17Beta-estradiol Stimulates Glucose Uptake Through Estrogen Receptor and AMP-activated Protein Kinase Activation in C2C12 Myotubes

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Background: Previous studies have shown that 17beta-estradiol activates AMP-activated protein kinase (AMPK) in rodent muscle and C2C12 myotubes and that acute 17beta-estradiol treatment rapidly increases AMPK phosphorylation possibly through non-genomic effects but does not stimulate glucose uptake. Here, we investigated whether 24-hour 17beta-estradiol treatment stimulated glucose uptake and regulated the expression of genes associated with glucose and energy metabolism through the genomic effects of estrogen receptor (ER) in C2C12 myotubes.

Methods: C2C12 myotubes were treated with 17beta-estradiol for 24 hours, and activation of AMPK, uptake of glucose, and expression of genes encoding peroxisome proliferator-activated receptor γ coactivator 1 α , carnitine palmitoyltransferase 1 β , uncoupling protein 2, and glucose transporter 4 were examined. Furthermore, we investigated whether AMPK inhibitor (compound C) or estrogen receptor antagonist (ICI182,780) treatment reversed 17beta-estradiol-induced changes.

Results: We found that 24-hour treatment of C2C12 myotubes with 17beta-estradiol stimulated AMPK activation and glucose uptake and regulated the expression of genes associated with glucose and energy metabolism. Treatment of C2C12 myotubes with the estrogen receptor antagonist (ICI182,780) reversed 17beta-estradiol-induced AMPK activation, glucose uptake, and changes in the expression of target genes. Furthermore, treatment with the AMPK inhibitor (compound C) reversed 17beta-estradiol-induced glucose uptake and changes in the expression of target genes.

Conclusion: Our results suggest that 17beta-estradiol stimulates AMPK activation and glucose uptake and regulates the expression of genes associated with glucose and energy metabolism in C2C12 myotubes through the genomic effects of ER.

Key words: 17Beta-estradiol, Estrogen receptor, AMP-activated protein kinase, Glucose uptake

Introduction

Postmenopausal women with estrogen deficiency are at a high risk of developing type 2 diabetes¹ and metabolic syndrome, both of which are characterized by a combination of risk factors such as abdominal obesity, insulin resistance, glucose intolerance, dyslipidemia, and hypertension.²⁻⁴ Long-term estrogen therapy prevents diabetes in postmenopausal women.⁵ Estrogen exerts physiological effects through the genomic effects of two subtypes of estrogen receptors (ERs), namely, ER- α and ER- β , which belong to nuclear receptor family of transcription factors.⁶ 17Beta-estradiol is the major physiological type of estrogen that shows similar affinity to both the ER

subtypes.⁶ However, molecular mechanisms underlying the direct metabolic effects of 17beta-estradiol-ER interaction on glucose metabolism are not clearly understood.

In humans, skeletal muscles are primarily responsible for glucose disposal from the circulating blood.⁷ Skeletal muscles account for approximately 70%–85% of whole body glucose disposal.⁸ Two major mechanisms stimulate glucose uptake by skeletal muscles. One is insulin signaling pathway that stimulates glucose uptake by activating PI-3 kinase/Akt pathway. Insulin enhances glucose uptake by skeletal muscles by translocating glucose transporter (GLUT) 4 to the cell membrane through the phosphorylation of Akt.⁹ The other mechanism is AMP-activated protein kinase (AMPK) pathway.¹⁰

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Received Nov. 4, 2016 Reviewed Nov. 7, 2016 Accepted Nov. 8, 2016

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AMPK activation is suggested to improve glucose uptake by muscles, and phosphorylation of AMPK and its substrate acetyl-CoA carboxylase (ACC) increases fatty acid oxidation in metabolic tissues.^{11,12} Previous studies have shown that 17beta-estradiol activates AMPK in rodent muscles¹³⁻¹⁵ and C2C12 myotubes.^{14,16} Moreover, acute (≤ 10 minutes) treatment with 17beta-estradiol rapidly increases Akt and AMPK phosphorylation possibly through non-genomic effects but does not stimulate glucose uptake or enhance insulin sensitivity in skeletal muscles (rat soleus) *ex vivo*.¹⁵

Here, we investigated whether 24-hour treatment with 17beta-estradiol stimulates glucose uptake and regulates the expression of genes associated with glucose and energy metabolism through the genomic effects of ER in C2C12 myotubes.

Methods

1. Materials

Estrogen (17beta-estradiol) was obtained from Sigma (St. Louis, USA). ICI182.780, an ER- α/β -nonspecific antagonist, was obtained from Tocris Bioscience (Ellisville, USA). Compound C, an AMPK inhibitor, and AICAR, an AMPK activator, were purchased from Calbiochem (San Diego, USA). 2-[³H]-deoxy-D-glucose (6.0 Ci/mmol) was obtained from DuPont-Net (Boston, USA). Dulbecco's modified Eagle's medium (DMEM), phenol red-free DMEM, fetal bovine serum (FBS), charcoal-stripped FBS, trypsin-EDTA, and antibiotics (penicillin and streptomycin) were obtained from Gibco BRL (Grand Island, USA). The following primary antibodies were used in this study: anti-AMPK α antibody, anti-phosphorylated Thr172-AMPK α antibody, anti-ACC antibody, anti-phosphorylated Ser79-ACC antibody (Cell Signaling Technology Inc., Beverly, MA, USA), and anti- β -actin antibody (internal control; Sigma). Horseradish peroxidase-conjugated sheep anti-mouse and donkey anti-rabbit immunoglobulin antibodies were obtained from Amersham Pharmacia Biotech-

nology (Tokyo, Japan). All other chemical were of analytical grade or complied with the standards needed for cell culture experiments.

2. Cell culture and differentiation

C2C12 myoblasts were obtained from American Type Culture Collection (Manassas, USA). The cells were maintained in DMEM supplemented with 10% heat-inactivated FBS, penicillin (100 U/mL), and streptomycin (100 mg/mL) at 37°C in an incubator containing 5% CO₂. At 90% confluency, the medium was replaced with a differentiation medium (for C2C12 myotubes) containing DMEM supplemented with 1% FBS and antibiotics. Before performing the experiments, the cells were starved for 12 hours in 1% FBS medium, followed by incubation with 10 μ M ICI182.780, 20 μ M compound C, 500 μ M AICAR, 10 nM insulin, or 10⁻⁷ M 17beta-estradiol alone or in combination. The cells were treated with ICI182.780 or compound C 30 minutes before treatment with 17beta-estradiol for indicated time and at an indicated dose.

3. Real-time polymerase chain reaction

Total RNA was isolated from C2C12 myotubes by using TRIzol reagent (Invitrogen Corp., San Diego, USA), according to the manufacturer's instructions. Equal amounts of the total RNA were reverse transcribed to cDNA by using ImProm-II^{TM} reverse transcriptase (Promega, Madison, USA) and an oligo(dT)₁₅ primer (Promega). Sequences of primers against genes encoding peroxisome proliferator-activated receptor γ coactivator 1 α (*PGC-1 α*), muscle-type carnitine palmitoyltransferase 1 (*CPT1 β*), uncoupling protein 2 (*UCP2*), and insulin-sensitive glucose transporter 4 (*GLUT4*) are listed in Table 1. Real-time polymerase chain reaction (RT-PCR) was performed in a 20- μ L reaction mixture containing 1 μ g cDNA, 10 pmol forward primer, 10 pmol reverse primer, and 10 μ L SYBR Green Premix Master (Roche, Diagnostic, Indianapolis, USA) by using DNA Engine Opticon System (MJ Research Inc., USA). Amplification was per-

Table 1. Real-time PCR primer sequences

Genes	Primers				
	Forward (5'-3')	Reverse (5'-3')	Size (bp)	PCR condition (annealing T _m)	GeneBank accession No.
<i>PGC-1α</i>	TATGGTTTCATCACCTACCG	CGTCCACAAAAGTACAGCTC	110	52°C, 30 s	MN-008904
<i>GLUT4</i>	GTGTGTGAGCGAGTGCTTTCC	TGGAGACTGATGCGCTCTAACC	218	57°C, 30 s	MN-009204
<i>UCP2</i>	AGATACATGAACCTCTGCCTTGGG	GGCAGAGGATGAAGAAAAAGAC	275	53°C, 30 s	MN-011671
<i>CPT1β</i>	CCCATAAGAACAAGACCTCC	GATGATTGGGATACTGTTTTGGG	249	53°C, 30 s	MN-009948
<i>β-actin</i>	ACGCCAGGTCATCACTATT	AATGTAGTTTCATGGATGCC	106	52-57°C, 30 s	MN-007393

formed using the following parameters: initial denaturation at 95°C for 5 minutes, followed by 40 cycles of denaturation at 95°C for 1 minute, annealing at 50°C–57°C for 30 seconds, and extension at 72°C for 1 minute. The housekeeping gene β -actin was used as a control. The mRNA expression levels of target genes are represented as threshold cycle (C_t) values normalized to the β -actin mRNA value.

4. Western blotting

Whole-cell lysates were prepared by lysing the cells in Proprep protein extraction solution (Intron Biotechnology, Seoul, Korea) containing 10 mM sodium phosphate (pH 7), 1% Triton X-100, 0.1% sodium dodecyl sulfate (SDS), 2 mM EDTA, 150 mM NaCl, 50 mM NaF, 0.1 mM sodium vanadate, 4 μ g/mL leupeptin, and 1 mM PMSF. Protein concentration of the lysates was measured using a protein assay kit (Bio-Rad, Hercules, USA). Equal amounts of the lysates were resolved by performing SDS-polyacrylamide gel electrophoresis (SDS-PAGE), and separated proteins were transferred onto nitrocellulose membranes (Invitrogen Corp.). The membranes were washed with phosphate-buffered saline (PBS)-Tween (PBST) solution containing 5% nonfat dry milk at room temperature and were incubated for 2 hours with the previously mentioned primary antibodies under the same condition. Next, the membranes were washed with PBST and were incubated for 1 hour with horseradish peroxidase-conjugated sheep anti-mouse and donkey anti-rabbit secondary immunoglobulin antibodies (dilution, 1:500) under the same condition. After washing again with PBST, specific signals were detected using an enhanced chemiluminescence detection system (Amersham Pharmacia Biotech). The band densities were quantified using Image J software (<http://rsb.info.nih.gov/ij/>).

5. 2-[^{3} H]-deoxy-D-glucose uptake

C2C12 myotubes derived by differentiating C2C12 myoblasts were grown in a 12-well plate and were serum starved for 3 hours. The cells were stimulated with drugs in Krebs–Ringer phosphate-HEPES buffer containing 25 mM HEPES, 118 mM NaCl, 4.8 mM KCl, 1.3 mM CaCl_2 , 1.2 mM KH_2PO_4 , 1.3 mM MgSO_4 , 5 mM NaHCO_3 , 20 mM glucose, and 0.07% BSA. After 30-minute or 24-hour incubation, the cells were treated with 20 μ l substrate (10.2 mM L-glucose and 0.5 μ Ci 2-[^{3} H]-deoxy-D-glucose) for 30 minutes. Next, the cells were washed three times with ice-cold PBS and were solubilized with 0.1% SDS plus 0.5 N NaOH. Radioactivity of the cells was measured by performing liquid scintillation counting. Results were corrected for nonspecific uptake, which was determined according to 2-[^{3} H]-deoxy-D-glucose uptake. Nonspecific binding was less than 10% of the total uptake.

6. Statistical analysis

All data are presented as mean $\pm$ standard deviation (SD) of multiple determinations. Statistical differences were analyzed using one-way ANOVA followed by Tukey test. All statistical analyses were performed using SPSS statistical software package (version 14.0; Chicago, USA). P -value of <0.05 was considered statistically significant.

Results

1. 17Beta-estradiol activates AMPK in C2C12 myotubes

In C2C12 myotubes not treated with insulin, 17beta-estradiol (10^{-7} M) treatment stimulated the phosphorylation of AMPK and its substrate ACC within 10 minutes (Fig. 1). Next, we investigated the effect of AMPK inhibitor compound C (20 μ M) on estrogen-induced

Figure 1. Estrogen stimulates AMP-activated protein kinase (AMPK) signaling pathway. Representative western blot analyses of phospho-AMPK, total-AMPK, phospho-acetyl-CoA carboxylase (ACC), and total-ACC in C2C12 myotubes. The phospho-protein-to-protein ratios are represented as the mean $\pm$ SD are represented as the percentage of expression.

* $P < 0.01$; [†] $P < 0.05$, compared with the expression at zero time.

AMPK activation (Fig. 2). We found that treatment with compound C reversed 17beta-estradiol-induced activation of AMPK and its substrate ACC.

2. 17Beta-estradiol activates AMPK through an ER in C2C12 myotubes

Treatment of C2C12 myotubes not exposed to insulin with the ER- α/β -nonspecific antagonist ICI182.780 (10 μ M) reversed estrogen-induced activation of AMPK and ACC (Fig. 3), indicating that 17beta-estradiol activated AMPK through an ER in C2C12 myotubes.

3. 17Beta-estradiol regulates the expression of genes associated with glucose and energy metabolism by activating the ER-AMPK pathway in C2C12 myotubes

Real-time PCR was performed to determine the effects of 24-hour treatment with estrogen on the expression of genes associated with glucose and energy metabolism. Results of RT-PCR showed that the expression of *PGC-1 α* , *CPT1 β* , *UCP2*, and *GLUT4* was significantly increased at 24 hours after 17beta-estradiol (10^{-7} M) treatment of C2C12 myotubes not exposed to insulin (Fig. 4). However, treatment with the ER- α/β -nonspecific antagonist ICI182.780 (10 μ M) reversed

Figure 2. The AMP-activated protein kinase (AMPK) inhibitor, Compound C attenuated estrogen-induced signaling in C2C12 myotubes. Representative western blot analysis of the molecular change of AMP-activated protein kinase (AMPK) and the phospho-protein-to-protein ratios in C2C12 myotubes. The phospho-protein-to-protein ratios are represented as the mean $\pm$ SD and are represented as the percentage of expression. * $P < 0.01$.

Figure 3. The estrogen receptor antagonist, ICI 182.780, attenuated estrogen-induced signaling in C2C12 myotubes. Representative western blot analysis of the molecular change of AMP-activated protein kinase (AMPK) signaling and the phospho-protein-to-protein ratios in C2C12 myotubes. The phospho-protein-to-protein ratios are represented as the mean $\pm$ SD and are represented as the percentage of expression. * $P < 0.01$.

Figure 4. Estrogen alters the expression of genes related to glucose and energy metabolism in C2C12 myotubes. The expression of peroxisome proliferator-activated receptor γ coactivator 1 α (*PGC-1 α*), muscle-type carnitine palmitoyltransferase 1 (*CPT1 β*), uncoupling protein 2 (*UCP2*), and insulin-sensitive glucose transporter 4 (*GLUT4*) in the C2C12 myotubes. The AMP-activated protein kinase (AMPK) inhibitor (Compound C) and estrogen receptor antagonist (ICI 182.780) attenuated estrogen-induced signaling in C2C12 myotubes. The mRNA expression levels were represented as the threshold cycle (Ct) value normalized to β -actin mRNA value. * $P < 0.01$.

Figure 5. Estrogen stimulates glucose uptake in C2C12 myotubes. C2C12 myotubes were pretreated with or without 500 μ M AICAR (AMP-activated protein kinase activator), 10 μ M ICI182.780, 20 μ M compound C, 100 nM insulin, and estrogen alone or in combinations. ICI182.780 and compound C were 30 min prior to estrogen for the indicated time. Glucose uptake was measured as described in materials and methods. Data are shown as the means $\pm$ SD.

* $P < 0.01$, $^{\dagger}P < 0.001$, as compared with control cells.

estrogen-induced increase in the expression of *PGC-1 α* , *CPT1 β* , *UCP2*, and *GLUT4* at 24 hours after 17beta-estradiol (10^{-7} M) treatment in C2C12 myotubes not exposed to insulin. Treatment with the AMPK antagonist compound C (20 μ M) also reversed estrogen-induced increase in the expression of *PGC-1 α* , *CPT1 β* , *UCP2*, and *GLUT4* at 24 hours after 17beta-estradiol (10^{-7} M) treatment in C2C12 myotubes not exposed to insulin. These results indicate that 17beta-estradiol regulates the expression of genes associated with glucose and energy metabolism in C2C12 myotubes by activating the ER-AMPK pathway and without activating insulin-induced signaling.

4. 17Beta-estradiol stimulates glucose uptake in C2C12 myotubes by activating the ER-AMPK pathway

Next, we determined glucose uptake by C2C12 myotubes. We observed that 30-minute treatment with insulin stimulated glucose uptake, whereas 30-minute treatment with 17beta-estradiol (10^{-7} M) and AICAR (AMPK activator) did not stimulate glucose uptake in C2C12 myotubes (Fig. 5A). Moreover, treatment with the ER- α/β -nonspecific antagonist ICI182.780 (10 μ M) and AMPK antagonist compound C (20 μ M) did not change the results. However, 24-hour treatment with insulin, 17beta-estradiol (10^{-7} M), and AICAR stimulated glucose uptake in C2C12 myotubes (Fig. 5B). Moreover, treatment with the ER- α/β -nonspecific antagonist ICI182.780 (10 μ M) and AMPK antagonist compound C (20 μ M) reversed 10^{-7} M 17beta-

estradiol treatment-induced glucose uptake after 24 hours. These results suggest that 24-hour treatment of C2C12 myotubes with 17beta-estradiol stimulates glucose uptake and regulates the expression of genes associated with glucose and energy metabolism, including *GLUT4*, through the genomic effects of the ER-AMPK pathway.

Discussion

Previous researches have shown that acute stimulation of C2C12 myotubes with 17beta-estradiol increases AMPK phosphorylation^{14,16}, suggesting that estrogen replacement therapy exerts beneficial effects against type 2 diabetes mellitus in postmenopausal women by stimulating glucose uptake by skeletal muscles.¹⁵ However, it is unclear whether estrogen stimulates glucose uptake in skeletal muscles or myocytes. Moreover, it is unclear whether 17beta-estradiol-induced activation of AMPK signaling increases muscle glucose uptake.

AMPK stimulates GLUT4 translocation independently of the PI-3 kinase/Akt pathway.¹⁷ Other studies suggest that AMPK functions upstream of the Akt pathway¹⁸ and that AMPK is activated by the PI-3 kinase pathway.¹⁹ In any case, AMPK stimulates glucose uptake in muscles. Previous studies have reported that estrogen stimulates Akt and AMPK activation but does not stimulate glucose uptake in muscles or myotubes.¹⁵ We postulated that estrogen did not stimulate glucose uptake through non-genomic action by AMPK. We sug-

gest that estrogen stimulates glucose uptake through genomic action by AMPK. Therefore, we determined whether 24-hour treatment with 17beta-estradiol stimulated glucose uptake and regulated the expression of genes associated with glucose and energy metabolism through the genomic effects of ER, a known transcription factor, in C2C12 myotubes.

Our results showed that acute (≤ 10 minutes) treatment with 17beta-estradiol rapidly increased AMPK phosphorylation possibly through the non-genomic effects but did not stimulate glucose uptake in C2C12 myotubes. However, 24-hour treatment with 17beta-estradiol stimulated glucose uptake and regulated the expression of genes associated with glucose and energy metabolism in C2C12 myotubes, suggesting that 17beta-estradiol exerted its effects through the genomic effects of the ER-AMPK pathway. However, we could not determine the mechanism through which 17beta-estradiol regulated the expression of genes associated with glucose and energy metabolism by activating the ER-AMPK pathway in C2C12 myotubes. This is the limitation of the present study. Results of the present study suggest that 17beta-estradiol stimulates glucose uptake by increasing GLUT4 expression through the genomic effects of the ER-AMPK pathway. These results might contribute to our overall understanding of the role of 17beta-estradiols in regulating glucose metabolism and in preventing the development of type 2 diabetes in postmenopausal women.

Results of the present study showed that 24-hour treatment with 17beta-estradiol increased the expression of genes associated with glucose and energy metabolism, such as *PPGC-1 α* , *CPT1 β* , *UCP2*, by activating the 17beta-estradiol-ER-AMPK pathway. These results suggest that 17beta-estradiol exerts beneficial effects on glucose and energy metabolism in C2C12 myotubes through the genomic effects of the ER-AMPK pathway and without activating insulin-induced signaling. *PGC-1 α* is involved in mitochondrial metabolism and biogenesis, thermogenesis, adipocyte differentiation, and glucose metabolism.^{20,21} *CPT1 β* controls mitochondrial β -oxidation of fatty acids.²² Mitochondrial *UCP2* is involved in physiological processes associated with glucose and lipid metabolism.²³ We selected *PGC-1 α* , *CPT1 β* , *UCP2*, and *GLUT4* for analysis in the present study based on these findings.

In conclusion, the results of the present study suggest that 17beta-estradiol stimulates AMPK activation and glucose uptake and regulates glucose and energy metabolism in C2C12 myotubes through

the genomic effects of ER.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

This research was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2012-0002286) of the Korean Government.

References

1. Lindheim SR, Buchanan TA, Duffy DM, Vijod MA, Kojima T, Stanczyk FZ, et al. Comparison of estimates of insulin sensitivity in pre- and postmenopausal women using the insulin tolerance test and the frequently sampled intravenous glucose tolerance test. *J Soc Gynecol Investig* 1994;1:150-4.
2. Liu S, Mauvais-Jarvis F. Minireview: estrogenic protection of β -cell failure in metabolic diseases. *Endocrinology* 2010;151:859-64.
3. Schneider JG, Tompkins C, Blumenthal RS, Mora S. The metabolic syndrome in women. *Cardiol Rev* 2006;14:286-91.
4. Park YW, Zhu S, Palaniappan L, Heshka S, Carnethon MR, Heymsfield SB. The metabolic syndrome: prevalence and associated risk factor findings in the US population from the Third National Health and Nutrition Examination Survey, 1988-1994. *Arch Intern Med* 2003;163:427-36.
5. Pentti K, Tuppurainen MT, Honkanen R, Sandini L, Kröger H, Alhava E, et al. Hormone therapy protects from diabetes: the Kuopio osteoporosis risk factor and prevention study. *Eur J Endocrinol* 2009;160:979-83.
6. Faulds MH, Zhao C, Dahlman-Wright K, Gustafsson JÅ. The diversity of sex steroid action: regulation of metabolism by estrogen signaling. *J Endocrinol* 2012;212:3-12.
7. DeFronzo RA, Jacot E, Jequier E, Maeder E, Wahren J, Felber JP. The effect of insulin on the disposal of intravenous glucose. Results from indirect calorimetry and hepatic and femoral venous catheterization. *Diabetes* 1981;30:1000-7.
8. Yki-Järvinen H, Young AA, Lamkin C, Foley JE. Kinetics of glu-

- cose disposal in whole body and across the forearm in man. *J Clin Invest* 1987;79:1713-9.
9. Wang Q, Somwar R, Bilan PJ, Liu Z, Jin J, Woodgett JR, et al. Protein kinase B/Akt participates in GLUT4 translocation by insulin in L6 myoblasts. *Mol Cell Biol* 1999;19:4008-18.
  10. Czech MP, Corvera S. Signaling mechanisms that regulate glucose transport. *J Biol Chem* 1999;274:1865-8.
  11. Lage R, Diéguez C, Vidal-Puig A, López M. AMPK: a metabolic gauge regulating whole-body energy homeostasis. *Trends Mol Med* 2008;14:539-49.
  12. Schimmack G, Defronzo RA, Musi N. AMP-activated protein kinase: Role in metabolism and therapeutic implications. *Diabetes Obes Metab* 2006;8:591-602.
  13. Riant E, Waget A, Cogo H, Arnal JF, Burcelin R, Gourdy P. Estrogens protect against high-fat diet-induced insulin resistance and glucose intolerance in mice. *Endocrinology* 2009;150:2109-17.
  14. D'Eon TM, Souza SC, Aronovitz M, Obin MS, Fried SK, Greenberg AS. Estrogen regulation of adiposity and fuel partitioning. Evidence of genomic and non-genomic regulation of lipogenic and oxidative pathways. *J Biol Chem* 2005;280:35983-91.
  15. Rogers NH, Witczak CA, Hirshman MF, Goodyear LJ, Greenberg AS. Estradiol stimulates Akt, AMP-activated protein kinase (AMPK) and TBC1D1/4, but not glucose uptake in rat soleus. *Biochem Biophys Res Commun* 2009;382:646-50.
  16. D'Eon TM, Rogers NH, Stancheva ZS, Greenberg AS. Estradiol and the estradiol metabolite, 2-hydroxyestradiol, activate AMP-activated protein kinase in C2C12 myotubes. *Obesity (Silver Spring)* 2008;16:1284-8.
  17. Russell RR 3rd, Bergeron R, Shulman GI, Young LH. Translocation of myocardial GLUT-4 and increased glucose uptake through activation of AMPK by AICAR. *Am J Physiol* 1999;277:H643-9.
  18. Ouchi N, Kobayashi H, Kihara S, Kumada M, Sato K, Inoue T, et al. Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. *J Biol Chem* 2004;279:1304-9.
  19. Zou MH, Kirkpatrick SS, Davis BJ, Nelson JS, Wiles WG 4th, Schlattner U, et al. Activation of the AMP-activated protein kinase by the anti-diabetic drug metformin in vivo. Role of mitochondrial reactive nitrogen species. *J Biol Chem* 2004;279:43940-51.
  20. Puigserver P, Spiegelman BM. Peroxisome proliferator-activated receptor-gamma coactivator 1 alpha (PGC-1 alpha): transcriptional coactivator and metabolic regulator. *Endocr Rev* 2003;24:78-90.
  21. Knutti D, Kralli A. PGC-1, a versatile coactivator. *Trends Endocrinol Metab* 2001;12:360-5.
  22. Baldán A, Relat J, Marrero PF, Haro D. Functional interaction between peroxisome proliferator-activated receptors-alpha and Mef-2C on human carnitine palmitoyltransferase 1beta (CPT1beta) gene activation. *Nucleic Acids Res* 2004;32:4742-9.
  23. Diano S, Horvath TL. Mitochondrial uncoupling protein 2 (UCP2) in glucose and lipid metabolism. *Trends Mol Med* 2012;18:52-8.