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# Beta Interferon Regulation of Glucose Metabolism Is PI3K/Akt Dependent and Important for Antiviral Activity against Coxsackievirus B3

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**ABSTRACT**

**An effective type I interferon (IFN)-mediated immune response requires the rapid expression of antiviral proteins that are necessary to inhibit viral replication and virus spread. We provide evidence that IFN-β regulates metabolic events important for the induction of a rapid antiviral response: IFN-β decreases the phosphorylation of AMP-activated protein kinase (AMPK), coincident with an increase in intracellular ATP. Our studies reveal a biphasic IFN-β-inducible uptake of glucose by cells, mediated by phosphatidylinositol 3-kinase (PI3K)/Akt, and IFN-β-inducible regulation of GLUT4 translocation to the cell surface. Additionally, we provide evidence that IFN-β-regulated glycolytic metabolism is important for the acute induction of an antiviral response during infection with coxsackievirus B3 (CVB3). Last, we demonstrate that the antidiabetic drug metformin enhances the antiviral potency of IFN-β against CVB3 both *in vitro* and *in vivo*. Taken together, these findings highlight an important role for IFN-β in modulating glucose metabolism during a virus infection and suggest that the use of metformin in combination with IFN-β during acute virus infection may result in enhanced antiviral responses.**

**IMPORTANCE**

**Type I interferons (IFN) are critical effectors of an antiviral response. These studies describe for the first time a role for IFN-β in regulating metabolism—glucose uptake and ATP production—to meet the energy requirements of a robust cellular antiviral response. Our data suggest that IFN-β regulates glucose metabolism mediated by signaling effectors similarly to activation by insulin. Interference with IFN-β-inducible glucose metabolism diminishes the antiviral response, whereas treatment with metformin, a drug that increases insulin sensitivity, enhances the antiviral potency of IFN-β.**

Type I interferons (alpha and beta interferons [IFN-α/β]) are pleiotropic cytokines that were originally identified for their ability to interfere with viral replication (1) and are now recognized for their potent immunomodulatory effects (2–4). Engagement of their cognate heterodimeric receptor, comprised of IFNAR1 and IFNAR2, initiates signaling that culminates in the expression of interferon-stimulated gene (ISG)-associated proteins, critical for antiviral activity. Given the rapid replication of viruses, in the order of several hours (5–8), the IFN-α/β response must be equally fast and robust, with rapid production of IFN-β and the subsequent activation of signaling cascades downstream of IFNAR1 and IFNAR2 within hours of infection (9–12). IFNAR activation by IFN results in the induction of ISGs (13–15). This rapid response initiated by IFN-αs and IFN-β is governed by a series of signaling effectors that are intermediates in the JAK/STAT, mitogen-activated protein kinase (MAPK), and phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR) pathways, which coordinately regulate the transcriptional and translational expression of ISGs (3, 16).

Previously, we and others have shown signaling effectors in the PI3K/mTOR pathway to be critical in governing an effective IFN-α/β-mediated antiviral response. Cells lacking p85α and -β (p85α/β) or Akt1 and -2 (Akt1/2) showed defective antiviral responses and reduced IFN-α/β-inducible ISG protein expression (17–19). Pharmacological inhibition of PI3K, Akt, or mTOR inhibits IFN-β-mediated suppression of hepatitis C virus (HCV) in a cell-based replicon system (20). Additionally, cells lacking repressors of IFN-α/β-mediated translational regulation, namely, TSC2 or 4E-BP1, show enhanced responsiveness to IFN-α/β and greater inducible expression of ISG proteins (21, 22). In mice lacking the translational suppressor 4E-BP1, we also showed enhanced IFN-β antiviral potency in infection with coxsackievirus B3 (CVB3) (22).

Since protein synthesis consumes a large proportion of cellular ATP, cellular processes are required to maintain energy homeostasis during the induction of translation. AMP-activated protein kinase (AMPK), an important sensor of cellular ATP flux, is invoked to balance energy-consuming pathways, mediated by regulation of mTOR and glucose uptake (23). Indeed, various growth factors (insulin, platelet-derived growth factor [PDGF], insulin-like growth factor 1 [IGF-1], and vascular endothelial growth factor [VEGF]) and cytokines (interleukin-3 [IL-3], IL-5, IL-6, IL-7, granulocyte-macrophage colony-stimulating factor [GM-CSF], tumor necrosis factor-alpha [TNF-α], and CCL5) that signal through PI3K/Akt/mTOR have been shown to regulate glucose metabolism, specifically through the PI3K/Akt/mTOR pathway (24–36). Cognizant that IFN-α/β engage PI3K/Akt/mTOR signal-

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ing to upregulate protein synthesis, we undertook studies to investigate any influence that IFN-β may exert on glucose metabolism in the context of protection from viral infection. Our data suggest IFN-β mobilization of metabolic events. Given the common signaling effectors between IFN-β and insulin, downstream from their respective cell surface receptors, we examined the effects of metformin, an insulin sensitizer, during an acute viral infection with CVB3. Our data reveal that IFN-β treatment engages mechanisms that meet the energy requirements of cells, thereby enabling a IFN-β-induced antiviral response, and that metformin enhances the antiviral effects of IFN-β.

## MATERIALS AND METHODS

**Cells, virus, and reagents.** Recombinant mouse IFN-β was provided by Darrin Baker, Biogen Idec (Cambridge, MA, USA). Human insulin was purchased from Eli Lilly. Immortalized mouse embryonic fibroblast (MEF) cultures derived from transgenic mice are described elsewhere, p85a$^{-/-}$ β$^{-/-}$ MEFs in references 18, 37, 38, and 39, Akt1$^{-/-}$/2$^{-/-}$ in references 19, 40, and 41, TSC2$^{-/-}$ MEFs in references 21, 42, and 43, and AMPKa1$^{-/-}$/a2$^{-/-}$ MEFs in references 44 and 45. Cells were cultured in RPMI 1640 (Sigma) supplemented with 10% fetal calf serum (FCS) (HyClone) and antibiotics. Coxsackievirus B3-CG (CVB3) was available in the laboratory as a stock of $1.3 \times 10^9$ PFU/ml. Monoclonal anti-phospho-AMPK (Thr172) was purchased from Cell Signaling, and monoclonal anti-alpha-tubulin was purchased from Sigma (Mississauga, ON, Canada). Monoclonal anti-phosho-STAT1 (Tyr 701) and monoclonal anti-ISG15 were purchased from Cell Signaling Technology (Danvers, Massachusetts). Monoclonal anti-GLUT4 (clone 3G10A3) was purchased from Abcam (Cambridge, United Kingdom). Metformin was purchased from Sigma (St. Louis, MO). 2-Deoxy-D-glucose (2-DG) was purchased from Sigma (Mississauga, ON, Canada). 2-[1,2-$^3$H(*N*)]-deoxy-D-glucose was purchased from PerkinElmer (Waltham, MA, USA).

**Cell lysis and immunoblotting.** Cells were cultured in medium containing 2% FCS for 16 h and then left untreated or treated for the times indicated below either with 10 mM 2-DG in the absence or presence of 1,000 U/ml IFN-β or with 1,000 U/ml IFN-β alone, after which the medium was aspirated and the cells lysed with radioimmunoprecipitation (RIPA) buffer (Cell Signaling) containing a protease and phosphatase inhibitor cocktail (Cell Signaling). 5× Laemmli-reducing buffer was added, and samples boiled for 10 min. An amount of 30 μg of protein lysate was resolved on a 12% SDS–PAGE gel, transferred overnight to an Immobilon polyvinylidene difluoride (PVDF) membrane, and blocked in TBST containing 5% bovine serum albumin (BSA) (wt/vol) and 0.1% Tween 20 (vol/vol). The blots were then probed with the antibodies indicated below and visualized by chemiluminescence (Bio-Rad).

**Glucose uptake assay.** Subconfluent cell monolayers were cultured in 6-well plates in 2% FCS medium for 16 h at 37°C in 5% $CO_2$ and then treated with vehicle, IFN-β, or insulin at the doses and for the times indicated below. The cells were washed twice with Krebs Ringer HEPES (KRH) buffer, followed by the addition of 1 ml of KRH containing 0.5 μCi/ml 2-[1,2-$^3$H(*N*)]-deoxy-D-glucose (29.8 Ci/mmol). The cells were then incubated at 37°C for 10 min, and $^3$H-2-deoxy-D-glucose ($^3$H-2-DG) uptake was terminated quickly by placing plates on ice and washing 3 times with ice-cold phosphate-buffered saline (PBS). The cells were then lysed by the addition of 500 μl of Milli-Q water followed by freezing and thawing. $^3$H-2-DG uptake was measured in a liquid scintillation counter (PerkinElmer).

**Intracellular ATP determination.** Subconfluent monolayers of MEFs were cultured in 10-mm plates in 2% FCS medium for 16 h prior to treatment with murine IFN-β (mIFN-β) or 2-DG. The cells were treated with 10 mM 2-DG or control medium for 30 min prior to the addition of mIFN-β for 1 h. The medium was aspirated, and the cells immediately lysed by the addition of 2.5% trichloroacetic acid (TCA), 4 mM EDTA. The cell lysates were then diluted 10 times with 100 mM Tris, 2 mM EDTA, pH 7.75, and assayed for intracellular ATP using an ATP bioluminescent assay kit (Sigma).

**GLUT4 measurement.** Subconfluent MEF monolayers were cultured in 2% medium for 16 h. The cells were then trypsinized and resuspended in 2% FCS medium at a density of $10^6$ cells/ml. Cells were kept in fluorescence-activated cell sorting (FACS) tubes for 2 h at 37°C in 5% $CO_2$. IFN-β or insulin was then added to the cells for the times indicated below, after which the cells were fixed with 2% formalin in 2% serum containing FACS buffer and subsequently washed with FACS buffer before being stained with anti-GLUT4 antibody. Alexa Fluor 488-conjugated goat anti-rabbit antibody was used as a secondary antibody. Cell fluorescence was measured using a BD FACSCalibur flow cytometer and analyzed using BD CellStar software.

**CVB3 infection of MEFs.** MEFs were cultured in 2% FCS medium for 16 h. IFN-β was added 6 h prior to infection with CVB3 at a multiplicity of infection (MOI) of 1 (1 PFU/cell). After 8 h of incubation with virus, the cells were washed twice with PBS and viral titers measured by plaque assay using HeLa cells, as described previously (22, 46). For those experiments where the influence of 2-DG on IFN-β-inducible antiviral effects was evaluated, 2-DG was added either 30 min prior to IFN-β treatment or at specified times following IFN-β treatment and remained in the medium for the duration of virus infection. In experiments evaluating the effect of metformin on IFN-β, metformin (10 mM) was added 30 min prior to treatment with the doses of IFN-β indicated below and remained in the medium for the duration of virus infection. Quantitation of differences between untreated and IFN-β-treated cells in each group was calculated by dividing the viral titers determined in untreated cells by the titers determined in treated cells and expressing this value as a fold reduction.

***In vivo* studies.** Female C57Bl/6J mice aged 8 to 12 weeks were ordered from Taconic or The Jackson Laboratory and housed in pathogen-free conditions. All procedures were approved by the Toronto General Research Institute Animal Care Committee. One day prior to infection, treated mice were administered metformin *ad libitum* at a dose of 200 mg/kg of body weight/day, based on previous measurements of daily water consumption. Water consumption was found to be equivalent in metformin-treated and control animals. Normal drinking water was given to the mice at the time of infection. Prior to CVB3 infection, mice were administered an intraperitoneal injection of $10^5$ U of mIFN-β. Four hours later, mice were infected by intraperitoneal injection with a sublethal dose of CVB3 ($10^3$ PFU). At 3 days postinfection, mice were euthanized and tissues aseptically harvested and frozen in liquid nitrogen. After 3 freeze-thaw cycles, viral titers were determined by plaque assay in HeLa cells as described previously (22, 46).

**Statistical analysis.** Statistical significance was measured by analysis of variance. *P* values of ≤0.05 were considered statistically significant. Data are expressed as means ± standard errors.

## RESULTS

**Effects of IFN-β on AMPK phosphorylation and intracellular ATP.** Since AMP-activated protein kinase (AMPK) is a central sensor and regulator of cellular ATP stores, we undertook at the outset studies to determine any effects that IFN-β would exert on AMPK activation, by examining phosphorylation of AMPK on Thr172. As anticipated, IFN-β treatment of wild-type (WT) MEFs resulted in the rapid tyrosine phosphorylation of STAT1 (Fig. 1A). A simultaneous decrease in AMPK activation, i.e., Thr172 phosphorylation, was observed (Fig. 1A). Next, we examined the effects of IFN-β treatment on ATP production, and the data in Fig. 1B show a dose-dependent increase in IFN-β-inducible ATP production. This IFN-β-inducible ATP is inhibited in the presence of the nonmetabolized analog of glucose, 2-DG (Fig. 1B).

**IFN-β induces glucose uptake mediated by regulation of the PI3K/Akt signaling cascade.** As glucose is a major source of cel-

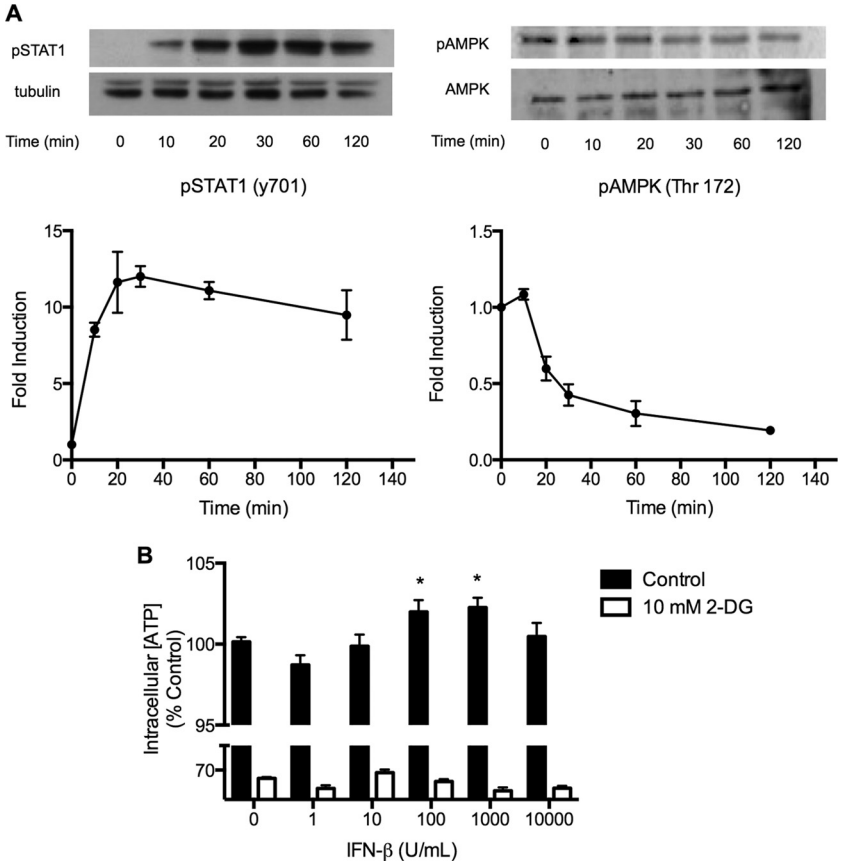


**FIG 1** IFN-β reduces AMPK phosphorylation and increases intracellular ATP. (A) MEFs were treated with 1,000 U/ml IFN-β for the indicated times. Cells were harvested, and protein lysates were resolved by SDS-PAGE and immunoblotted with anti-phospho-AMPKα (Thr172) or anti-phospho-STAT1 (Tyr701) antibodies. Membranes were stripped and reprobed with anti-AMPKα or anti-α-tubulin antibody for loading. Phosphorylation is shown relative to that of untreated cells and normalized for loading. Data are representative of two independent experiments (±standard errors of the means [±SEM]). (B) MEFs were pretreated with medium or 10 mM 2-DG for 30 min prior to treatment with the indicated doses of IFN-β for 1 h. Cells were lysed, and intracellular ATP quantified by a bioluminescence assay. Quantification is shown relative to the results for control-treated samples. Data are representative of 4 independent experiments (±SEM). *, $P < 0.05$.

lular ATP, we next investigated the influence of IFN-β treatment on glucose uptake. In time course experiments, we identified a biphasic enhancement of glucose uptake by IFN-β-treated cells (Fig. 2A). Using $^3$H-2-DG, we observed a rapid spike in $^3$H-2-DG uptake within minutes of IFN treatment, followed by a sustained decrease in uptake over a period of hours. Subsequent studies revealed that the influence of IFN-β treatment on glucose uptake is dose dependent, albeit less potent than the effects observed for 100 nM insulin treatment (Fig. 2B).

To identify potential IFN-regulated signaling effectors that might contribute to the regulation of glucose uptake, we employed a panel of MEFs with targeted disruption of elements of the PI3K/Akt/mTOR signaling cascade (Fig. 2C). Earlier published studies have shown that MEFs with targeted disruption of the p85 subunits of PI3K or Akt1/2 fail to respond to the antiviral effects of IFN when challenged with virus (18, 19). In contrast, targeted disruption of TSC1/2 results in enhanced responsiveness to the antiviral effects of IFN (21). In contrast to wild-type MEFs that respond to IFN-β treatment with a modest but rapid uptake of 2-DG, cells that lacked the p85α/β subunits of PI3K or Akt1/2 had decreased $^3$H-2-DG uptake (Fig. 2C) in response to IFN-β treatment. Cells lacking either TSC2 or AMPKα1/2 remained responsive to treatment with IFN-β in terms of $^3$H-2-DG uptake (Fig. 2C).

Glucose uptake is mediated by cell surface glucose transporters (47). Among these, GLUT4 is responsive to insulin treatment. Notably, insulin also regulates glucose uptake mediated by PI3K signaling (31, 48). Accordingly, we examined the effects of IFN-β treatment on cell surface expression of GLUT4 and observed a modest yet reproducible increase in expression by 1 h (Fig. 2D).

**Inhibition of glycolysis affects the antiviral activity of IFN-β.** To investigate the importance of glycolytic metabolism during an IFN-induced antiviral response, we next examined the effects of 2-DG treatment on an IFN-induced anti-CVB3 response. When cells were treated with IFN-β in the presence or absence of 2-DG, we observed a dose-dependent blunting of the IFN-β-inducible antiviral response in the presence of 2-DG (Fig. 3A). 2-DG treatment alone also inhibits viral replication. To further demonstrate the importance of glycolytic metabolism during the earliest stages of an IFN-induced antiviral response, we added 2-DG at various times relative to IFN-β treatment and examined the antiviral response (Fig. 3B and C). The results indicate that inhibition of glycolysis by 2-DG inhibits an IFN response in a time-dependent manner, specifically, during the earliest induction phase of the IFN response (Fig. 3C). Additionally, the expression of the IFN-β-inducible antiviral protein ISG15 was also sensitive to glycolytic inhibition by 2-DG (Fig. 3D). Given that the IFN-β dose em-

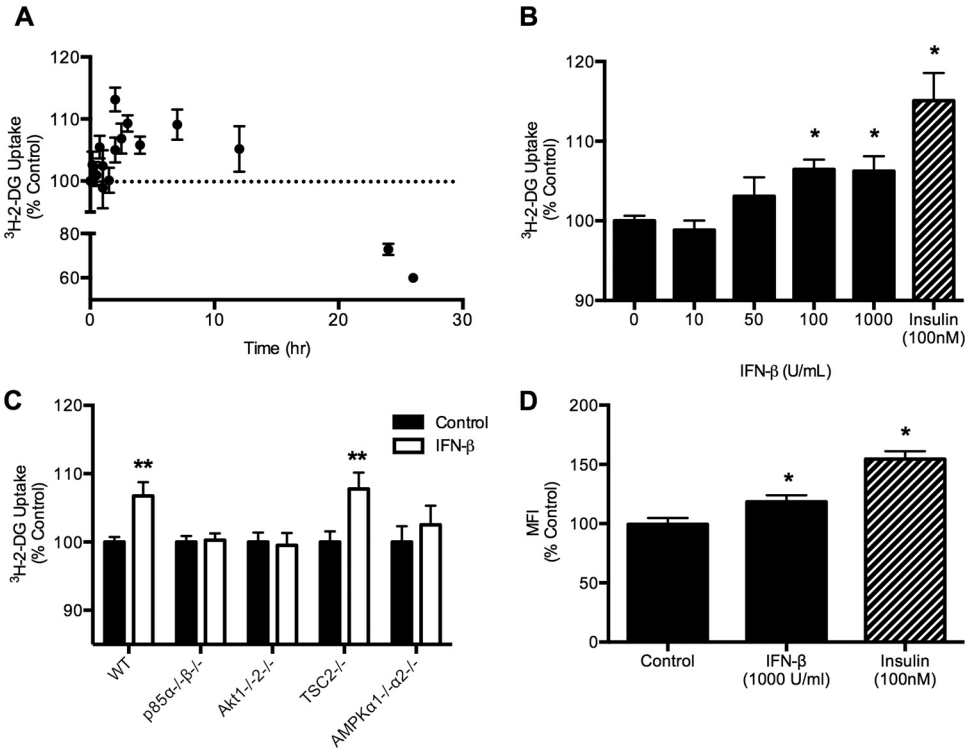


**FIG 2** IFN-β influences glucose uptake. (A) MEFs were treated with medium or 1,000 U/ml IFN-β for the indicated times. At time zero, cells were washed and then incubated with 0.5 μCi $^3$H-2-deoxy-D-glucose for 10 min. Reactions were quenched, and radioactivity measured by liquid scintillation counting. Data are shown relative to the results for control-treated samples at each time point and were combined from 3 independent experiments (±SEM). (B) MEFs were treated with the indicated doses of IFN-β or 100 nM insulin for 1 h. Uptake was measured as described above. Data are shown relative to the results for control-treated samples and were combined from 3 independent experiments (±SEM). *, $P < 0.05$. (C) MEFs were treated with medium or 1,000 U/ml IFN-β for 1 h. Uptake was measured as described above. Data were combined from 3 independent experiments (±SEM). **, $P < 0.05$. (D) Serum-starved MEFs were treated with medium, 1,000 U/ml IFN-β, or 100 nM insulin for 1 h. Cells were fixed with 2% paraformaldehyde, stained for surface GLUT4 expression, analyzed by FACS, and quantified for mean fluorescence intensity (MFI). Data are shown relative to the results for medium-treated control and were collected from 4 independent experiments (±SEM).*, $P < 0.05$.

ployed, $10^3$ U/ml, induces a robust antiviral response *in vitro*, the inhibitory effect of blocking glycolysis underscores the relevance of glycolysis to an IFN-induced antiviral response.

**Treatment with metformin enhances the antiviral activity of IFN-β.** Metformin, an antidiabetic drug, increases insulin sensitivity, activates AMPK, and enhances GLUT4 translocation to the cell surface (49, 50). Accordingly, we next examined the effects of combination treatment with IFN-β and metformin against CVB3 infection of MEFs. As shown by the results in Fig. 4A, treatment of MEFs with a combination of metformin and IFN-β led to an enhanced antiviral response, greater than that of either treatment alone.

In a final series of experiments, given our preceding data that suggest a role for IFN-β in regulating metabolic events that would meet the energy needs of a cell to invoke an antiviral response, we examined the effect of combination treatment with IFN-β and metformin on CVB3 infection in mice. Our earlier published studies identified that IFN-β treatment is protective against infection with the cardiotropic CVB3 (22, 46). When infected with CVB3, mice exhibit signs of infection, i.e., reduced activity and ruffled fur. Heart viral titers indicate acute virus infection, with the peak viral burden at 3 days postinfection and then progressive clearance of the virus from the heart (22). Mice were allowed *ad libitum* access to metformin in their water supply. We observed no difference in water consumption whether metformin was included in the water or not. Mice were either left untreated or treated with IFN-β and then challenged with CVB3. Three days postinfection, all mice were euthanized, blood and various tissues aseptically harvested, and viral titers measured. The results in Fig. 4B demonstrate that combination treatment with IFN-β and metformin significantly reduced heart, liver, spleen, and serum viral titers compared with the results for treatment with IFN-β or metformin alone. A similar trend was observed, although less pronounced, in the pancreata of infected mice.

## DISCUSSION

Type I IFNs exert their immunomodulatory influence in a wide variety of cell types and, in the context of virus infections, do so rapidly to inhibit virus replication and limit virus spread. This antiviral activity is mediated by transcriptional and posttranscriptional signaling proteins, including STATs, MAPKs, and PI3K (16). In recent years, the role of type I IFNs in regulating PI3K/mTOR-mediated posttranscriptional effects has become better defined, with a significant area of focus on translational regulation (18–21, 37, 51–53). It has become increasingly apparent that mTOR is a central sensor of metabolic stresses and, in addition to translation, regulates processes such as autophagy and lipid and carbohydrate metabolism, thereby maintaining cellular energy

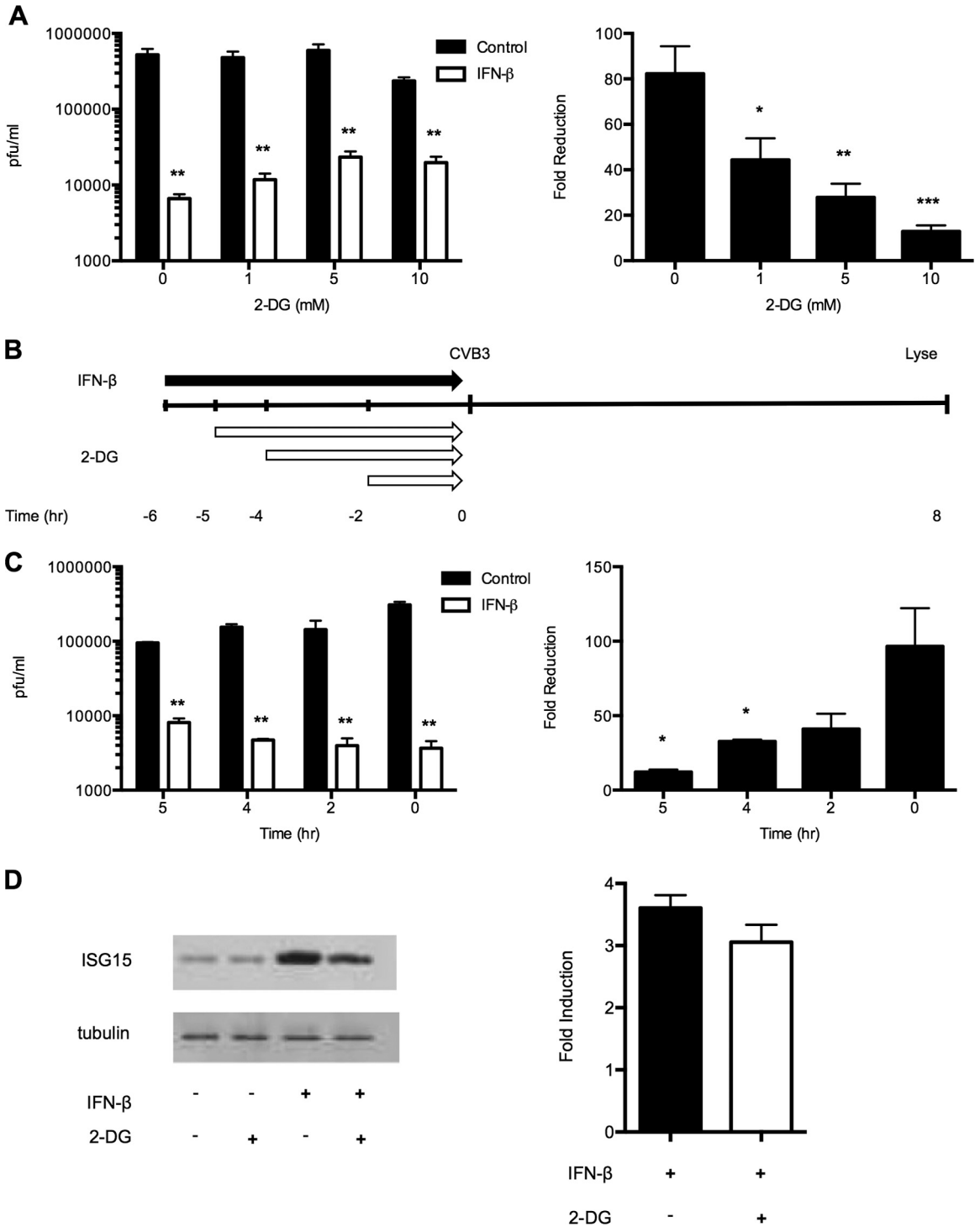


**FIG 3** Glucose metabolism is critical for induction of a IFN-β-mediated antiviral response. (A) MEFs were pretreated with medium or indicated doses of 2-DG 30 min prior to addition of medium or 1,000 U/ml IFN-β for 6 h. Cells were then infected with CVB3 at an MOI of 1. Cells were washed and lysed by freeze-thaw after 8 h, and viral titers determined by plaque assay. Data are shown as PFU/ml, and antiviral effect indicated as fold reduction relative to the viral titer for medium-treated cells. Data are from 3 independent experiments (±SEM). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$. (B, C) MEFs were treated with medium or 1,000 U/ml IFN-β 6 h prior to infection with CVB3 (MOI of 1). At the indicated times following IFN-β treatment, 2-DG was added. Following an 8-h infection, cells were washed and lysed. Time points (hr) for 2-DG treatment prior to infection are indicated. Data are representative of 2 independent experiments (±SEM). IFN-β-inducible antiviral effect is quantified as fold reduction relative to the viral titer for control-treated cells. *, $P < 0.05$; **, $P < 0.01$. (D) MEFs were pretreated with medium or 10 mM 2-DG 30 min prior to the addition of medium or 1,000 U/ml IFN-β for 6 h. Cells were harvested, and protein lysates were resolved by SDS-PAGE and immunoblotted with anti-ISG15 and anti-α-tubulin antibodies. Expression is shown relative to the results for untreated cells and normalized for loading. Data are representative of technical triplicates and 3 independent experiments (±SEM).

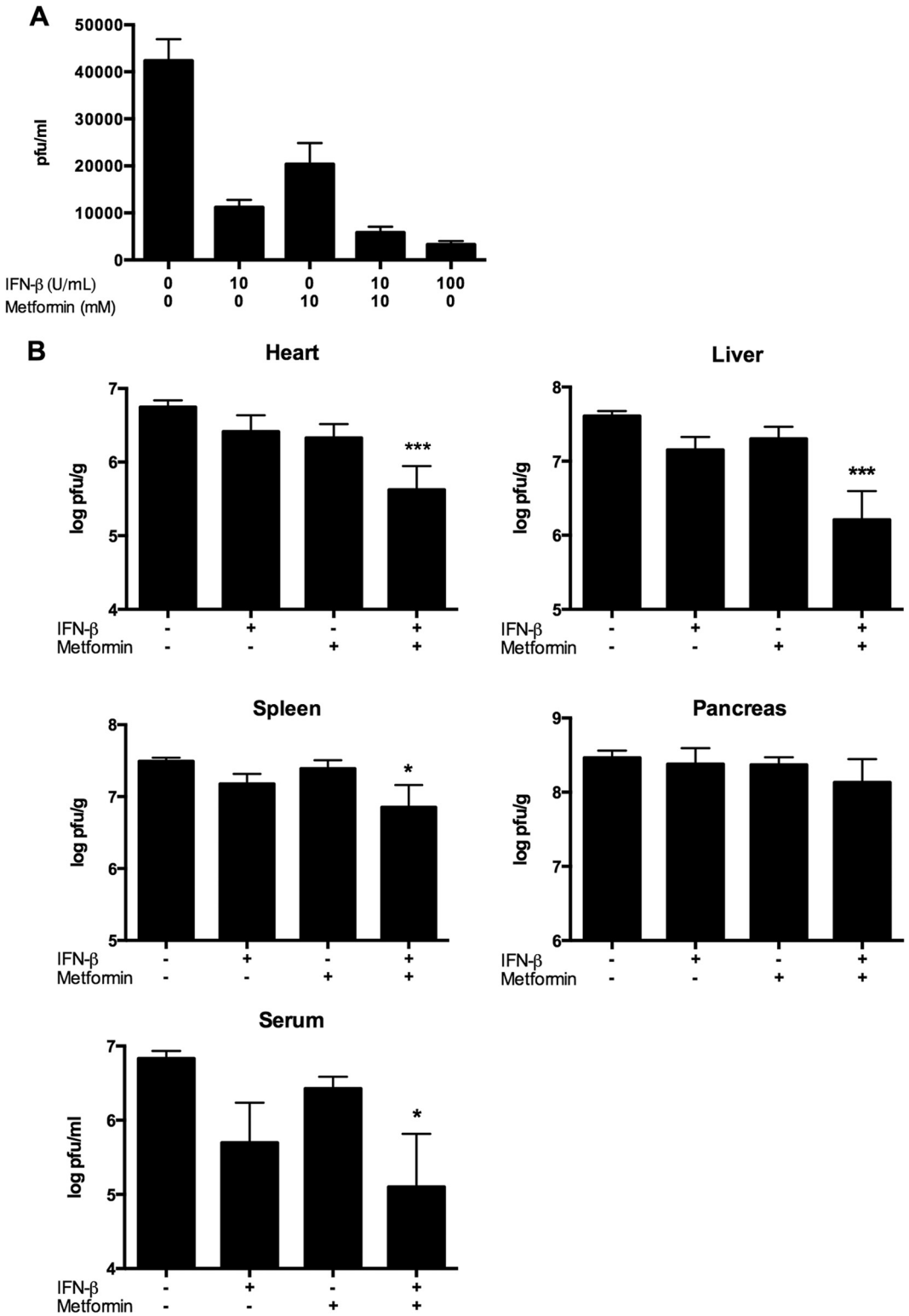


**FIG 4** Metformin enhances antiviral effect of IFN-β during infection with CVB3. (A) MEFs were left untreated or treated with 10 mM metformin 30 min prior to treatment with indicated doses of IFN-β. Following 6 h of treatment, cells were infected with CVB3 for 8 h. Data were combined from 3 independent experiments and are shown as mean PFU/ml (±SEM). (B) Mice were administered metformin in drinking water *ad libitum* prior to treatment with IFN-β for 4 h prior to infection with CVB3. At 3 days following infection, mice were sacrificed and tissues collected for determination of viral titers. Data were combined from 5 independent experiments and are shown as mean log PFU/g (±SEM). Data from 15 mice were collected for each treatment group. *P* values are given relative to the results for control-treated samples. *, $P < 0.05$; ***, $P < 0.001$.

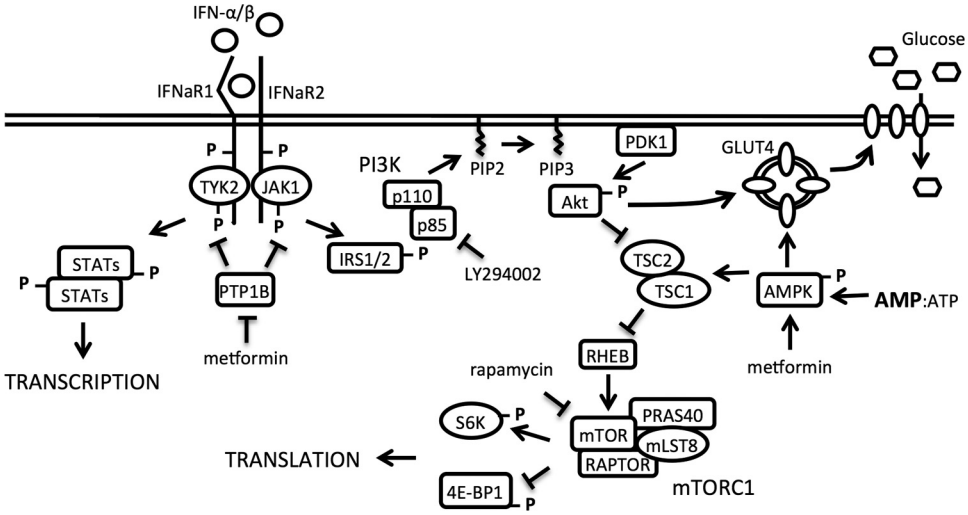


**FIG 5** Schematic of IFN-β-mediated regulation of PI3K/Akt/mTOR signaling and pharmacological agents active in this pathway.

homeostasis (54). Here, we report on the influence of IFN-β on glucose metabolism in the context of virus infection.

Given the high energy demands of IFN-inducible protein synthesis, we anticipated an effect on AMPK activation and cellular ATP synthesis accompanying treatment with IFN-β. Indeed, IFN-β treatment reduced AMPK phosphorylation at Thr172, with a concurrent increase in STAT1 phosphorylation at Tyr701, which is indicative of a IFN-β-inducible cell response. Since AMPK is a sensitive indicator of the cytosolic AMP/ATP ratio (55), activated by phosphorylation in the presence of low ATP concentrations, we infer that the decrease in Thr172 phosphorylation we identified upon IFN-β treatment is associated with an increase in ATP production. Indeed, IFN-β treatment of MEFs resulted in an increase in ATP production. It is unlikely that IFN-β directly regulates AMPK phosphorylation; rather, it is likely that IFN-β induces an effect which indirectly influences AMPK activation through changes in the AMP/ATP ratio. IFN-β-mediated changes in ATP levels were abrogated in the presence of the nonmetabolizable glucose analog 2-DG. This inhibition of glycolytic-derived ATP provides evidence that IFN-β influences glucose metabolism. In support of this, we demonstrate that IFN-β promotes a dose-dependent uptake of $^{3}$H-2-DG by cells.

For IFNs to be most effective as antivirals, it is crucial that cells respond rapidly in terms of producing antiviral proteins that will inhibit viral replication. Accumulating data implicate IFN-α/β in the regulation of translation of host protein synthesis and the corresponding expression of antiviral proteins (18, 19, 21). Our data suggest that there is a rapid and robust uptake of glucose by cells, within minutes of IFN-β treatment, consistent with meeting the energy demands of protein synthesis. Moreover, the nature of the biphasic response, whereby glucose uptake is initially increased, followed by a suppression, is in agreement with the paradigm of type I IFN-mediated antiproliferative effects (56–71). Specifically, in uninfected cells, the early translation of antiviral proteins is followed by a progressive shutdown of protein synthesis that would disable cell growth and, upon infection, inhibit viral protein synthesis. Indeed, this biphasic response is consistent with a scenario where virus replicates rapidly and infection spreads. An infected cell produces and secretes IFN-β in response to viral replication prior to viral progeny egress, thereby activating the antiviral response in neighboring uninfected cells (9–11). Transiently, uninfected cells rapidly increase their metabolism to support the synthesis of antiviral proteins, such as 2′-5′-oligoadenylate synthetase (2′-5′-OAS), protein kinase R (PKR), and RNase L, followed by the subsequent downregulation of metabolism. Upon viral spread, IFN-β-primed cells respond to viral RNA by secreting additional IFN-α, thereby inhibiting further viral replication and spread.

In contrast, when astrocytes are exposed to low concentrations of IFN-α2a, IFN-α2b, or IFN-β (<5 U/ml), no significant changes in glucose consumption are observed over 2 h, and yet chronic exposure to low-dose IFN reduces glucose uptake (71). This model of low-dose, chronic IFN exposure was intended to reflect the systemically low plasma concentrations of type I IFN in HCV-infected individuals over the duration of a chronic infection. In contrast, our studies reflect a scenario of localized virus infection where cells in close proximity experience high concentrations of IFN-α/β produced by tissue-resident cells or plasmacytoid dendritic cells during an acute immune response to virus infection. In other studies, Navarro et al. examined the effects of type I IFN treatment on glucose metabolism in primary mesenteric and splenic lymphocytes after 48 h and likewise showed a suppression of glucose uptake (72). Notably, in the earliest IFN experiments of Isaacs and Lindenmann, conducted in chicken embryo cells, they identified a modest IFN-inducible effect on lactate production after 4 h, an indicator of glycolysis (73).

A number of studies have confirmed the roles of PI3K and Akt signaling in regulating glucose uptake induced by growth factors or cytokines in adipocytes, skeletal muscle cells, and lymphocytes (24–35). Our strategy was to examine the contribution of different effector intermediates in the PI3K/Akt/mTOR signaling cascade to the IFN-β-inducible regulation of glucose uptake that we observed, specifically, by using MEFs with targeted disruption of certain genes (Fig. 5). A striking effect was observed in cells null for either p85α/β or Akt1/2. The lack of either of these two signaling effectors was sufficient to completely ablate IFN-β-inducible glucose uptake. Consistent with the negative regulatory role that TSC2 exerts on mTOR activity, IFN-β-inducible glucose uptake

in TSC2$^{-/-}$ cells was unaffected. MEFs lacking mLST8, a nonessential component of mTORC1, exhibited a partial reduction in IFN-β-inducible glucose uptake, suggestive of a role for mTORC1 in regulating glucose uptake. Surprisingly, in cells lacking AMPKα1/2, an upstream negative regulator of mTOR through TSC2 (74), we observed only a partial reduction in responsiveness to IFN-β-inducible glucose uptake. This may be attributed to the other role that AMPK has in influencing GLUT4 translocation to the cell surface (49, 75). Consistent with our findings of IFN-β regulation of glucose uptake, the surface expression of GLUT4 was also increased upon treatment with IFN-β. PI3K and Akt activation are associated with GLUT4 translocation to the cell surface (31, 48, 76), providing further support for a potential mechanism whereby IFN activation of these effectors enhances the expression of glucose transporters required for glucose uptake.

Previous publications have identified that treatment of cells with 2-DG reduces the replication of a variety of viruses, including CVB3 (77–83). Limiting the energy supplies in an infected cell would affect protein synthesis and the assembly of viral progeny. In contrast, a rapid burst of energy will enable an early robust IFN response, as we show, and yet the biphasic nature of the effect we observe supports the subsequent inhibition of cell growth and viral replication.

Clinical studies have drawn attention to a correlation between insulin and IFN sensitivities in individuals who are infected with hepatitis C virus (84). The expression levels of TNF-α are often increased in HCV-infected livers. TNF-α upregulates the activity of the phosphatase, PTP-1B, which is responsible for the downregulation of insulin and type I IFN signaling (85). In the same study, metformin, an inhibitor of PTP-1B, was used effectively to restore insulin and IFN sensitivities in mouse livers expressing high levels of TNF-α. Indeed, metformin is used to treat insulin resistance in patients with type 2 diabetes (86). Moreover, earlier studies demonstrated the negative regulatory effects of PTP-1B on JAK/STAT signaling (87–90). We therefore reasoned that metformin may be administered along with IFN-β to enhance antiviral potency during a virus infection. Coxsackieviruses encompass a group of cardiotropic viruses that can cause acute myocarditis and lead to dilated cardiomyopathy (91). While it is not a standard treatment for viral myocarditis, the administration of IFN-α/β has been shown to improve cardiac function (92, 93). Interestingly, patient TNF-α expression levels are measured in the serum and heart during acute virus myocarditis, reflective of an inflammatory response to infection (94–97). Given our data, it is intriguing to speculate that this TNF may influence endogenous type I IFN signaling in the heart, exacerbating infection. In our study, we provide evidence that metformin enhances the antiviral effects of low-dose IFN-β treatment of MEFs challenged with CVB3. Similarly, treating mice with IFN-β and metformin prior to infection with CVB3 enhanced the antiviral effects of IFN-β, most notably reducing viral titers in the hearts, livers, spleens, and sera of infected mice. We speculate that the antiviral effects of metformin alone may be associated with the promotion of endogenous type I IFN activity.

Viewed together, our data provide new evidence that IFN-β modulates glucose metabolism through a PI3K/Akt-dependent mechanism and that this regulation of metabolism appears important for the induction of an effective antiviral response. Additionally, we provide evidence for the application of metformin to enhance the antiviral activity of IFN-β.

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