

ORIGINAL ARTICLE

Glucose represses PPAR α gene expression via AMP-activated protein kinase but not via p38 mitogen-activated protein kinase in the pancreatic β -cell

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Abstract

Background: Peroxisome proliferator-activated receptor α (PPAR α) regulates the expression of fatty acid metabolism genes and is thought to play a role in the regulation of insulin secretion and lipid detoxification. We have examined the mechanism whereby glucose decreases PPAR α gene expression in the pancreatic β -cell.

Methods: INS832/13 β -cell and isolated rat islets were incubated at 3 and 20 mM glucose for 18 h in the absence or presence of adenosine monophosphate (AMP)-activated protein kinase (AMPK) activators and inhibitors, as well as p38 mitogen-activated protein kinase (p38 MAPK) inhibitors. In another set of experiments, INS832/13 were infected with an adenovirus expressing a dominant-negative form of AMPK. PPAR α expression levels were measured by reverse transcription polymerase chain reaction and Western blot.

Results: Elevated glucose reduced the abundance of the PPAR α transcript and protein, and its target genes acyl-coenzyme A (CoA) oxidase (ACO) and uncoupling protein 2 (UCP-2) in INS832/13 β -cell and isolated rat islets. Glucose reduced AMPK activity, while the AMPK activators 5-amino-4-imidazolecarboxamide riboside and metformin increased PPAR α expression and suppressed the action of glucose. By contrast, the AMPK inhibitor compound C mimicked the glucose effect. A dominant negative form of AMPK α reduced the PPAR α , ACO and UCP-2 transcripts to the same extent as elevated glucose. Pharmacological evidence indicated that glucose-regulated PPAR α expression does not involve p38 MAPK, a target of AMPK in several cell types.

Conclusions: The results indicate that glucose represses PPAR α gene expression via AMPK, but not via p38 MAPK in the β -cell.

Keywords: adenosine monophosphate-activated protein kinase, fat oxidation, p38 mitogen-activated protein kinase, pancreatic β -cell, peroxisome proliferator-activated receptor α .

Introduction

Peroxisome proliferator-activated receptor α (PPAR α) belongs to a nuclear receptor family of ligand-activated transcription factors that bind fatty acids and their metabolites.¹ PPAR α regulates the expression of

genes of fatty acid metabolism.^{2–4} We have shown that exposure of β -cells to elevated glucose rapidly decreases PPAR α gene expression. The mechanism by which glucose regulates PPAR α expression requires phosphorylation of the sugar, but not de novo protein synthesis.⁵ We proposed that the reduction in PPAR α

gene expression caused by elevated glucose, together with chronic elevated malonyl-CoA levels and reduced adenosine monophosphate (AMP)-activated protein kinase (AMPK) activity, play a role in β -cell "glucolipotoxicity".^{6,7}

AMPK forms a heterotrimer composed of a catalytic α subunit and non-catalytic β and γ subunits. AMPK is a "fuel gauge" activated in response to adenosine triphosphate (ATP) depletion switching off ATP consuming pathways by inhibiting enzymes involved in glycogen, fatty acid and cholesterol synthesis, and promoting ATP generating pathways by activating enzymes of fatty acid oxidation and glycolysis.^{8–10} Evidence for glucose regulation of AMPK was first recognized because of glucose-induced changes in the phosphorylation of the AMPK substrate, acetyl-CoA carboxylase (ACC), in FaO hepatoma and HIT-T15 β -cells.¹¹ Low glucose concentrations that reduce the ATP/adenosine diphosphate (ADP) ratio activate AMPK in pancreatic INS1 cells.¹² By contrast, AMPK activity is inhibited at high glucose in HIT-T15,¹² MIN6 and INS1¹³ β -cells. Hydroxymethylglutaryl-CoA reductase and ACC were the first known targets of AMPK,¹⁴ and many additional enzymes regulated by AMPK have been identified. Activation of AMPK also reduces ACC gene expression and prevents the glucose-activation of L-type pyruvate kinase (L-PK)¹³ and fatty acid synthase.^{15,16} Phosphoenolpyruvate carboxykinase and glucose-6-phosphatase¹⁷ gene expression are decreased following AMPK activation with 5-amino-4-imidazolecarboxamide riboside (AICAR). It has been proposed that AMPK might also regulate hepatic nuclear factor 4 (HNF4) α ^{18–20} and the carbohydrate-response-element-binding protein (CREBP),²¹ which play key roles in the regulation of hepatic metabolic genes. Moreover, the transcriptional activity of several nuclear receptors is inhibited by AMPK via the phosphorylation of p300, a co-activator of PPAR receptors.^{20,22} A study showed that AMPK suppresses the expression and activation of sterol regulatory element-binding proteins 1c (SREBP1c), a key transcription factor regulating genes of fatty acid biosynthesis.²³ Finally, Jäger et al.²⁴ and others^{25,26} showed that AMPK regulates PPAR γ co-activator (PGC)-1 α transcription, a key factor for mitochondrial energy metabolism, mediated by upstream stimulatory factor (USF)-1 in skeletal muscle.^{27,28}

The p38 mitogen-activated protein kinase (p38 MAPK) family is composed of four isoforms and is involved in biological processes such as cell growth and differentiation, as well as in inflammatory processes, energy metabolism, and cell death.^{29–33} p38 MAPK are activated in response to a variety of extracellular and stress stimuli. Observations from heart cells,^{34–36}

hepatocytes,³⁷ L6 myotube cells,³⁸ cardiac fibroblast³⁹ and skeletal muscle cells⁴⁰ indicated that under stress conditions, such as ischemia or in response of cytokines, p38 MAPK is activated by AMPK to regulate in turn the expression of target genes.^{29,31,32,41–44}

Here we have studied the mechanism by which glucose regulates PPAR α expression levels and show that AMPK, but not p38 MAPK, plays a key role in this process.

Methods

Materials

Moloney murine leukemia virus (MMLV) reverse transcriptase was purchased from Invitrogen (Carlsbad, CA, USA), Taq DNA polymerase from Life Technologies (Rockville, MD, USA) and Pd(N)₆ hexamer from Amersham/Pharmacia (Uppsala, Sweden). Protran nitrocellulose membranes for protein analysis were from Schleicher & Schuell (Dassel, Germany) and the bicinchoninic acid (BCA) protein assay was from Pierce (Rockford, IL, USA). The enhanced chemiluminescence (ECL) detection kit for Western blot and T4 polynucleotidekinase were from Amersham/Pharmacia. AICAR and metformin were purchased from Sigma-Aldrich (Oakville, Canada). The AMPK inhibitor, compound C, was kindly provided by Merck (Rahway, NJ, USA). The horseradish peroxidase-conjugated goat anti-rabbit immunoglobulin antibody and zeta-probe membranes were obtained from Bio-Rad (Hercules, CA, USA). The p38 MAPK inhibitors SB 202190, SB 203580 and PD 169316, and the inactive derivative SB 202474 were purchased from Calbiochem (San Diego, CA, USA). Antibodies against AMPK and phospho-AMPK, ACC and phospho-ACC, and p38 and phospho-p38 MAPK were from Cell Signaling Technology (Danvers, MA, USA). Antibodies against PPAR α and tubulin were from Abcam (Cambridge, MA, USA).

Cell culture and treatment

INS832/13⁴⁵ cells were cultured in RPMI-1640 medium (Wisent, St-Bruno, Canada) containing 11 mM glucose supplemented with 10% fetal calf serum, 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (pH 7.4), 1 mM sodium pyruvate, and 50 μ M β -mercaptoethanol (complete medium) at 37°C in a humidified atmosphere containing 5% CO₂. Cells were seeded in 60-mm tissue culture dishes (3 \times 10⁶ cells/dish) and incubated for 24 h. On the next day, cells were preincubated for 18 h in complete culture medium containing 3 mM glucose. They were then incubated in complete culture medium at 3 mM (3G) and 20 mM (20G)

glucose for 18 h, with or without 1 mM AICAR, 2 mM metformin or 20 μ M compound C. Since we studied the role of AMPK in the effect of elevated glucose on PPAR α expression, we chose 3G and 20G as the low and high glucose concentrations, respectively, since AMPK activity is high at 3G and low at 20G. In another set of experiments, cells were seeded as above, pre-incubated for 18 h in complete culture medium containing 3 mM glucose and incubated in complete culture medium at 3G and 20G glucose for 18 h with or without the following p38 inhibitors: SB 202190, SB 203580, and PD 169316. SB 202474, an inactive compound structurally related to SB 202190, served as a negative control. Stock solutions of the inhibitors were made in dimethyl sulfoxide (DMSO) at a concentration of 1–25 mg/mL.

Reverse transcription polymerase chain reaction analysis

Total RNA was extracted from cells by the guanidium/thiocyanate phenol/chloroform extraction method.⁴⁶ First strand complementary DNA (cDNA) was synthesized from 5 μ g of total RNA in 50 μ L (final volume) of a buffer containing the Pd(N)₆ random primers and MMLV reverse transcriptase. Amplification of rat PPAR α and β -actin was carried out using the primers and experimental conditions described in Roduit et al.⁵ using a PTC-100 thermocycler (MJ Research, Watertown, MA, USA). To ensure that polymerase chain reactions (PCR) were in the linearity of the amplification, the number of cycles used for each gene under study was selected according to a pilot experiment performed with cDNAs derived from cells with similar expression levels. Ten microliters of the PCR products were subjected to electrophoresis on 1.2% agarose gels, followed by Southern blotting on a zeta-probe membrane. Specific β -actin and PPAR α DNA probes were radiolabeled using T4 polynucleotide kinase and γ -³²P-ATP (Amersham/Pharmacia), and used to hybridize the Southern blots as described previously.⁵ The blots were extensively washed and analyzed by autoradiography. For each gene studied, only one specific signal at the expected size was detected. The hybridization signals were quantitated by densitometric analysis of the autoradiograms. Expression levels were normalized for the β -actin transcript. Acyl-coenzyme A (CoA) oxidase (ACO) and uncoupling protein-2 (UCP-2) messenger RNA (mRNA) levels were determined by real time quantitative PCR (RT-qPCR) on the same cDNAs prepared for PPAR α gene expression (see above). RT-qPCR was performed using a Rotor-Gene 3000 (Corbett Robotics, San Francisco, CA, USA) and the PCR products were quantified using the FastStart DNA

Master PLUS SYBR green kit (Roche Diagnostics, Laval, Canada) according to the manufacturer's instructions. Expression levels were normalized for the β -actin transcript. The primer sequences were: β -actin, forward (5'-GTGCCCATCTATGAGGGTTACGCG-3'), reverse (5'-GGAACCGCTCATTGCCGATAGTG-3'); ACO, forward (5'-GCCCTCAGCTATGGTATTAC-3'), reverse (5'-AGGAACTGCTCTCACAATGC-3'); and UCP-2, forward (5'-CCTTGCCACTTCACTTCTGCC-3'), reverse (5'-ATCCCAAGCGGAGGAAGGAAG-3'). After amplification, the PCR products were subjected to electrophoresis on 1.0% agarose gels to confirm the specificity of the RT-qPCR.

AMPK assay

For assay of AMPK, the medium was removed and 1.0 mL of ice-cold lysis buffer [50 mM Tris/HCl, pH 7.4 at 4°C, 250 mM mannitol, 50 mM NaF, 1 mM sodium pyrophosphate, 1 mM ethylene diamine tetraacetic acid (EDTA), 1 mM ethylene glycol tetraacetic acid (EGTA), 1 mM dithiothreitol (DTT), 0.1 mM benzamidine, 0.1 mM phenylmethylsulphonyl fluoride (PMSF), 5 μ g/mL soybean trypsin inhibitor, 1% (v/v) Triton X-100] was added. The cells were scraped off with a cell scraper and the lysate transferred to a microcentrifuge tube. Extracts were vortex-mixed and centrifuged (14 000 g; 3 min; 4°C). INS832/13 cell extracts were taken for assay at this stage. Peptide kinase(s) with the amino acid sequence Ser–Ala–Met–Ser (SAMS) were assayed in crude cell lysates as follows. Reaction mixtures containing 5 μ L of assay buffer (62.5 mM Na HEPES, pH 7.0, 62.5 mM NaCl, 62.5 mM NaF, 1.25 mM sodium pyrophosphate, 1.25 mM EDTA, 1.25 mM EGTA, 1 mM DTT, 0.1 mM benzamidine, 0.1 mM PMSF, 5 μ g/mL soybean trypsin inhibitor), 5 μ L of 1 mM AMP in assay buffer, 5 μ L of 1 mM substrate peptide in assay buffer, and 5 μ L of cell extract resuspended in assay buffer were prepared on ice, and the reaction was initiated by the addition of 5 μ L of [γ -³²P]ATP solution (1 mM [γ -³²P]ATP (250–500 c.p.m./pmol), 25 mM MgCl₂). Assays were then conducted as described previously by Davies et al.⁴⁷

Immunoblot analysis

After treatments, the cell extracts were prepared and immunoblots performed for AMPK α , phosphorylated AMPK α (pAMPK α), ACC and phosphorylated ACC (pACC), according to Hamilton et al.⁴⁸ The detection of p38 MAPK phosphorylation was performed according to the manufacturer's protocol (Cell Signaling Technology).

Adenovirus production and cells infection

An adenovirus that expresses the dominant negative (DN) form of AMPK was employed in these studies.³⁴ INS832/13 cells were infected in complete medium with adenovirus encoding the DN-AMPK under the control of the cytomegalovirus promoter (AdCMV-DN-AMPK) or AdCMV- β -galactosidase (as the control) at 10 pfu/cell for 18 h. Cells were then washed with phosphate-buffered saline and further cultured for 18 h prior to incubation at 3G or 20G.

Statistical analysis

Statistical significance was calculated with the unpaired two-tailed Student's *t*-test or two-way analysis of variance (ANOVA) with appropriate post-hoc test.

Results

Pharmacological evidence for the implication of AMPK in glucose-induced down regulation of PPAR α gene expression

As reported in the β -cell lines HIT-T15 and INS1,^{11,49} Fig. 1a shows that AMPK activity is decreased in

INS832/13 cells incubated at high glucose for 18 h. We confirmed in the same series of experiments that glucose reduced the expression level of the PPAR α transcript (Fig. 1b) and protein (Fig. 1d,e).

To assess the possible role of AMPK in the glucose modulation of the PPAR α gene, we tested the effects of the AMPK activators AICAR⁵⁰ and metformin.²³ At low glucose, both AMPK activators induced the PPAR α transcript by twofold (Fig. 1b). The high glucose-induced decrease of PPAR α expression was abolished by the AMPK activators both at the mRNA (Fig. 1b) and protein (Fig. 1d,e) levels. In contrast, the AMPK inhibitor compound C mimicked the glucose effect on PPAR α mRNA (Fig. 1c). Compound C reduced PPAR α mRNA level by 65% at low glucose, and the effect of elevated glucose was not additive to that of compound C.

Studies with a DN form of AMPK

AdCMV-DN-AMPK allows the expression of a DN form of the AMPK α 1 subunit.^{34,51} This α 1 subunit is mutated in its T172 phosphorylation site, rendering it inactive. It competes with endogenous α subunits for the binding of endogenous non-catalytic β and γ

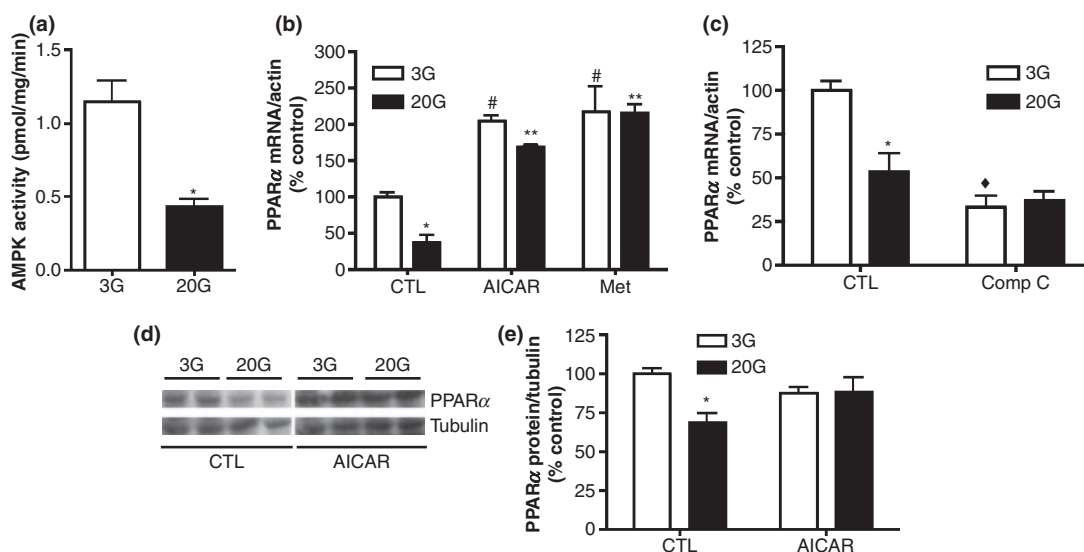


Figure 1 Adenosine monophosphate-activated protein kinase (AMPK) activators and inhibitors modulate the action of glucose on peroxisome proliferator-activated receptor α (PPAR α) gene expression. (a) INS832/13 cells were incubated for 18 h at 3 mM (3G) or 20 mM (20G) glucose. Protein lysate from treated cells were analyzed for AMPK activity using the SAMS peptide kinase assay. In panels b and c, cells were incubated for 18 h at low (3G) or high (20G) glucose in the presence or the absence (CTL) of 1 mM 5-amino-4-imidazolecarboxamide riboside (AICAR) or 2 mM metformin (Met) (b, d, and e), or in the presence of 20 μ M of compound C (Comp C) (c). PPAR α and β -actin messenger RNA (mRNA) levels were measured by reverse transcription polymerase chain reaction (RT-PCR) (b and c), and PPAR α and β -tubulin protein levels were measured by Western blots (d and e). Means \pm SEM of three experiments in triplicate for RT-PCR experiments and in duplicate for Western blot experiments. (a) **P* < 0.02 vs 3G control; (b) **P* < 0.02 vs 3G control, ***P* < 0.001 vs 20G control, #*P* < 0.0001 vs 3G control for AICAR and #*P* < 0.01 vs 3G control for metformin; (c) **P* < 0.05 vs 3G control; ♦*P* < 0.01 vs 3G control; (e) **P* < 0.05 vs 3G control.

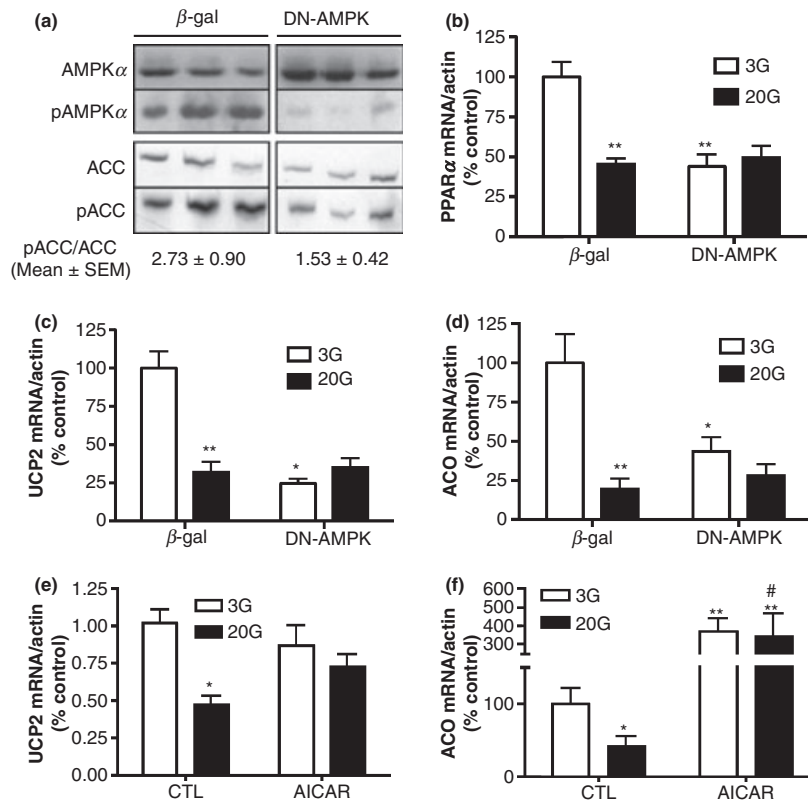


Figure 2 Effects of 5-amino-4-imidazolecarboxamide riboside (AICAR) and a dominant negative adenosine monophosphate-activated protein kinase (AMPK) on the expression of the peroxisome proliferator-activated receptor α (PPAR α) transcript and its target genes. INS 832/13 cells were preincubated for 24 h in RPMI-1640 medium containing 11 mM glucose prior to infection with 10 pfu/cell of AdCMV-DN-AMPK (DN-AMPK) and AdCMV- β -gal (β -gal). After 18 h cells were washed with PBS and incubated for 30 h at 3 mM glucose prior analysis by Western blot (a), or incubated for 18 h at 3 mM glucose and then 18 h at 3 mM (3G) or 20 mM (20G) glucose (b–d). (a) Protein lysate of the treated cells were blotted with specific antibodies against AMPK α , phosphorylated AMPK α (pAMPK α), acetyl-coenzyme A carboxylase (ACC), and phosphorylated ACC (pACC). The pACC/ACC and pAMPK/AMPK ratios are representative of AMPK activity. (b–d) PPAR α , uncoupling protein 2 (UCP-2), acyl-coenzyme A oxidase (ACO) and β -actin messenger RNA (mRNA) levels were measured by real time quantitative polymerase chain reaction. (e, f) INS832/13 cells were incubated for 18 h at 3G or 20G in the presence or the absence (CTL) of 1 mM AICAR. UCP-2, ACO and β -actin mRNA levels were measured by RT-qPCR. Means \pm SEM of three experiments in triplicate. (b) ** P < 0.01 vs 3G control; (c) * P < 0.005 vs 3G control, ** P < 0.01 vs 3G control; (d) * P < 0.01 vs 3G control, ** P < 0.005 vs 3G control; (e) * P < 0.05 vs 3G control; (f) * P < 0.05 vs 3G control; ** P < 0.01 vs 3G control, # P < 0.001 vs 20G control.

subunits, thus causing more rapid turnover and degradation of both the α 1 and α 2 subunits.⁵¹ Cells were infected with AdCMV-DN-AMPK and AdCMV- β -galactosidase (β -gal) as a control. Figure 2a shows little change in total AMPK α expression after infection with AdCMV-DN-AMPK. However, as we have previously documented, the degradation of the native α subunit being enhanced,⁵¹ this band now largely represents the DN gene product. Most importantly, we observed an absence of AMPK172 α phosphorylation, indicating AMPK inactivity with expression of the DN construct (see row pAMPK α). The effectiveness of the DN-AMPK in inhibiting AMPK activity is also apparent from the observed pACC/ACC ratio, which reflects AMPK activity. Infection of cells with AdCMV-

DN-AMPK reduced the pACC/ACC ratio (control virus [β -gal]: 2.73 \pm 0.9; AdCMV-DN-AMPK 1.53 \pm 0.42; mean \pm SEM of n = 3; P < 0.01). Chronic exposure of INS832/13 cells infected with the control adenovirus (β -gal) to elevated glucose resulted in a 40–50% decrease in PPAR α transcript (Fig. 2b). In the presence of AdCMV-DN-AMPK, the PPAR α mRNA level was low at both concentrations of glucose. The DN-AMPK thus mimicked the glucose effect on PPAR α gene expression.

To assess the biological consequence of AMPK-induced changes in PPAR α expression, the expression of the ACO and UCP-2 transcripts was determined in cells following infection with AdCMV-DN-AMPK or AdCMV- β -gal (Fig. 2c,d). Inactivation of AMPK

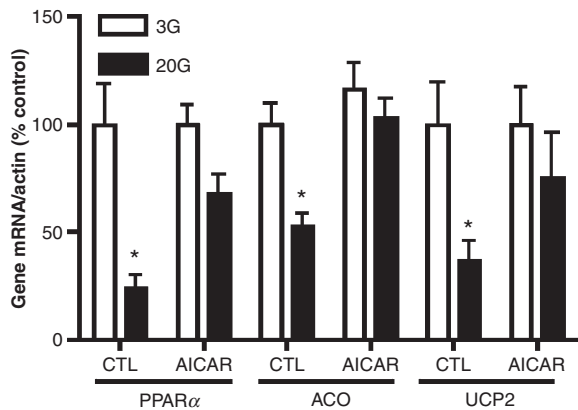


Figure 3 Effect of 5-amino-4-imidazolecarboxamide riboside (AICAR) on the expression of the peroxisome proliferator-activated receptor α (PPAR α), acyl-coenzyme A oxidase (ACO) and uncoupling protein 2 (UCP-2) transcripts in isolated rat islets. Islets were incubated for 18 h at 3 mM (3G) or 20 mM (20G) glucose in the presence or the absence (CTL) of 1 mM AICAR. Means \pm SEM of four separate experiments carried out in triplicate. * P < 0.05 vs 3G control. mRNA, messenger RNA.

decreased ACO and UCP-2 mRNA to the same extent as elevated glucose and abolished the action of the sugar. Conversely, AMPK activation by AICAR increased the expression of the ACO transcript at low glucose, suppressed the repressive effect of high glucose (Fig. 2f), and suppressed the inhibitory effect of high glucose on the UCP-2 transcript (Fig. 2e).

Studies with isolated rat islets

The expression of the PPAR α , ACO and UCP-2 transcripts^{2,5} were determined in isolated rat islets incubated at low and high glucose in the absence or presence of AICAR (Fig. 3). The abundance of all three transcripts was decreased in the presence of elevated glucose. Activation of AMPK with AICAR reversed the action of glucose.

p38 MAPK is not implicated in the glucose-induced repression of the PPAR α gene

Since p38 MAPK is a target of AMPK and that p38 MAPK modulates the transcription of many genes,^{29,31,32,41–44,52} it was reasonable to hypothesize that glucose modulates the expression of the PPAR α gene via AMPK and p38 MAPK. Figure 1a shows that high glucose decreased the phosphorylation state of p38 MAPK, consistent with the view that p38 MAPK is a target of AMPK in INS832/13 cells. The positive control anisomycin, an activator of p38 MAPK, increased the ratio of phospho-p38/p38; however, none of the p38

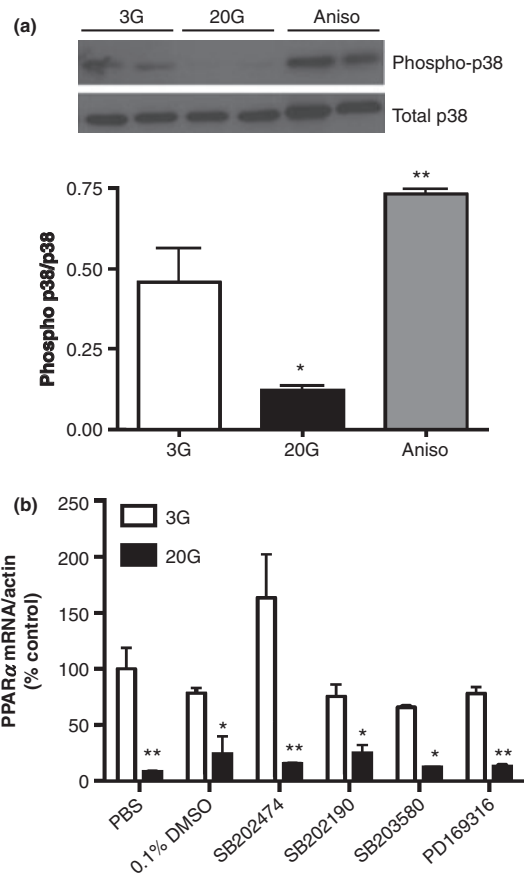


Figure 4 Glucose-induced changes in the expression of the peroxisome proliferator-activated receptor α (PPAR α) gene is not mediated by p38 mitogen-activated protein kinase (p38 MAPK). (a) INS832/13 cells were serum-starved for 1 h in RPMI-1640 medium and then incubated for 10 min at 3 mM (3G) or 20 mM (20G) glucose without or with the p38 activator anisomycin (Aniso). Protein lysates from treated cells were analyzed for phosphorylation of p38 by Western blot. Means \pm SEM of three separate experiments carried out in duplicates. (b) INS832/13 cells were incubated for 18 h at 3G or 20G in the absence (PBS and 0.1% DMSO) or presence of various p38 MAPK inhibitors (SB202190, SB203580, PD169316) and the inactive compound SB202474. The final concentration of inhibitors was 5 μ M, except for PD169316 (1 μ M). Means \pm SEM of three independent experiments carried out in triplicates. (a) * P < 0.02 vs 3G control, ** P < 0.0001 vs 20G; (b) * P < 0.05 vs 3G control, ** P < 0.02 vs 3G control.

MAPK inhibitors used at supramaximal concentration ($> 10 \times IC_{50}$)^{53–56} decreased PPAR α mRNA at low or high glucose (Fig. 4b), indicating that p38 MAPK is not involved in the regulation of PPAR α expression by glucose.

Discussion

The results show that AMPK is involved in the regulation of PPAR α gene expression and of its target genes

by glucose in the β -cell. Thus, using a pharmacological approach with two chemically unrelated AMPK activators (AICAR and metformin) we have observed that the activation of AMPK leads to an increase in the expression of the PPAR α gene and suppresses the action of glucose. By contrast, when AMPK activity is reduced by a pharmacological agent (compound C) or via the expression of a DN-AMPK, the expression of the PPAR α transcript at low glucose is reduced to a similar extent as elevated glucose, and the action of elevated glucose is not additive to that of DN-AMPK or compound C. Furthermore, AMPK activation or inhibition also modified the expression of the two PPAR α target genes ACO and UCP-2 and counteracted the action of glucose. We reported before that glucose-induced repression of the PPAR α transcript is associated with a similar effect at the protein level and the DNA binding activity of PPAR α .⁵ We also reported that glucose induces the down-regulation of PPAR α in normal rat pancreatic islets.⁵ In addition, we showed that PPAR α protein levels parallel changes of the AMPK transcript in response to glucose and AICAR.

AMPK activation by AICAR has been reported to inhibit glucose-induced stimulation of L-PK, fatty acid synthase, Spot 14 (S14) and ACC gene expression.^{16,18} Using constitutively active and DN forms of the kinase, Woods et al.⁵⁷ have suggested that AMPK is involved in the inhibition of expression of some genes modulated by glucose, in particular L-PK, fatty acid synthase,¹⁵ and S14. The results of the present study provide additional support to the view that the down-regulation of key transcriptional factor and metabolic genes by glucose are mediated via AMPK. Further experiments are needed to clarify the detailed mechanisms of such regulation. Mandrup et al.⁵⁸ reported that glucose regulates PPAR α gene expression in INS-1E cells via a mechanism implicating the AMPK α 2 subunit and possibly protein phosphatase 2A. The present work is in accordance with this study and extends it by showing that glucose regulates both PPAR α expression and its target genes in normal islet tissue, and also by showing that the AMPK target p38MAPK is not implicated in this process.

Much support has been adduced for a role of AMPK in the regulation of muscle fatty acid oxidation^{6,8} via inhibition of ACC activity, which causes a reduction in malonyl-CoA levels, thereby increasing metabolic flux through carnitine palmitoyltransferase-1 that catalyses the limiting step of fat oxidation. Evidence that AMPK activates malonyl-CoA decarboxylase (MCD) during muscle contraction, thus, leading to a decrease in malonyl-CoA levels and a rise in fat

oxidation has been documented,⁵⁹ although others have not observed such an effect.⁶⁰ Overall these considerations suggest that fat oxidation is under the control of multiple enzymes (CPT-1, ACC, MCD) and transcriptional factors (PPAR α , SREBP1c and PGC1 α), which are regulated by AMPK.⁶ A study reported the presence of a PPAR responsive element in the MCD promoter,⁶¹ and suggested that PPAR α may regulate malonyl-CoA levels and fat oxidation via changes in MCD expression.⁶¹ Using PPAR α deficient-mice, we provided evidence that PPAR α regulates fat oxidation and glucose-induced insulin secretion via lipid signaling during the fed and fasted states.⁶² Whether PPAR α regulates fat oxidation in the β -cell in part via MCD gene expression remains to be clarified. It can be hypothesized that AMPK plays a role in the regulation of β -cell fatty acid oxidation through additional enzymes/proteins that are modulated by PPAR α in particular ACO and UCP-2⁵ and as well as other PPAR α target genes implicated in fatty acid oxidation. Thus, PPAR α provides an attractive link between enhanced AMPK activity and fat oxidation in various tissues.

A role for AMPK in the regulation of PPAR α expression is possibly of importance with respect to β -cell failure in diabetes.⁶ Thus, we have postulated that glucose-induced down-regulation of PPAR α expression may contribute to β -cell "glucolipototoxicity"^{7,63} via decreased expression of genes involved in fat oxidation. The results also provide additional support for the view that AMPK controls lipid partitioning not only via short term changes, such as the phosphorylation state of ACC and MCD, but also via long-term actions through the regulation of gene expression, in particular PPAR α .

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Disclosure

The authors have nothing to disclose from a financial point of view. The authors state that the manuscript has never been published before and is not submitted elsewhere.

References

- Willson TM, Brown PJ, Sternbach DD, Henke BR. The PPARs: From orphan receptors to drug discovery. *J Med Chem.* 2000; **43**: 527–50.
- Mandard S, Muller M, Kersten S. Peroxisome proliferator-activated receptor alpha target genes. *Cell Mol Life Sci.* 2004; **61**: 393–416.
- Alaynick WA. Nuclear receptors, mitochondria and lipid metabolism. *Mitochondrion.* 2008; **8**: 329–37.
- Jump DB, Botolin D, Wang Y, Xu J, Christian B, Demeure O. Fatty acid regulation of hepatic gene transcription. *J Nutr.* 2005; **135**: 2503–6.
- Roduit R, Morin J, Masse F et al. Glucose down-regulates the expression of the peroxisome proliferator-activated receptor-alpha gene in the pancreatic beta-cell. *J Biol Chem.* 2000; **275**: 35799–806.
- Ruderman N, Prentki M. AMP kinase and malonyl-CoA: Targets for therapy of the metabolic syndrome. *Nat Rev Drug Discov.* 2004; **3**: 340–51.
- Prentki M, Nolan CJ. Islet beta cell failure in type 2 diabetes. *J Clin Invest.* 2006; **116**: 1802–12.
- Winder WW, Hardie DG. AMP-activated protein kinase, a metabolic master switch: Possible roles in type 2 diabetes. *Am J Physiol.* 1999; **277**: E1–10.
- Kemp BE, Mitchelhill KI, Stapleton D, Michell BJ, Chen ZP, Witters LA. Dealing with energy demand: The AMP-activated protein kinase. *Trends Biochem Sci.* 1999; **24**: 22–5.
- Hardie DG. AMPK: A key regulator of energy balance in the single cell and the whole organism. *Int J Obes (Lond).* 2008; **32** (Suppl. 4): S7–12.
- Louis NA, Witters LA. Glucose regulation of acetyl-CoA carboxylase in hepatoma and islet cells. *J Biol Chem.* 1992; **267**: 2287–93.
- Salt IP, Johnson G, Ashcroft SJ, Hardie DG. AMP-activated protein kinase is activated by low glucose in cell lines derived from pancreatic beta cells, and may regulate insulin release. *Biochem J.* 1998; **335**(Pt 3): 533–9.
- da Silva Xavier G, Leclerc I, Salt IP et al. Role of AMP-activated protein kinase in the regulation by glucose of islet beta cell gene expression. *Proc Natl Acad Sci U S A.* 2000; **97**: 4023–8.
- Hardie DG, Carling D. The AMP-activated protein kinase: Fuel gauge of the mammalian cell? *Eur J Biochem.* 1997; **246**: 259–73.
- Van de Casteele M, Kefas BA, Ling Z, Heimberg H, Pipeleers DG. Specific expression of Bax-omega in pancreatic beta-cells is down-regulated by cytokines before the onset of apoptosis. *Endocrinology.* 2002; **143**: 320–6.
- Foretz M, Carling D, Guichard C, Ferre P, Foufelle F. AMP-activated protein kinase inhibits the glucose-activated expression of fatty acid synthase gene in rat hepatocytes. *J Biol Chem.* 1998; **273**: 14767–71.
- Lochhead PA, Salt IP, Walker KS, Hardie DG, Sutherland C. 5-Aminoimidazole-4-carboxamide riboside mimics the effects of insulin on the expression of the 2 key gluconeogenic genes PEPCK and glucose-6-phosphatase. *Diabetes.* 2000; **49**: 896–903.
- Leclerc I, Lenzner C, Gourdon L, Vaulont S, Kahn A, Viollet B. Hepatocyte nuclear factor-4alpha involved in type 1 maturity-onset diabetes of the young is a novel target of AMP-activated protein kinase. *Diabetes.* 2001; **50**: 1515–21.
- Hong YH, Varanasi US, Yang W, Leff T. AMP-activated protein kinase regulates HNF4alpha transcriptional activity by inhibiting dimer formation and decreasing protein stability. *J Biol Chem.* 2003; **278**: 27495–501.
- Leff T. AMP-activated protein kinase regulates gene expression by direct phosphorylation of nuclear proteins. *Biochem Soc Trans.* 2003; **31**: 224–7.
- Kawaguchi T, Osatomi K, Yamashita H, Kabashima T, Uyeda K. Mechanism for fatty acid “sparing” effect on glucose-induced transcription: regulation of carbohydrate-responsive element-binding protein by AMP-activated protein kinase. *J Biol Chem.* 2002; **277**: 3829–35.
- Yang W, Hong YH, Shen XQ, Frankowski C, Camp HS, Leff T. Regulation of transcription by AMP-activated protein kinase: Phosphorylation of p300 blocks its interaction with nuclear receptors. *J Biol Chem.* 2001; **276**: 38341–4.
- Zhou G, Myers R, Li Y et al. Role of AMP-activated protein kinase in mechanism of metformin action. *J Clin Invest.* 2001; **108**: 1167–74.
- Jäger S, Handschin C, St-Pierre J, Spiegelman BM. AMP-activated protein kinase (AMPK) action in skeletal muscle via direct phosphorylation of PGC-1alpha. *Proc Natl Acad Sci U S A.* 2007; **104**: 12017–22.
- Terada S, Goto M, Kato M, Kawanaka K, Shimokawa T, Tabata I. Effects of low-intensity prolonged exercise on PGC-1 mRNA expression in rat epitrochlearis muscle. *Biochem Biophys Res Commun.* 2002; **296**: 350–4.
- Suwa M, Egashira T, Nakano H, Sasaki H, Kumagai S. Metformin increases the PGC-1alpha protein and oxidative enzyme activities possibly via AMPK phosphorylation in skeletal muscle in vivo. *J Appl Physiol.* 2006; **101**: 1685–92.
- Irrcher I, Ljubcic V, Hood DA. Interactions between ROS and AMP kinase activity in the regulation of PGC-1alpha transcription in skeletal muscle cells. *Am J Physiol Cell Physiol.* 2009; **296**: C116–23.
- Irrcher I, Ljubcic V, Kirwan AF, Hood DA. AMP-activated protein kinase-regulated activation of the PGC-1alpha promoter in skeletal muscle cells. *PLoS ONE.* 2008; **3**: e3614.
- Zarubin T, Han J. Activation and signaling of the p38 MAP kinase pathway. *Cell Res.* 2005; **15**: 11–8.

30. Ashwell JD. The many paths to p38 mitogen-activated protein kinase activation in the immune system. *Nat Rev Immunol.* 2006; **6**: 532–40.
31. Bradham C, McClay DR. p38 MAPK in development and cancer. *Cell Cycle.* 2006; **5**: 824–8.
32. Lluís F, Perdiguero E, Nebreda AR, Muñoz-Canoves P. Regulation of skeletal muscle gene expression by p38 MAP kinases. *Trends Cell Biol.* 2006; **16**: 36–44.
33. Cao WH, Xiong Y, Collins QF, Liu HY. p38 mitogen-activated protein kinase plays a critical role in the control of energy metabolism and development of cardiovascular diseases. *Zhong Nan Da Xue Xue Bao Yi Xue Ban.* 2007; **32**: 1–14.
34. Pelletier A, Joly E, Prentki M, Coderre L. Adenosine 5'-monophosphate-activated protein kinase and p38 mitogen-activated protein kinase participate in the stimulation of glucose uptake by dinitrophenol in adult cardiomyocytes. *Endocrinology.* 2005; **146**: 2285–94.
35. Li J, Miller EJ, Ninomiya-Tsuji J, Russell RR 3rd, Young LH. AMP-activated protein kinase activates p38 mitogen-activated protein kinase by increasing recruitment of p38 MAPK to TAB1 in the ischemic heart. *Circ Res.* 2005; **97**: 872–9.
36. Capano M, Crompton M. Bax translocates to mitochondria of heart cells during simulated ischaemia: Involvement of AMP-activated and p38 mitogen-activated protein kinases. *Biochem J.* 2006; **395**: 57–64.
37. Xi X, Han J, Zhang JZ. Stimulation of glucose transport by AMP-activated protein kinase via activation of p38 mitogen-activated protein kinase. *J Biol Chem.* 2001; **276**: 41029–34.
38. Cheng Z, Pang T, Gu M et al. Berberine-stimulated glucose uptake in L6 myotubes involves both AMPK and p38 MAPK. *Biochim Biophys Acta.* 2006; **1760**: 1682–9.
39. Du JH, Xu N, Song Y et al. AICAR stimulates IL-6 production via p38 MAPK in cardiac fibroblasts in adult mice: A possible role for AMPK. *Biochem Biophys Res Commun.* 2005; **337**: 1139–44.
40. Yoon MJ, Lee GY, Chung JJ, Ahn YH, Hong SH, Kim JB. Adiponectin increases fatty acid oxidation in skeletal muscle cells by sequential activation of AMP-activated protein kinase, p38 mitogen-activated protein kinase, and peroxisome proliferator-activated receptor alpha. *Diabetes.* 2006; **55**: 2562–70.
41. D'Addario M, Arora PD, McCulloch CA. Role of p38 in stress activation of Sp1. *Gene.* 2006; **379**: 51–61.
42. Zdanov S, Debacq-Chainiaux F, Remaclé J, Toussaint O. Identification of p38MAPK-dependent genes with changed transcript abundance in H₂O₂-induced premature senescence of IMR-90 hTERT human fibroblasts. *FEBS Lett.* 2006; **580**: 6455–63.
43. Zhang S, Liu H, Liu J, Tse CA, Dragunow M, Cooper GJ. Activation of activating transcription factor 2 by p38 MAP kinase during apoptosis induced by human amylin in cultured pancreatic beta-cells. *FEBS J.* 2006; **273**: 3779–91.
44. Stassen M, Klein M, Becker M et al. p38 MAP kinase drives the expression of mast cell-derived IL-9 via activation of the transcription factor GATA-1. *Mol Immunol.* 2007; **44**: 926–33.
45. Hohmeier HE, Mulder H, Chen G, Henkel-Rieger R, Prentki M, Newgard CB. Isolation of INS-1-derived cell lines with robust ATP-sensitive K⁺ channel-dependent and -independent glucose-stimulated insulin secretion. *Diabetes.* 2000; **49**: 424–30.
46. Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987; **162**: 156–9.
47. Davies SP, Carling D, Hardie DG. Tissue distribution of the AMP-activated protein kinase, and lack of activation by cyclic-AMP-dependent protein kinase, studied using a specific and sensitive peptide assay. *Eur J Biochem.* 1989; **186**: 123–8.
48. Hamilton SR, Stapleton D, O'Donnell JB Jr et al. An activating mutation in the gamma1 subunit of the AMP-activated protein kinase. *FEBS Lett.* 2001; **500**: 163–8.
49. Chen L, Alam T, Johnson JH, Hughes S, Newgard CB, Unger RH. Regulation of beta-cell glucose transporter gene expression. *Proc Natl Acad Sci U S A.* 1990; **87**: 4088–92.
50. Corton JM, Gillespie JG, Hawley SA, Hardie DG. 5-Aminoimidazole-4-carboxamide ribonucleoside. A specific method for activating AMP-activated protein kinase in intact cells? *Eur J Biochem.* 1995; **229**: 558–65.
51. Crute BE, Seefeld K, Gamble J, Kemp BE, Witters LA. Functional domains of the alpha1 catalytic subunit of the AMP-activated protein kinase. *J Biol Chem.* 1998; **273**: 35347–54.
52. Jeong HW, Hsu KC, Lee JW et al. Berberine suppresses proinflammatory responses through AMPK activation in macrophages. *Am J Physiol Endocrinol Metab.* 2009; **296**: E955–64.
53. Singh RP, Dhawan P, Golden C, Kapoor GS, Mehta KD. One-way cross-talk between p38(MAPK) and p42/44(MAPK). Inhibition of p38(MAPK) induces low density lipoprotein receptor expression through activation of the p42/44(MAPK) cascade. *J Biol Chem.* 1999; **274**: 19593–600.
54. Davies SP, Reddy H, Caivano M, Cohen P. Specificity and mechanism of action of some commonly used protein kinase inhibitors. *Biochem J.* 2000; **351**: 95–105.

55. Nath R, McGinnis K, Dutta S, Shivers B, Wang KK. Inhibition of p38 kinase mimics survival signal-linked protection against apoptosis in rat cerebellar granule neurons. *Cell Mol Biol Lett*. 2001; **6**: 173–84.
56. Young PR, McLaughlin MM, Kumar S et al. Pyridinyl imidazole inhibitors of p38 mitogen-activated protein kinase bind in the ATP site. *J Biol Chem*. 1997; **272**: 12116–21.
57. Woods A, Azzout-Marniche D, Foretz M et al. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol Cell Biol*. 2000; **20**: 6704–11.
58. Ravnskjaer K, Boergesen M, Dalgaard LT, Mandrup S. Glucose-induced repression of PPAR α gene expression in pancreatic beta-cells involves PP2A activation and AMPK inactivation. *J Mol Endocrinol*. 2006; **36**: 289–99.
59. Saha AK, Schwarsin AJ, Roduit R et al. Activation of malonyl-CoA decarboxylase in rat skeletal muscle by contraction and the AMP-activated protein kinase activator 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside. *J Biol Chem*. 2000; **275**: 24279–83.
60. Habinowski SA, Hirshman M, Sakamoto K et al. Malonyl-CoA decarboxylase is not a substrate of AMP-activated protein kinase in rat fast-twitch skeletal muscle or an islet cell line. *Arch Biochem Biophys*. 2001; **396**: 71–9.
61. Lee GY, Kim NH, Zhao ZS, Cha BS, Kim YS. Peroxisomal-proliferator-activated receptor α activates transcription of the rat hepatic malonyl-CoA decarboxylase gene: A key regulation of malonyl-CoA level. *Biochem J*. 2004; **378**: 983–90.
62. Gremlich S, Nolan C, Roduit R et al. Pancreatic islet adaptation to fasting is dependent on peroxisome proliferator-activated receptor α transcriptional up-regulation of fatty acid oxidation. *Endocrinology*. 2005; **146**: 375–82.
63. Prentki M, Joly E, El-Assaad W, Roduit R. Malonyl-CoA signaling, lipid partitioning, and glucolipototoxicity: Role in beta-cell adaptation and failure in the etiology of diabetes. *Diabetes*. 2002; **51** (Suppl. 3): S405–13.