

Supplementary Material

**Empagliflozin induces the transcriptional program
for nutrient homeostasis in skeletal muscle in normal mice**

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Short title: Effects of Empagliflozin on Skeletal Muscle

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1. Methods

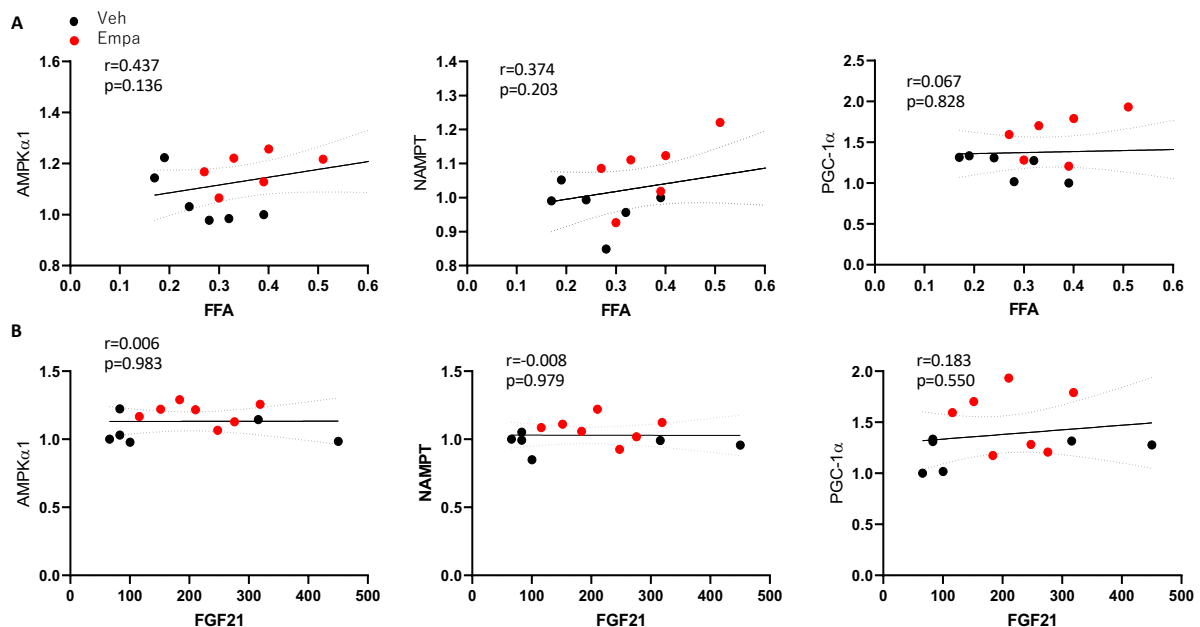
Cell culture

C2C12 cells were grown in Dulbecco's modified Eagle medium (DMEM) supplemented with 20% fetal bovine serum (growth medium, GM). For differentiation into multinucleate myotubes, nearly confluent cultures (60%–70% confluency) were switched to nutrient poor medium (DMEM supplemented with 2% horse serum (differentiation medium, DM) ¹. Extensive myoblast fusion was obtained after 24 to 36 h. 96 h after switching to DM, cells were cultured in serum free DMEM containing glucose of 5.6mM (100mg/dL) for 24 h and stimulated with bOHB of indicated concentrations for 24 h before harvest.

2. Results

Quantitative relationships between serum FFA or FGF21 and the transcripts of the nutrient sensors and PGC-1 α .

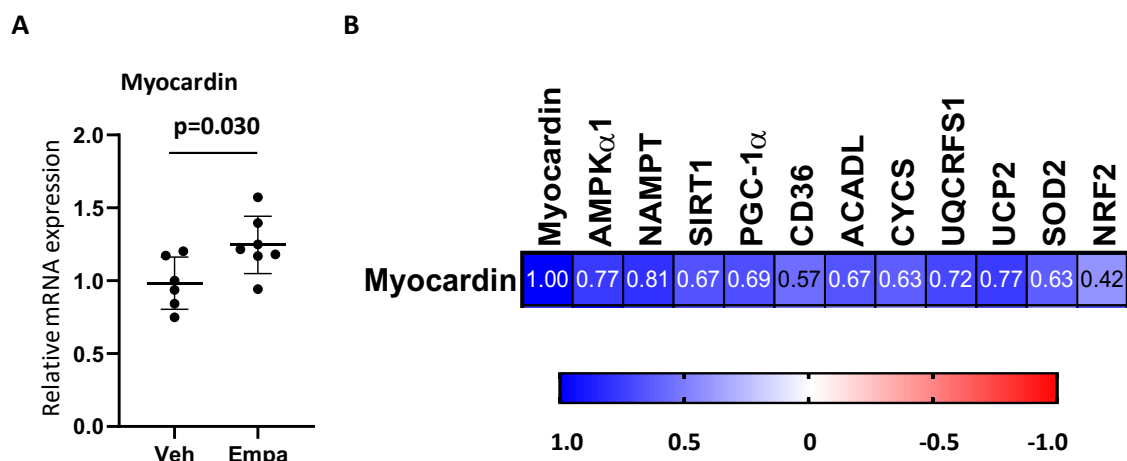
Pearson's correlation analysis showed that neither FFA nor FGF21 correlated the transcript levels of the nutrient sensors, AMPK α 1 and NAMPT, and coactivator PGC-1 α (Supl.Fig.S1).



Suppl. Fig. S1. Correlations between serum FFA or FGF21 and the transcripts of the nutrient sensors and PGC-1 α . Relative transcript levels for the AMPK α 1, NAMPT, and PGC-1 α genes are shown in the y-axis while serum FFA (A) or FGF21 (B) concentrations are shown in the x-axis. A linear regression line, Pearson's correlation coefficient and p-value are shown.

Effects of empagliflozin on myogenic gene expression.

We examined the effects of empagliflozin on the transcript levels of the genes encoding transcription factors or coactivators, including, MyoD, MEF2A, MEF2C, myocardin, myocardin-related transcription factor (MRTF-A, MRTF-B) in skeletal muscle. Among the genes tested, the myocardin transcripts significantly increased in empagliflozin-treated mice compared with that in vehicle-treated mice ($p=0.030$) (Suppl.Fig.S2A). In addition, the transcripts for the myocardin gene significantly correlated with those for nutrient sensors, PGC-1 α , and mitochondrial metabolism genes (Suppl.Fig.S2B).

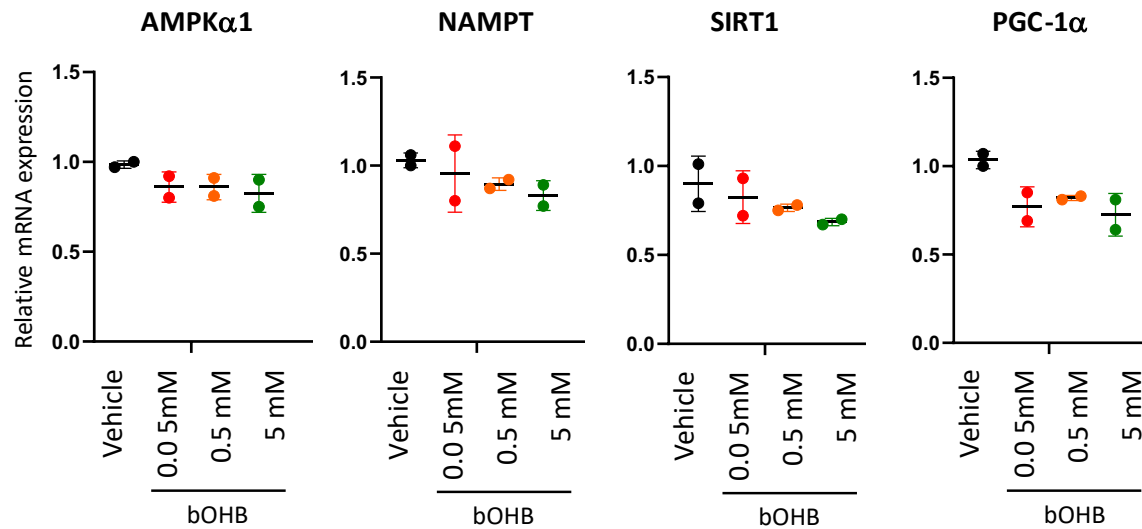
**Suppl. Fig. S2. Effects of empagliflozin on myocardin gene expression in skeletal muscle.**

(A) Relative transcript levels for the myocardin gene in quadriceps muscle from vehicle-treated and empagliflozin-treated mice. Values represent mean $\pm$ SD (vehicle-treated, $n=6$; empagliflozin-treated, $n=7$).

(B) Pearson's correlation coefficient between the relative transcript levels for the myocardin and many genes involved in multiple biological processes.

Effects of bOHB on the transcripts of the nutrient-sensing genes and PGC-1 α gene in differentiated C2C12 cells.

Effects of bOHB on the transcript levels for the AMPK α 1, NAMT, SIRT1, and PGC-1 α genes in the differentiated C2C12 cells were examined by qPCR. Results showed that bOHB tended to decrease the transcript levels for each of these genes in a dose-dependent manner in the single experiment (Suppl.Fig.S3).



Suppl. Fig. S3. Effects of bOHB on the expression of the genes for nutrient sensors and PGC-1α in differentiated C2C12 cells.

Differentiated C2C12 cells were stimulated with bOHB of indicated concentrations for 24 h. Transcript levels for each of the AMPKα1, NAMPT, SIRT1 and PGC-1α genes were expressed as relative levels of vehicle-treated cells. The transcripts were normalized to those of 36B4 gene.

Suppl. Table 1

List of primer sets used for qPCR

Primers set	Forward	Reverse
AMPKα1	5'-tcatgtggctgggtgtgtaagata-3'	5'-ggcattccattcatcatgtcagag-3'
NAMPT	5'-tgtcctgcatgggtatagggcatag-3'	5'-agccgggtggaattcacacaa-3'
SIRT1	5'-gcagacgtggtaatgtccaaacag-3'	5'-acatcttggcagttattgtggtgaa-3'
PGC-1α	5'-ttgacagctgcattcattatcacc-3'	5'-aacacttgagcaagcattcgaca-3'
PPARα	5'-ctcagggtaccactacggagttcac-3'	5'-tgaatcttcagctccgatcac-3'
PPARδ	5'-cagctcacaggcagagttgcta-3'	5'-caagccactgaagcagcaga-3'
Myocardin	5'-ggaagccatcctgtgcttgag-3'	5'-tcctgggaattgattggtctgtc-3'
KLF15	5'-aagtttccaagaaccagca-3'	5'-gacaactcatctgagcgggaaa-3'
TFAM	5'-gcagcaggcactacagcgatac-3'	5'-ctgagctccgagtcctgaacac-3'
PKD4	5'-gggtctcaataggtcacctgtgta-3'	5'-aagtgggcctgggcatttag-3'
CD36	5'-tgctggagctgttattggtg-3'	5'-tcttgatgtgcaaaacca-3'
ACADL	5'-acttgggaagagcaagcgta-3'	5'-ttccgtttccacaaaaag-3'
CPT1b	5'-gtcgtcttctcaaggcttg-3'	5'-aagaaagcagcagcttcgat-3'
CYCS	5'-cattaccctgggtgtggttca-3'	5'-atgccataatacagcagccattag-3'
UQCRRS1	5'-gtgaagcgacccttctgtg-3'	5'-gcctcactgcttctttagaagatt-3'
SLC25A4	5'-tctgtccaggcatcatcatctac-3'	5'-ctgggcaatcatccagctca-3'
UCP2	5'-actctgccttgggcccagta-3'	5'-gctgctcataggtgacaaacatc-3'
UCP3	5'-ctctgcactgtatgctgaagatg-3'	5'-cacgtccaagctcccaga-3'
SOD2	5'-gagaatctcagtgctcactcgtgtc-3'	5'-ggaaccctaaatgctgccagtc-3'
NRF2	5'-tccgtgccatcagtcagtc-3'	5'-attgtgccttcagcgtgcttc-3'
36B4	5'-atccctgacgcaccgccgtga-3'	5'-tgcctctgcttggagcccacgtt-3'

Reference

- 1 Sartorelli, V. *et al.* Acetylation of MyoD directed by PCAF is necessary for the execution of the muscle program. *Mol Cell* **4**, 725-734 (1999). [https://doi.org/10.1016/s1097-2765\(00\)80383-4](https://doi.org/10.1016/s1097-2765(00)80383-4)