods

Cell culture

%

C2C12 myoblasts were cultured for 48 h to reach confluence, and

%

becco's modified Eagle's medium, fetal bovine serum and horse

TRPV1 gene silencing

braries to ensure the specificity of the target. Two oligonucleotides

M547-569 construct, sense, 5′-TGACAGATAGCCTGAAGC AGTTCAAGAGACTGCTTCAGGCTATCTGTCTTTTTTC-3′, antisense, 5′-TCGAGAAAAAAGACAGATAGCCTGAAGCAGT CTCTTGAACTGCTTCAGGCTATCTGTCA-3′;

M1294-1316 construct, sense, 5′-

-3′, antisense, 5′-TCGAGAAAAAAGCGCATCTTCTACTTCAACTC TCTTGAAGTTGAAGTAGAAGATGCGCA-3′.

in vitro

Immunoblot analysis

absence of iRTX (1 μM) or BAPTA (10 μM) for 24 h before total

% %

placed at −20 °C for 20 min and centrifuged at 12 000 × g at 4 °C

USA). A total of 50-μg portions of the protein were resolved on

difluoride membranes. After transfer, the membranes were blocked

the membranes were incubated overnight at 4 °C with antibod ies for TRPV1 (Alamone, Israel), PGC-1α(Cell signaling, USA),

surfeit 1, cytochrome C1 and β-actin (Santa Cruz, USA). After

chemiluminescence and quantified using a Gel Doc 2000 Imager

trol β-actin.

Immunofluorescence

C2C12 myotubes or cryosectioned muscle tissues were fixed

%

%

%

cific antibodies (Alamone) overnight at 4 °C. The cells or tissues were then washed three times and incubated with fluorescent dye-

Intracellular free calcium imaging

%

Individual cells were defined as the region of interest, and fluores

with capsaicin with or without pretreatment with iRTX (1 μM) for

increases in fluorescence intensity at 340 nm and 380 nm.

Mitochondrial staining

. Briefly, C2C12 myotubes were incubated in the prewarmed (37 °C) growth medium containing 200 nM

Assessment of ATP production

ATP Bioluminescence Assay kit (Genmed Scientifics Inc., Shang

Generation of TRPV1 transgenic mice

fertilized oocytes from C57BL/6J × CBA F1 mice, and the oocytes

pups identified five pups as founders. This job was completed by

generations. Transgenic mice were identified among the offspring

5′-GCGTGGATAGCGGTTTGA-3′; 5′-CGACTCCTGGATGTGAAGATG-3′

mice were sacrificed at 6-month old.

Animal care

n

%

4 months, and then sacrificed for blood and tissue examinations.

%

Exercise endurance tests

Oxygen consumption measurement

Int J Biochem Cell Biol **37**

et al. Regulation of muscle fiber

PLoS Biol

2

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278

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nitine palmitoyltransferase-1 in skeletal muscle is sufficient

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et al. Muscle fiber type is *Am J Physiol Endocrinol Metab* **282**

et al. fiber composition is related to adiposity and *in vitro* *Am J Physiol* **268**

et al. Muscle-specific expres *J Appl Physiol* **104**

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Cell Metab **6**

method for the simultaneous identification of skeletal muscle fiber types I, IIA, IIB and IIC. *Stain Technol* **65**

Diabetes **57**

Supplementary information
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