Supporting Information

Extracellular Visfatin Activates Gluconeogenesis in HepG2 Cells Through the Classical PKA/CREB-Dependent Pathway

Authors
Y. J. Choi¹,*, S.-E. Choi²,*, E. S. Ha¹, Y. Kang², S. J. Han¹, D. J. Kim¹, K. W. Lee¹, H. J. Kim¹

Affiliations
¹Department of Endocrinology and Metabolism, Ajou University School of Medicine, Suwon, Republic of Korea
²Department of Physiology, Ajou University School of Medicine, Suwon, Republic of Korea

Correspondence
H. J. Kim, MD, PhD
Department of Endocrinology and Metabolism
Ajou University School of Medicine
San 5 Wonchon-dong, Yeongtong-gu,
Suwon, 443-721
Republic of Korea
Tel.: +82/31/219 4498
Fax: +82/31/219 4497
jinkim@ajou.ac.kr

*These authors contribute equally to this work.
Fig. S1  A No significant effects were observed after treatment with only extracellular visfatin (100 ng/mL). Data are expressed as the levels of NAD relative to 0-h controls (1). B Co-treatment with nicotinamide (10 nM) and visfatin (10 ng/mL) for 3 h showed a significantly additive effect compared with nicotinamide only. Data are expressed as the levels of NAD relative to untreated controls (1). †p<0.01, *p<0.05, **p<0.005 vs. untreated control.
**Measurement of total cellular NAD**

Levels of total NAD (tNAD, NAD+, and NADH) were measured by using NAD+/NADH Quantification Kit (Biovision, Mountain View, CA). HepG2 cells were seeded in six-well plates and incubated for 24 h. Visfatin-treated cells were then harvested by scraping, and washed with phosphate-buffered saline (PBS), and homogenized in assay buffer (supplied with the kit). Cellular protein was removed using deprotein kit. To quantify NADH, total NAD was decomposed via heating at 60°C for 30 min. NAD cycling mix was added to both samples, and the samples were incubated at room temperature for 5 min before the addition of NADH developer solution. Samples were left to incubate at room temperature for 1-2 h before the addition of stop solution. Samples were then read on a spectrophotometer at 450 nm and quantity of tNAD was then determined on the basis of NADH standard curve. Protein content was determined in each sample via the Bradford method, and values are expressed relative to protein content.