Original Article

AMP Kinase Activation Increases Glucose Uptake, Decreases Apoptosis, and Improves Pregnancy Outcome in Embryos Exposed to High IGF-I Concentrations

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Women with polycystic ovarian syndrome are at increased risk of miscarriage. Although evidence exists that metformin reduces this risk, the mechanism is unknown. This study tests the hypothesis that AMP kinase (AMPK) activation with metformin directly improves insulin signaling within the blastocyst, leading to improved pregnancy outcomes. Murine embryos were exposed to 200 nmol/l IGF-I, similar to the concentrations that can occur during polycystic ovary syndrome (PCOS). Resulting blastocysts were compared with embryos cocultured with excess IGF-I plus metformin and embryos cultured in control medium for the following: AMPK phosphorylation, insulin-stimulated glucose uptake, and apoptosis. Study and control blastocysts were also transferred into control animals. On embryonic day (E) 14.5, resulting fetuses were examined for size and rates of fetal implantation and resorption. Compared with control blastocysts, blastocysts exposed to high concentrations of IGF-I showed a decrease in AMPK activation and insulin-stimulated glucose uptake and an increase in the number of apoptotic nuclei. Blastocysts cocultured in metformin and excess IGF-I performed as well as controls in all studies. 5-Aminoimidazole-4-carboxamide 1-β-D-ribofuranoside, another AMPK activator, also prevented the effects of excess IGF-I on blastocysts. Implantation rates and fetal size at day 14.5 were significantly lower among IGF-I–exposed embryos transferred into control mothers compared with control embryos transferred into control mothers. Both of these parameters were reversed by coincubation with metformin and IGF-I before transfer. Activation of embryonic AMPK may be the mechanism responsible for the improved pregnancy outcomes seen in PCOS patients taking metformin. *Diabetes* 56:2228–2234, 2007

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AICAR, 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside; AMPK, AMP kinase; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; E, embryonic day; hCG, human chorionic gonadotropin; HTF, human tubal fluid; IGFBP-1, IGF binding protein-1; PCOS, polycystic ovary syndrome; TBST, Tris-buffered saline with Tween; TUNEL, transferase-mediated dUTP nick-end labeling.

Women with polycystic ovary syndrome (PCOS) experience oligomenorrhea/anovulation, clinical or biochemical hyperandrogenism, and polycystic ovaries (1). The clinical presentation is variable, but many women with PCOS also exhibit obesity, hyperinsulinemia with insulin resistance, and infertility with recurrent pregnancy loss (2–4). The etiology of this recurrent pregnancy loss remains unclear. Both high concentrations of androgens and gonadotropins have been suggested, but only small, correlative clinical investigations have been conducted (5,6). Bioactive levels of the insulin-like growth factor, IGF-I, are also increased in PCOS patients because of an insulin-induced decrease in the production of IGF binding protein-1 (IGFBP-1) (7,8).

The preimplantation blastocyst stage embryo is an insulin-sensitive tissue (9,10). Prior studies have shown that the murine blastocyst responds to insulin or IGF-I by increasing glucose uptake and that this event occurs via the IGF-I receptor. IGF-I receptor signaling induces translocation of GLUT8 to the plasma membrane of the trophectoderm cells of the embryo (11). Expression of GLUT8 and translocation to the plasma membrane are critical for embryo survival (12,13). On exposure to high concentration of IGF-I or insulin, the preimplantation blastocyst responds by downregulating the IGF-I receptor, similar to what occurs in other cell systems in response to chronic insulin stimulation (14,15). The blastocyst becomes insulin resistant. As a result, insulin-stimulated glucose uptake is reduced, intraembryonic glucose levels drop, and apoptosis is triggered. The combination of decreased insulin/IGF-I receptor signaling and decreased glucose uptake are responsible for this apoptosis (10,16). The pregnancy outcome of these embryos exposed to IGF-I/insulin when transferred into surrogate mice is poor. Higher rates of miscarriages or resorptions are seen, and implantation rates are lower (17). These animal findings are consistent with that seen in women with hyperinsulinemia. Women with PCOS, type 2 diabetes, and obesity all experience increased rates of early pregnancy loss. We hypothesize that alterations in maternal physiology due to hyperinsulinemia directly impair the embryo's insulin signaling pathways and, as a result, hinder its ability to transport glucose at a critical stage in development, leading to pregnancy failure.

Metformin has been used to treat anovulation in women with PCOS. Recent studies have suggested that metformin may also decrease the higher miscarriage rates in these

women (18–20). The mechanism for this event is unclear. Studies have shown that metformin activates AMP kinase (AMPK) in rat hepatocytes and diabetic human muscle (21,22). Additional work has shown that this activation stimulates muscle glucose uptake (23,24) and meiotic maturation in mouse oocytes (25). We hypothesize that metformin and AMPK activation at the blastocyst stage could trigger glucose uptake into the embryo, improve insulin signaling, and rescue embryos from apoptosis due to insulin resistance. These results would support the use of metformin in PCOS patients and in patients with other insulin-resistant conditions to improve pregnancy outcome.

RESEARCH DESIGN AND METHODS

Embryo recovery and culture. Embryos were recovered as previously described (26). Briefly, 3-week-old female mice (B6 × SJL F1; The Jackson Laboratories, Bar Harbor, ME) were given free access to food and water and were maintained on a 12-h light/dark cycle. Female mice were superovulated with an intraperitoneal injection of 10 IU/animal pregnant mare serum gonadotropin (Sigma, St. Louis, MO), followed 48 h later by 10 IU/animal human chorionic gonadotropin (hCG) (Sigma). Female mice were mated with males of proven fertility overnight after the hCG injection. Mating was confirmed by identification of a vaginal plug. Mice were killed 48 h after hCG injection to recover embryos at the two-cell embryo stage. Embryos were recovered by flushing dissected uterine horns and ostia with human tubal fluid (HTF) medium (Irvine Scientific, Santa Ana, CA) containing 0.25% BSA (fraction V; Sigma). Embryos were then cultured in vitro in the following treatment media for 72 h until blastocyst stage: *1*) control HTF medium; *2*) metformin (1,1-dimethylbiguanide hydrochloride) (25 μg/ml; Sigma); *3*) added IGF-I (200 nmol/l; Sigma); or *4*) metformin (25 μg/ml) with IGF-I (200 nmol/l).

For the dose of metformin, we chose 25 μg/ml (151 μmol/l). For the studies with 5-aminoimidazole-4-carboxamide 1-β-D-ribofuranoside (AICAR) (Toronto Research Chemicals, Toronto, CA), this chemical was used at a concentration of 1 μmol/l. For the studies with the A1 adenosine receptor antagonist, 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) (Sigma), this chemical was used at a concentration of 1 μmol/l with and without high-dose IGF-I. These experiments were conducted at least three times each. All procedures described above were reviewed and approved by the animal studies committee at Washington University and were performed in accordance with the institutional animal care and use committee's approval.

Embryo Western blot analysis. An average of 75 embryos were pooled, added directly to Laemmli sample buffer, subjected to SDS-PAGE, and transferred to nitrocellulose. Blots were blocked for 1 h at room temperature in 5% milk in Tris-buffered saline with Tween (TBST). The blots were probed overnight at 4°C in 1% milk in TBST with the following antibodies: rabbit anti–phospho-AMPK-α (Thr172) (Upstate Cell Signaling, Lake Placid, NY), rabbit anti–AMPK-α1 (Upstate Cell Signaling), or mouse anti–β-actin (Chemicon, Temecula, CA). Blots were washed three times for 10 min each with TBST. The appropriate horseradish peroxidase–conjugated secondary antibody (either goat anti-rabbit or goat anti-mouse) at a dilution of 1:10,000 was used for detection (Santa Cruz Biotechnology, Santa Cruz, CA) and visualized using SuperSignal West Dura following the manufacturer's instructions (Pierce, Rockford, IL). All experiments were conducted at least three times on separate pools of blastocysts to generate the figures shown.

Insulin-stimulated 2-deoxyglucose uptake in blastocysts. Nonradioactive insulin-stimulated 2-deoxyglucose uptake into single blastocysts was performed using microfluorometric assays combined with enzymatic cycling reactions as previously described (27). Briefly, two-cell embryos were cultured for 72 h in one of the four treatment groups described. Next, blastocysts from each condition were incubated in HTF medium at a final glucose concentration of 5.6 mmol/l with 500 nmol/l insulin (bovine pancreas; Sigma) for 30 min. 2-Deoxyglucose uptake was then measured as described previously (27) and expressed as millimoles per kilogram wet weight over a 15-min time interval. The rate is expressed as the difference between insulin-stimulated and basal values.

Evaluation of apoptosis by transferase-mediated dUTP nick-end labeling assay. Apoptosis was assayed using transferase-mediated dUTP nick-end labeling (TUNEL) as previously described (26). Embryos were fixed in 3% paraformaldehyde (Sigma) for 20 min and then permeabilized in 0.1% Tween-20 (Sigma) for 30 min. Apoptosis was assessed using the In Situ Cell Death Detection kit, TMR (Roche, Basel, Switzerland) according to the manufacturer's protocol. After the TUNEL assay was performed, the nuclei of the embryos were stained using 4 μmol/l To-Pro-3-iodide (Molecular Probes, Eugene, OR) for 20 min. A *Z*-series consisting of 4–11 sections was taken for each embryo using a Nikon C1 laser-scanning confocal microscope. The total numbers of nuclei and apoptotic nuclei per embryo were quantitated manually for each *Z*-series by a blinded observer. The percentage of TUNEL-positive nuclei was calculated by dividing the total number of apoptotic nuclei seen in sections, divided by the total number of nuclei visualized in a representative embryo cross-section. This experiment was performed at least three times with a minimum of five embryos per group.

Blastocyst transfer studies. Two-cell embryos were cultured in vitro to a blastocyst stage in *1*) control HTF medium; *2*) metformin (25 μg/ml); *3*) added IGF-I (200 nmol/l); or *4*) metformin (25 μg/ml) with IGF-I (200 nmol/l). Unhatched blastocysts were then transferred back into the uterine horn of pseudopregnant female recipient mice at 3.5 days postcoitum (28). Eight to 10 blastocysts were transferred to a single uterine horn for each recipient. The mice were killed, and the embryos were examined at E 14.5. Five independent experiments were performed. The percentage of fetal resorption per mouse was calculated by dividing the number of resorptions by the total number of implantation sites. The resorption percentage per mouse was then averaged for the control or the treated group over all five experiments. Fetus crown to rump lengths were also measured. All results are expressed as means ± SE.

Statistical analysis. For the evaluation of differences between groups in all experiments, ANOVA with a Bonferoni/Dunn post hoc test was used. Results are expressed as means ± SE of at least three separate experiments. Significance is defined as a $P < 0.05$.

RESULTS

Metformin stimulates phosphorylation of AMPK in blastocysts. To detect AMPK activity during the preimplantation stage, two-cell embryos were cultured for 72 h in vitro to a blastocyst stage in *1*) HTF medium with added IGF-I (200 nmol/l); *2*) HTF medium with added metformin (25 μg/ml); *3*) control HTF medium without any additive; or *4*) HTF medium with added AICAR (1 μmol/l). The concentration of IGF-I chosen was based on previous studies in which we had measured IGF-I levels in fallopian tube fluid from control rhesus monkeys (10). These levels ranged from 4 to 480 ng/ml or 1 to 66 nmol/l in controls. We chose a 130–200 nmol/l or 950-1500 ng/ml range as a two- to threefold elevated physiological range indicative of mild insulin resistance. Since that time, several papers have been published showing this two- to threefold elevation in fasting serum insulin levels and in IGF-I levels among PCOS patients (29,30). This study assumes that serum and tubal IGF-I levels are similar. Murine fallopian tube insulin and IGF-I have been detected (31,32); however, direct comparison of the serum and fluid values has not been done. The actual IGF-I concentration not bound to IGFBPs is not know in PCOS patients, and it is also not known whether this concentration changes in response to administration of metformin.

Metformin has a very low binding capacity to plasma proteins with a bioavailability of ~60%. It is not metabolized and is excreted in urine (33). Serum levels have only been reported at the 500 mg/day dose with a steady-state concentration of 7 μg/ml after 8 h (34). Because the clinical dose for PCOS is typically 1 g twice daily, we estimated that the serum concentration might be as high as four times greater than the 7 μg/ml dose. We chose an intermediate concentration of 25 μg/ml (151 μmol/l). Zhou et al. (22) had previously demonstrated in primary hepatocytes that AMPK was significantly activated by 100 μmol/l metformin, and in pilot studies, we showed that the concentration used in this study was not toxic as determined by TUNEL assay (data not shown). In addition, toxicology studies of mouse embryos after implantation used doses >100 μg/ml metformin, which caused embryo lethality; however, 25–50 μg/ml had no adverse effects on these later stage embryos (35).

The amount of phosphorylated AMPK protein in these blastocysts exposed to the different conditions was de-

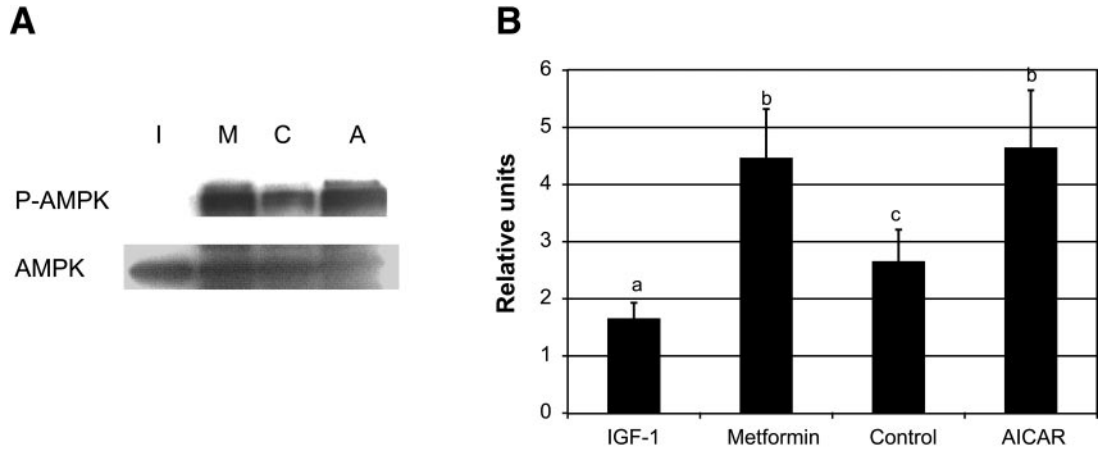


FIG. 1. Metformin exposure results in phosphorylation of AMPK. *A*: Blastocysts cultured in IGF-I (I); metformin (M); control HTF medium (C); or AICAR (A) were subjected to SDS-PAGE and Western blot analysis (n = 50 for each group for each experiment). For this set of experiments, membranes were first probed with antibodies specific for P-AMPK (*top*). They were then stripped and reprobed with antibodies specific for total AMPK (*bottom*). This blot is representative of the three experiments performed. *B*: Western blots were quantitated using NIH Image software (National Institutes of Health), and expression was normalized to total AMPK. Values with different letters were significantly different. IGF-I vs. control embryos, $P < 0.05$; IGF vs. metformin or vs. AICAR, $P < 0.01$; control vs. metformin or vs. AICAR, $P < 0.05$.

tected by Western immunoblot and normalized to the total AMPK protein amount in the same sample to measure AMPK activity. AICAR also stimulates AMPK activity and thus was used as a positive control along with metformin. Both AMPK activators increased the amount of phosphorylated AMPK when normalized to total AMPK protein (Fig. 1*A* and *B*). Exposure to high concentrations of IGF-I led to decreased active AMPK in the blastocyst-stage embryos.

Metformin improves glucose uptake in IGF-I–exposed blastocysts. Insulin-stimulated 2-deoxyglucose uptake was significantly decreased in single blastocysts cultured in 200 nmol/l IGF-I compared with control embryos (Fig. 2). This finding had previously been demonstrated in both in vitro and in vivo assays with high IGF-I and high insulin concentrations (10,17). Coculturing the embryos with 25 μg/ml metformin plus IGF-I for 72 h ameliorated the adverse effect of high IGF-I and improved insulin sensitivity and deoxyglucose uptake. No significant difference was seen between the embryos cultured in metformin plus IGF-I versus the control group. Likewise, insulin-stimulated glucose transport values for embryos cultured in metformin alone did not differ significantly from those values for control embryos cultured in control HTF medium.

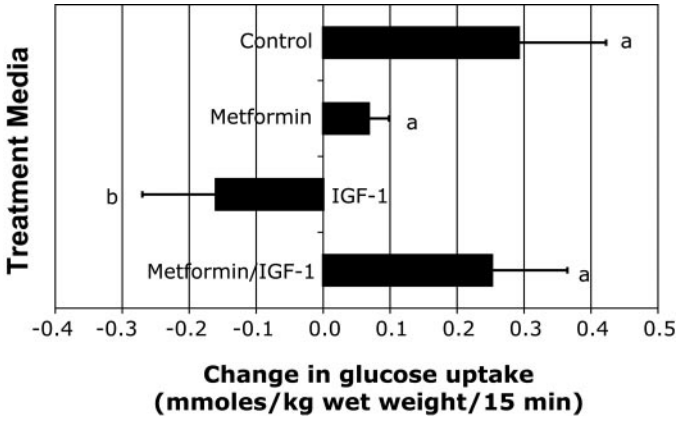


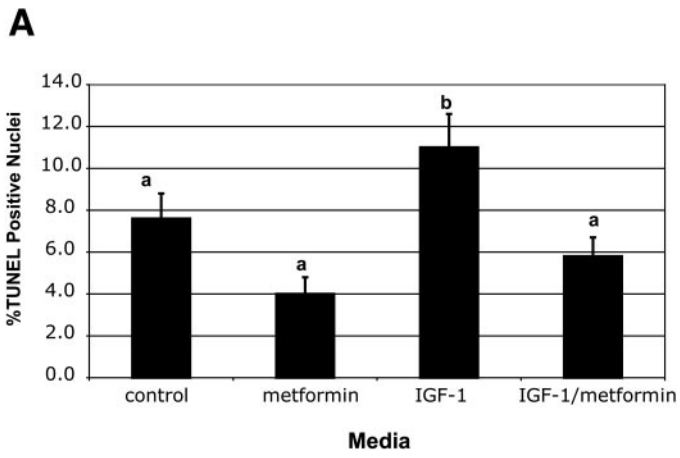
FIG. 2. Metformin improves glucose uptake in IGF-I–exposed blastocysts. Two-cell embryos were cultured for 72 h in control medium (n = 47), medium with metformin alone (n = 45; 25 μg/ml), medium with high concentrations of IGF-I (n = 55; 200 nmol/l), or medium with IGF-I plus metformin (n = 55). Insulin-stimulated 2-deoxyglucose uptake (stimulated rate minus basal rate) was measured in individual blastocysts from each condition. Values with different letters were significantly different. IGF-I vs. control embryos, $P < 0.05$. IGF plus metformin vs. IGF-I, $P < 0.01$.

Metformin prevents apoptosis in IGF-I–exposed blastocysts. Coculture of metformin with IGF-I prevented the adverse effect of 200 nmol/l IGF-I on apoptosis as measured by the percentage of TUNEL-positive nuclei per embryo. The percentage of TUNEL-positive nuclei fell in the embryos cocultured with metformin and IGF-I compared with embryos exposed to 200 nmol/l IGF-I alone (Fig. 3*A* and *B*). The TUNEL-positive percentage in the IGF-I plus metformin group of embryos was not significantly different from control or metformin alone. The apoptosis in the IGF-I–exposed embryos appeared to localized to the inner cell mass in the majority of blastocysts.

AICAR prevents IGF-I effects on glucose uptake and apoptosis. To test whether another known activator of AMPK had the same effect as metformin, AICAR was used. AICAR, added at a concentration of 1 μmol/l to the 200 nmol/l IGF-I culture medium, partially ameliorated the adverse effect of 200 nmol/l IGF-I alone on insulin-stimulated 2-deoxyglucose uptake. No significant difference was seen between the embryos cultured in AICAR plus IGF-I versus embryos cultured in AICAR alone (Fig. 4*A*). Significant differences were seen between those embryos cultured in control HTF versus those cultured in either AICAR alone or AICAR and IGF-I. Although it is not clear why AICAR alone reduces insulin sensitivity, these studies demonstrate that AICAR, like metformin, improves insulin sensitivity in blastocysts exposed to high concentrations of IGF-I.

The addition of AICAR to the 72-h culture in IGF-I reversed the adverse effect of 200 nmol/l IGF-I on apoptosis as measured by TUNEL-positive nuclei per embryo. The percentage of TUNEL-positive nuclei fell in embryos cocultured with AICAR compared with embryos exposed to 200 nmol/l IGF-I alone (Fig. 4*B*). TUNEL-positive percentage in the IGF-I plus AICAR group of embryos was not significantly different than control or AICAR alone.

AICAR is a nucleoside that is taken up, accumulates



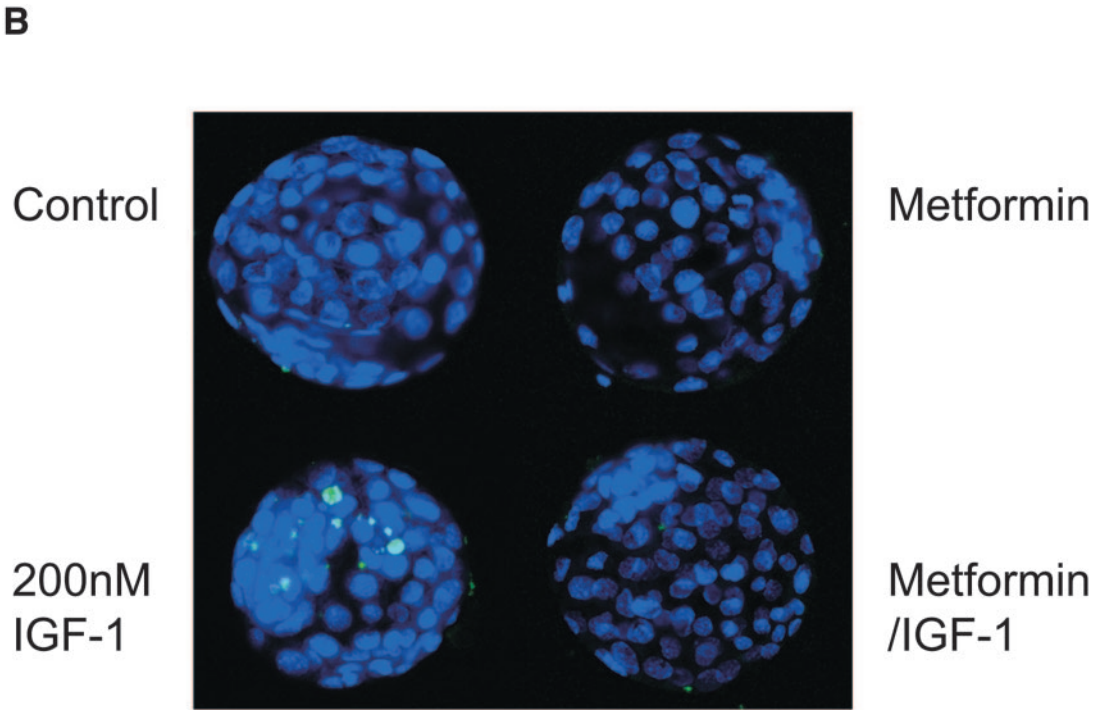


FIG. 3. Metformin reverses apoptosis in IGF-I–exposed blastocysts. *A*: Two-cell embryos were cultured in the same conditions as Fig. 1. Apoptosis was measured in individual blastocysts from each condition using the percentage of TUNEL-positive nuclei. Values with different letters were significantly different ($P < 0.001$). Control, $n = 21$; IGF-I, $n = 28$; IGF-I plus metformin, $n = 28$; metformin alone, $n = 21$. *B*: Immunofluorescent images of blastocysts described in *A*. Blue channel, nuclear dye; green channel, TUNEL reaction. (Please see http://dx.doi.org/10.2337/db07-0074 for a high-quality digital representation of this figure.)

inside the embryonic cell as the monophosphorylated nucleotide (ZMP), and activates AMPK. AICAR can also act as an adenosine analog. To test whether any adenosine receptor activation by AICAR was responsible for the positive effects of AICAR on blastocyst survival, a set of experiments was completed using the A1 adenosine receptor antagonist DPCPX at a dose of 1 μmol/l. DPCPX added to the AICAR plus IGF-I culture conditions did not have any effect on the ability of AICAR to reverse IGF-I–induced apoptosis in the blastocyst (Fig. 4*C*).

Metformin reverses the detrimental effects of IGF-I on implantation and crown-rump length. Embryos cultured in 200 nmol/l IGF-I for 72 h and then transferred into control mice demonstrated significantly lower implantation rates at E 14.5 compared with embryos cultured in control HTF (Fig. 5*A*). Metformin rescued the embryos cocultured in 200 mmol/l IGF-I with significantly higher implantation rates seen. Resorption rates were also decreased in the group with added metformin compared with the IGF-I alone group. In addition, the crown-rump lengths of the fetuses from the IGF-I group were significantly smaller than the control-treated fetuses. Once again, metformin rescued these embryos from growth retardation. The average crown-rump length of the metformin plus IGF-I group was significantly higher than the IGF-I alone group and not significantly different from the control group (Fig. 5*B*).

DISCUSSION

Previous models of high insulin or IGF-I environments have shown a detrimental effect on murine preimplantation embryos both in vitro and in vivo (9,10,17,36). High physiological levels of IGF-I and insulin induce a decrease in protein expression of the IGF-I receptor on the surface of the mouse blastocyst and decreased insulin/IGF-I signaling in the embryo (10,17). Downregulation of the IGF-I receptor occurs in response to a high ligand concentration, as seen with other in vitro and in vivo model systems of hyperinsulinemia and/or high IGF-I (14,15,37). This phenomenon is also accompanied by a decrease in insulin-stimulated glucose uptake, which is regulated by the IGF-I receptor (9). These findings are consistent with insulin resistance in the blastocyst. The resulting apoptosis seen

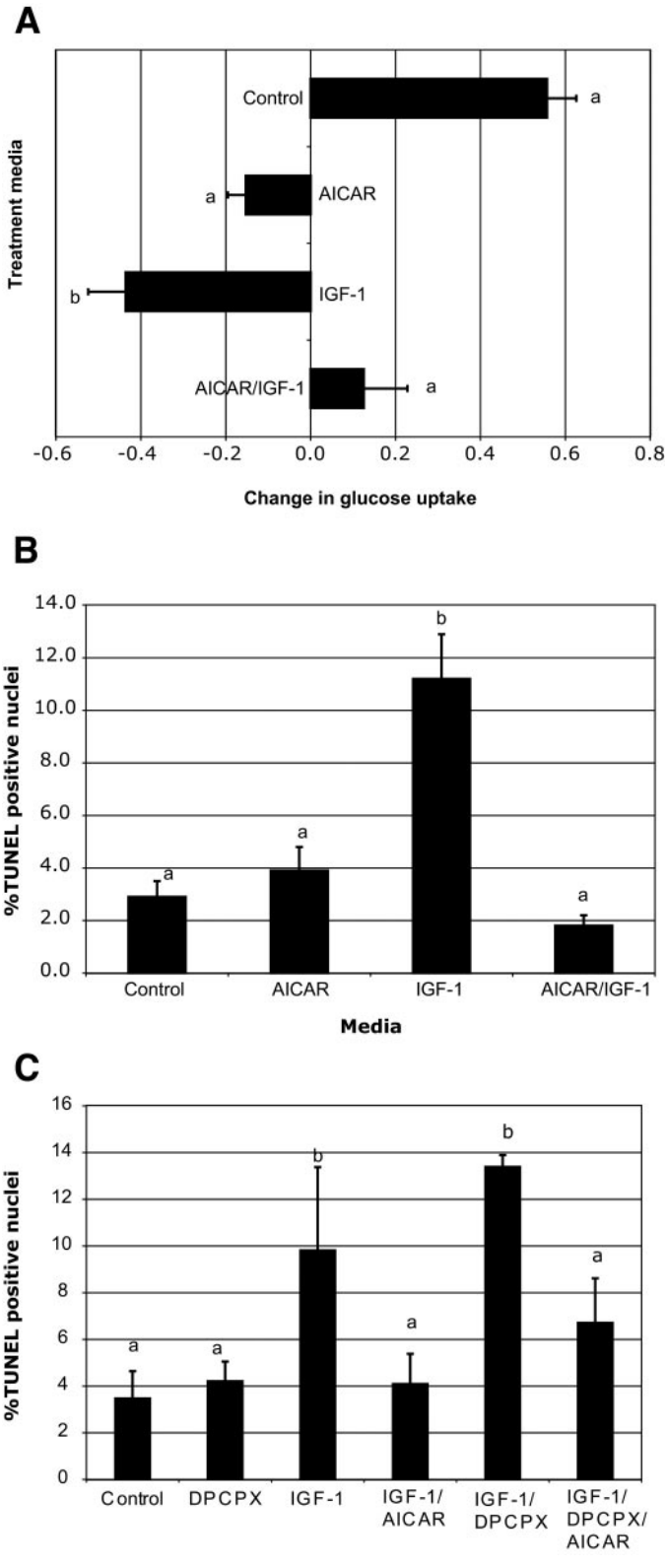


FIG. 4. AICAR prevents IGF-I effects on glucose uptake and apoptosis, and these events are not mediated by adenosine-like action. *A*: Two-cell embryos were cultured for 72 h in control medium (n = 27), medium with AICAR alone (n = 25; 1 μmol/l), medium with high concentrations of IGF-I (n = 23; 200 nmol/l), or medium with IGF-I plus AICAR (n = 15; 1 μmol/l). Values with different letters were significantly different (P < 0.001). *B*: Apoptosis was measured in individual blastocysts from each condition using the TUNEL assay. The percentage of TUNEL-positive nuclei was significantly lower in embryos cultured in control HTF (n = 31), AICAR alone (n = 30), or high IGF-I plus AICAR (n = 36) compared with high IGF-I alone (n = 34) (P < 0.001). *C*: Two-cell embryos were cultured for 72 h in control medium (n = 24), the A1 adenosine receptor antagonist DPCPX at a dose of 1 μmol/l (n = 18), IGF-I (200 nmol/l) (n = 21), IGF-I plus AICAR (1 μmol/l) (n = 19), or IGF-I plus AICAR and DPCPX (n = 20). Apoptosis was measured as in *B*. Values with different letters were significantly different. IGF-I plus DPCPX vs. IGF-I plus DPCPX plus AICAR, P = 0.01; IGF-I plus AICAR vs. IGF-I plus DPCPX, P < 0.001).

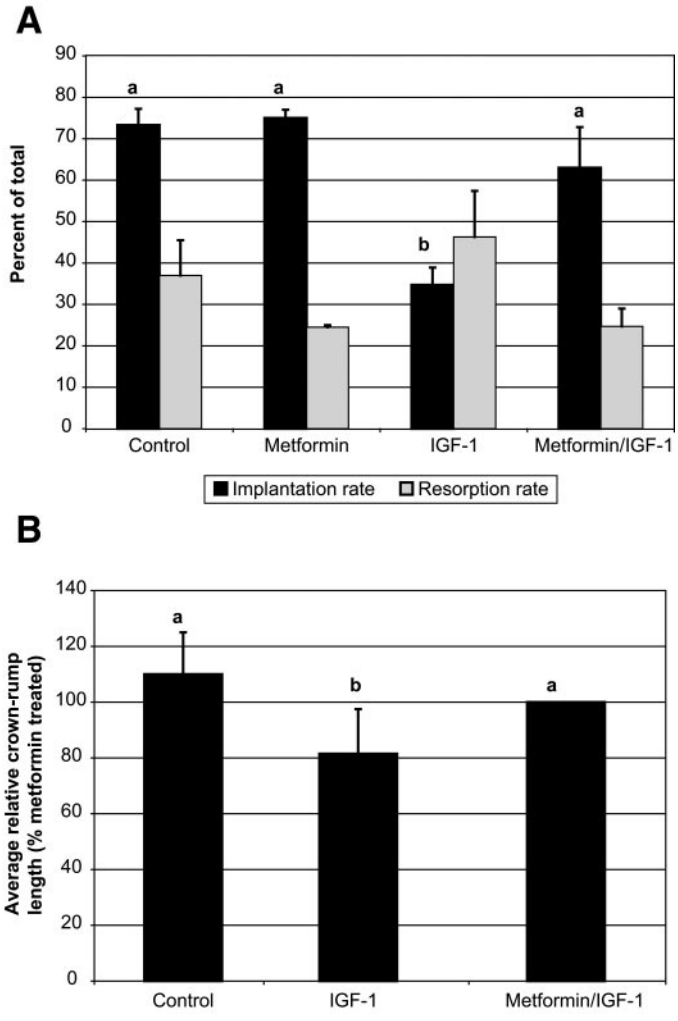


FIG. 5. Metformin reverses IGF-I effect on pregnancy outcome and crown-rump length. *A*: Embryos were cultured as described for Fig. 2 and then transferred into control mice. Values with different letters were significantly different. Control embryos (n = 8 experiments) had significantly higher implantation rates compared with embryos cultured in 200 nmol/l IGF-I and transferred (n = 8 experiments; P < 0.003). Metformin plus IGF-I (n = 8 experiments) reversed the adverse effect of IGF-I (P < 0.05). *B*: Crown-rump length at E14.5 was measured in embryos from the control group (n = 71 fetuses), the IGF-I group (n = 53), and the IGF-I plus metformin group (n = 61). Values with different letters were significantly different (P < 0.05).

in these blastocysts is attributed to decreased insulin signaling, which has been reported in other cell types (38), and to diminished glucose uptake, which is critical to embryo development and survival (39).

Pregnancy outcome of these IGF-I–induced apoptotic and insulin-resistant embryos is poor. In vivo studies using IGF-I–containing pellets and embryo transfer studies demonstrate higher rates of miscarriages and lower implantation rates among embryos exposed to higher concentrations of IGF-I or insulin (17). Targeting the cascade that links IGF-I levels to embryo loss may be helpful in treating recurrent pregnancy loss in women with PCOS.

Metformin has been shown to improve GLUT4-mediated glucose transport in insulin-resistant skeletal muscle and adipose tissue (24,38). Although GLUT4 is not expressed in the blastocyst, GLUT8 is the insulin-responsive transporter in the mouse blastocyst (11). Therefore, the cellular machinery involved in insulin-stimulated glucose transport in the blastocyst may be similar to that of skeletal muscle and adipose tissue, with GLUT8 acting as GLUT4. In this study, we show for the first time that metformin directly improves insulin signaling in the insulin-resistant mouse blastocyst. Yet the beneficial effects of metformin are not only with glucose uptake, but also with embryonic survival, because apoptosis at the blastocyst stage is decreased. As a result, embryo outcome is improved with a decrease in embryo resorption seen in transfer studies and an increase in embryo size as measured by crown-rump length. Metformin has been shown to improve insulin action by several different mechanisms, including generation of mitochondrial reactive nitrogen species (40), inhibition of fatty acid–induced nuclear factor-κB transactivation (41), and improving glucose transport (24), with all effects having in common activation of AMP-activated protein kinase or AMPK.

AMPK is a sensor of cellular energy that switches on ATP-generating catabolic processes while switching off ATP-requiring pathways. We demonstrate for the first time that preimplantation embryos experiencing decreased insulin sensitivity as a result of prolonged exposure to 200 nmol/l IGF-I have decreased AMPK activity, as measured by the ratio of P-AMPK to AMPK. Metformin coculture activates AMPK in the mouse blastocyst, which leads to improved embryo survival, implantation, and fetal size. This treatment also improves insulin sensitivity in the IGF-I–treated blastocyst. In addition, AICAR was used as a control for stimulation of AMPK and supported the hypothesis that the effects of metformin were due to AMPK activity. To test that the effect of AICAR was not via the adenosine-like action of the drug, we also used the A1 adenosine receptor antagonist DPCPX. Adding DPCPX did not alter the positive effect of AICAR on IGF-I–induced apoptosis. AMPK activity has not been described previously in mouse blastocysts; however, it has been identified in the mouse oocyte where AICAR and AMPK activation accelerate meiotic maturation (25).

Although the AMPK pathway has recently been linked to the regulation of whole-body energy homeostasis, historically, AMPK has been associated with sensing and regulating energy balance at a cellular level in systems as simple as single-cell organisms. Genes encoding AMPK are also found in plants and yeast *Saccharomyces cerevisiae* (42), which has an ortholog that responds to glucose deprivation. We identify the blastocyst as a multicellular developmental system that responds to AMPK activation by improving insulin sensitivity and promoting embryo survival.

Many women with hyperinsulinemia or insulin resistance who wish to conceive are already taking metformin to improve their patterns of ovulation (43,44). This study provides evidence to support the continued use of metformin after conception to improve pregnancy outcome in these women (19,20). Furthermore, these studies suggest that AMPK activation directly improves insulin-stimulated glucose uptake and decreases apoptosis at the level of the blastocyst and that this may be a mechanism for improved pregnancy outcomes in patients treated with metformin.

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