Chronic exposure to β-hydroxybutyrate impairs insulin action in primary cultures of adult cardiomyocytes

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Tardif, Annie, Nathalie Julien, Amélie Pelletier, Gaétan Thibault, Ashok K. Srivastava, Jean-Louis Chiasson, and Lise Coderre. Chronic exposure to β-hydroxybutyrate impairs insulin action in primary cultures of adult cardiomyocytes. Am J Physiol Endocrinol Metab 281: E1205–E1212, 2001.—Type 1 and type 2 diabetic patients often show elevated plasma ketone body concentrations. Because ketone bodies compete with other energetic substrates and reduce their utilization, they could participate in the development of insulin resistance in the heart. We have examined the effect of elevated levels of ketone bodies on insulin action in primary cultures of adult cardiomyocytes. Cardiomyocytes were cultured with the ketone body β-hydroxybutyrate (β-OHB) for 4 or 16 h, and insulin-stimulated glucose uptake was evaluated. Although short-term exposure to ketone bodies was not associated with any change in insulin action, our data demonstrated that preincubation with β-OHB for 16 h markedly reduced insulin-stimulated glucose uptake in cardiomyocytes. This effect is concentration dependent and persists for at least 6 h after the removal of β-OHB from the media. Ketone bodies also decreased the stimulatory effect of phorbol 12-myristate 13-acetate and pervanadate on glucose uptake. This diminution could not be explained by a change in either GLUT-1 or GLUT-4 protein content in cardiomyocytes. Chronic exposure to β-OHB was associated with impaired protein kinase B activation in response to insulin and pervanadate. These results indicate that prolonged exposure to ketone bodies altered insulin action in cardiomyocytes and suggest that this substrate could play a role in the development of insulin resistance in the heart. insulin resistance; ketone bodies; glucose uptake; protein kinase B; heart

DIABETIC SUBJECTS have an increased morbidity and mortality from cardiac disease. In men and women, diabetes raises the risk of developing heart disease by 2.4 and 3.5 times, respectively (40). Recent studies suggest that abnormal regulation of glucose uptake in the heart plays a role in cardiac dysfunction. In humans, a reduction in cardiac glucose uptake has been observed in obese and type 2 diabetic subjects (3). Diminished glucose utilization and contractile dysfunction have been also observed in the heart of db/db mice, a model of type 2 diabetes (2). These abnormalities were normalized by selective overexpression of a glucose transporter (GLUT-4) in the heart (2).

In addition to glucose, the heart also uses ketone bodies as an energy source. Studies have shown that acetacacetate reduces the oxidation of both fatty acids and lactate in the heart (25, 36). Potentially, ketone bodies could modulate glucose utilization and insulin action in the heart. Various pathophysiological situations such as diabetes and starvation are associated with hyperketonemia (25). Significant increases in ketone body concentration are also observed in chronic heart failure (27, 28) and after a high-fat diet (25). Interestingly, perfusion of the rat heart with ketone bodies as the sole substrate provoked a severe decline in contractile function, a condition that was normalized with the inclusion of glucose in the perfusate (41). In congestive heart failure, the increase in plasma ketone body concentration correlates with the severity of cardiac dysfunction (28).

The major glucose transporters expressed in the heart are GLUT-1 and GLUT-4. In this tissue, >80% of GLUT-1 resides at the plasma membrane, and it is explained that this transporter is primarily responsible for basal glucose uptake (13). In contrast, GLUT-4 is found primarily in intracellular vesicles (4, 13). Insulin stimulation of glucose uptake is accomplished by recruiting GLUT-4 and to a lesser extent GLUT-1 from their intracellular sites to the plasma membrane (13, 37, 46, 48).

Our understanding of the insulin-signaling cascade has increased dramatically in recent years, and many additional proteins involved in insulin action have been identified. One of these proteins is the serine/threonine kinase protein kinase B (PKB) or Akt. Overexpression of a constitutively active PKB increases GLUT-4 translocation in both muscle cells (15, 45) and adipocytes (22, 45). In addition, microinjection of either a PKB substrate peptide or PKB antibody reduces GLUT-4 in response to insulin by 50% (18). On the
other hand, transfection with dominant negative PKB mutants has yielded contradictory results (45). Recent studies have demonstrated, however, that in mice lacking the gene for PKB-β, the ability of insulin to reduce plasma glucose concentration is reduced (6). Thus the bulk of the data suggest that PKB plays a role in insulin-mediated glucose transport.

Because of the potential role of ketone bodies in the regulation of substrate utilization, we determined whether elevated concentrations of this substrate induce insulin resistance in the heart. To address this question, we have examined the effect of ketone bodies on insulin-stimulated glucose uptake and PKB activation in primary cultures of adult cardiomyocytes.

**EXPERIMENTAL PROCEDURES**

**Chemicals.** All cell culture solutions, supplements, and DNase I were from Sigma-Aldrich Canada (Oakville, ON). Collagenase was obtained from Worthington Biochemical (Lakewood, NJ). Human insulin (Humulin R) was from Eli Lilly Canada (Toronto, ON). Anti-GLUT-1 and GLUT-4 antibodies were from Research Diagnostics (Flanders, NJ) and Santa Cruz Biotechnology (Santa Cruz, CA), respectively. Polyclonal antibodies for phospho-PKB (Ser{sup 473}) and PKB-α were purchased from New England Biolabs (Beverley, MA). 2-[{sup 3}H]deoxyglucose (2-DG) was purchased from NEN Research Products (Boston, MA). The enhanced chemiluminescence detection system was from Amersham Pharmacia Biotechnology (Baie d’Urfe, QC, Canada). The bichinonic acid (BCA) protein assay kit was purchased from Pierce (Rockford, IL). All electrophoresis reagents were obtained from Boehringer-Mannheim (Laval, QC, Canada). Potassium dipherovonanadate was synthesized according to Ravishankar et al. (34).

**Isolation of adult rat cardiomyocytes.** All experiments conformed to guidelines of the Canadian Council of Animal Care and were approved by the Animal Care Committee of the Centre Hospitalier de l’Université de Montréal. Male Sprague-Dawley rats weighing 175–200 g were injected with heparin sulfate (500 units ip) 15 min before anesthesia with pentobarbital sodium (60 mg/kg ip). The heart was excised, and calcium-tolerant cardiomyocytes were isolated by the Langendorff method (retrograde perfusion), as described previously (43). During the whole procedure, the cells were washed two times with 4 ml of media containing no insulin or β-OBH. The cells were then incubated in 2 ml of media containing no insulin or β-OBH for 16 h. On the day of the study, the cells were washed two times with 4 ml of media containing no insulin or β-OBH. The cells were then incubated in 2 ml of media containing no insulin or β-OBH for 1 h. They were then treated with saline, 10−8 M insulin, or 100 μM perevanadate for 5 min. The reaction was stopped by two rapid washes with ice-cold KR buffer. Cells were lysed in buffer containing 25 mM Tris-HCl, pH 7.4, 25 mM NaCl, 1 mM sodium orthovanadate, 10 mM sodium fluoride, 10 mM sodium pyrophosphate, 2 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 2 mM benzamidine, 10 μg/ml aprotinin, 0.5 μg/ml leupeptin, 1% Triton X-100, and 0.1% SDS. The lysate was then centrifuged for 10 min at 12,000 g at 4 °C to remove insoluble material, and the resulting supernatant was used for immunoblotting.

**Gel electrophoresis and immunoblotting.** Samples were electrophoresed on a 10% SDS polyacrylamide gel and transferred to polyvinylidene difluoride membranes (Immobilon, Millipore, Bedford, MA) for Western blotting. The membranes were blocked for 1 h in 5% (wt/vol) nonfat dry milk in PBS. They were then incubated with an appropriate primary antibody. For the PKB experiments, the blots were first probed with anti-phosphorylated (Ser{sup 473}) PKB antibody and then reprobed with anti-α-PKB. This was followed by a second incubation with the appropriate secondary antibodies conjugated to horseradish peroxidase, and the antigen-antibody complex was detected with the enhanced chemiluminescence method. Quantitative analysis was performed using a scanning densitometer.

**Statistical analysis.** Statistical analysis was performed using one-way ANOVA for multiple comparisons. This was followed by a Tukey’s post hoc test. All data are reported as means ± SE. Glucose uptake data are means of at least five independent experiments. For the PKB experiments, densitometric analysis was performed on three independent experiments. A value of P < 0.05 was considered significant.

**RESULTS**

**Induction of insulin resistance by ketone bodies.** Because ketone bodies are increased under various pathophysiological situations, we examined whether an elevation in their concentration would decrease glucose uptake and create a state of insulin resistance in the...
heart. To address this question, primary cultures of adult cardiomyocytes were incubated with the ketone body β-OHB for either 4 or 16 h, and insulin-stimulated glucose uptake was evaluated. We chose β-OHB because it is the primary ketone body produced during hyperketonemia (25). Addition of 10⁻⁸ M insulin increased glucose uptake from to 7.2 to 15.2 Bq/mg protein in cardiomyocytes (P < 0.05; Fig. 1A). The strength of this effect was comparable to that in previous reports in the literature for these cells (9). Although exposure for 4 h to 5 mM β-OHB did not affect basal or insulin-mediated glucose uptake, our results demonstrated that incubation with β-OHB for 16 h inhibited the stimulatory effect of insulin on glucose uptake in cardiomyocytes (P < 0.05; Fig. 1B).

The development of insulin resistance induced by ketone bodies is dose dependent. Plasma ketone body concentrations vary greatly depending on the nutritional or pathophysiological state of the animal. In healthy individuals, ketone body concentrations are usually ≤0.2 mM. During hyperketonemia, however, these levels can increase up to 1 mM, while during ketoacidosis they reach levels between 3 and 20 mM (25). Similarly, in the rat, diabetes and starvation are associated with concentrations of β-OHB >1 mM (19). We determined the concentration of ketone body required to induce insulin resistance in cardiomyocytes. Cardiomyocytes were incubated with increasing concentrations of β-OHB for 16 h, and glucose uptake was evaluated (Fig. 2). Exposure to either 1 or 5 mM β-OHB inhibited insulin action in these cells (P < 0.05). Although not significant, a 20% decrease in the maximal insulin effect was observed at 0.5 mM β-OHB. Pre-treatment with 0.2 mM β-OHB has no effect on insulin action. In addition, incubation with increasing concentrations of β-OHB was associated with a progressive reduction in basal glucose transport. Although never significant, this effect was seen in most of the experiments (Fig. 3 and see Fig. 5). Thus the decreased response to insulin after pretreatment with ketone bodies was dose dependent and occurred at concentrations that are associated with physiological and pathological states in vivo. All subsequent experiments were done at 5 mM β-OHB.

Role of insulin in the reduction of glucose uptake induced by ketone bodies. Our preparations of adult cardiomyocytes were cultured in the presence of 10⁻⁷ M insulin to maintain cell survival. Desensitization of

Fig. 1. Incubation of cardiomyocytes with the ketone body β-hydroxybutyrate (β-OHB) for 4 (A) or 16 (B) h before glucose uptake. Data are from 5 (A) and 10 (B) independent experiments. *P < 0.05, basal vs. insulin-treated cells. †P < 0.05, different from insulin-stimulated control.

Fig. 2. Incubation of cardiomyocytes with increasing concentrations of β-OHB for 16 h before glucose uptake. Data shown are from 7 independent experiments. *P < 0.05, basal vs. insulin-treated cells. †P < 0.05, different from insulin-stimulated control (0 mM β-OHB).

Fig. 3. Incubation of control and β-OHB-treated cardiomyocytes with media containing either 10⁻⁷ or 10⁻¹¹ M insulin for 16 h before glucose uptake. Data shown are from 5 independent experiments. *P < 0.05, basal vs. insulin-treated cells. †P < 0.05, different from insulin-stimulated control.
the glucose uptake process by high insulin concentrations has been observed in both adipocytes (14, 42) and skeletal muscle (16) and could play a role in the development of ketone body-induced insulin resistance. Thus the reduction in insulin-stimulated glucose uptake that we observed with ketone bodies could potentially result from a combination of elevated concentrations of insulin and β-OHB. Adult cardiomyocytes were cultured with either $10^{-11}$ or $10^{-7}$ M insulin in the presence or absence of ketone bodies for 16 h, and insulin-mediated glucose transport was examined. We chose these concentrations because complete removal of the hormone from the media for long periods of time decreased insulin action in primary cultures of adult cardiomyocytes (data not shown and Ref. 9). As shown in Fig. 3, reducing the concentration of insulin in the media from $10^{-7}$ to $10^{-11}$ M caused a slight increase in the effect of insulin on cardiomyocytes. This protocol did not, however, prevent the development of insulin resistance, and the effect of the hormone was reduced by 50% both at $10^{-7}$ and $10^{-11}$ M insulin ($P < 0.05$).

**Resensitization of insulin-mediated glucose uptake after ketone body exposure.** To better understand the mechanism underlying the development of insulin resistance by ketone bodies, we next investigated the time course necessary to reestablish insulin action in insulin-resistant cells after removal of ketone bodies. Cardiomyocytes were exposed to 5 mM β-OHB for 16 h, as described above. The cells were washed two times with media containing no β-OHB and then incubated for a period of 4, 6, or 8 h before evaluation of glucose uptake. Our results demonstrated that removal of β-OHB for either 4 or 6 h did not improve insulin action in these cells. A period of 8 h was required to restore insulin responsiveness in β-OHB-pretreated cardiomyocytes (Fig. 4). These results indicate that insulin resistance persists for an extended period of time, even after the removal of β-OHB from the media.

**Effect of ketone bodies on the activation of glucose uptake by PMA and pervanadate.** We next examined whether ketone bodies alter the response to other glucose transport agonists. It has been shown that, in skeletal muscle, phorbol esters mediate glucose uptake by a mechanism distinct from insulin (17). Phorbol esters are functional analogs of diacylglycerol that are able to activate classical and novel protein kinase C (PKC). Acute activation of PKC was achieved with the phorbol ester PMA. As shown in Fig. 5A, exposure to 100 and 500 nM PMA increased glucose uptake from 12.3 to 17.5 and 20.7 Bq/mg protein, respectively. In adult cardiomyocytes, the effect of insulin and PMA on glucose uptake was not additive. Pretreatment with β-OHB for 16 h reduced the effect of 100 and 500 nM PMA on glucose uptake by 55 and 64%, respectively ($P < 0.05$).

Pervanadate is a powerful tyrosine phosphatase inhibitor that mimics insulin action in a variety of cells. It has been shown that pervanadate can bypass a defect in insulin signaling and stimulate glycogen synthesis in insulin-resistant skeletal muscle (26). Thus we examined whether this compound could stimulate glucose uptake in ketone body-treated cardiomyocytes. As shown in Fig. 5B, pervanadate increased glucose uptake from 21.2 to 31.6 Bq/mg protein. The maximal effect of pervanadate was reduced by 53% in β-OHB-treated cardiomyocytes ($P < 0.05$).
Effect of ketone bodies on GLUT-1 and GLUT-4 expression. The reduction in insulin-stimulated glucose uptake by ketone bodies could result from changes in the expression of the glucose transporter in cardiomyocytes. To address this possibility, we evaluated both GLUT-1 and GLUT-4 content in control and β-OHB-treated cardiomyocytes. As shown in Fig. 6, ketone bodies did not modify either GLUT-1 or GLUT-4 protein content in these cells.

Impairment in PKB activation in response to ketone bodies in adult cardiomyocytes. PKB is a serine/threonine kinase that has been implicated in insulin action. Studies by Tsiani et al. (44) have shown that pervanadate is a powerful activator of PKB. Because PKB activation also plays a key role in insulin action, we were interested in determining whether the activation of this enzyme by either insulin or pervanadate was altered in β-OHB-pretreated cardiomyocytes. To address this question, cardiomyocytes were pretreated with ketone bodies for 16 h, after which PKB activation in response to either insulin or pervanadate was evaluated. To ensure that these differences were not the result of unequal PKB expression, the relative levels of PKB were determined by immunoblotting. Ketone bodies did not affect PKB expression in cardiomyocytes (Fig. 7B). In control cells, insulin and pervanadate induce a four- to six-fold increase in PKB phosphorylation (Fig. 7A). Ketone body pretreatment decreases PKB phosphorylation in response to insulin and pervanadate by 84 and 69%, respectively (Fig. 7C). In addition to its effect on insulin and pervanadate action, β-OHB-treated cardiomyocytes show a tendency toward lower basal PKB phosphorylation.

DISCUSSION

Diabetic ketoacidosis is a major complication of type 1 diabetes. Less well known, however, is that plasma ketone body concentrations are also increased in poorly controlled type 2 diabetic patients, particularly in African-Americans (1, 25). Higher ketone body levels have also been observed during chronic heart failure and after consumption of a high-fat diet (25, 27, 28). Therefore, there exist a number of physiological and pathological situations where plasma ketone body concentrations are augmented. Because ketone bodies compete with various metabolic substrates such as fatty acids, lactate, or glucose for utilization by the heart (31, 36, 47), their contribution to the energy supply of the myocardium will increase significantly during hyperketonemia. Our results support a role for ketone bodies in the regulation of substrate utilization and demonstrate for the first time that chronic exposure to β-OHB induces insulin resistance in primary cultures of adult cardiomyocytes. We have demonstrated that 1) prolonged exposure to β-OHB decreases insulin responsiveness as evaluated by glucose transport and PKB activation, 2) the development of insulin resistance by β-OHB is concentration dependent, and 3) in ketone body-treated cardiomyocytes, impaired glucose uptake in response to insulin persists for at least 6 h after the removal of β-OHB. Together, these data suggest that ketone bodies can play an important role in the regulation of glucose uptake in cardiomyocytes.

Plasma ketone body concentrations can rise dramatically during diabetes, reaching levels up to 20 mM (25). Our results demonstrated that a diminution in insulin-mediated glucose transport occurs after prolonged exposure to ketone bodies (16 h) at concentra-

![Fig. 6. Total GLUT-4 (A) and GLUT-1 (B) immunoreactive protein in control and β-OHB-treated cardiomyocytes.](http://ajpendo.physiology.org/)

![Fig. 7. Protein kinase B (PKB) activation by insulin (I) and pervanadate (PV) in control and β-OHB-treated cardiomyocytes. B, basal. A: representative immunoblot of phospho-PKB. B: representative immunoblot of total PKB-α. C: calculation of the ratio of signal derived from scanning phosphorylated PKB over the signal derived from total PKB.](http://ajpendo.physiology.org/)
tions of 1 mM and greater. This could have clinical significance because it has been shown that, during diabetic ketoacidosis, plasma levels of ketone bodies usually remain elevated for >12 h after the beginning of treatment to reduce their levels (25). Thus the reduction in insulin responsiveness observed in β-OHB-pretreated cardiomyocytes in vitro occurs at concentrations and within a time frame that are consistent with a role for this substrate in the regulation of glucose transport during the etiology of diabetic complications. Because our measurements were done at supraphysiological insulin concentrations, we do not know whether ketone bodies also alter insulin sensitivity in these cells. Studies by Fischer et al. (12) have demonstrated that the inhibitory effect of various cardiac substrates on glucose transport are more pronounced at submaximal than at maximal insulin concentration. It would thus be very interesting to determine whether prolonged exposure to ketone bodies also alters insulin sensitivity in addition to its effect on insulin responsiveness.

Our results show that short-term exposure to β-OHB (4 h) does not modify insulin action in cardiomyocytes. Therefore, the inhibition of insulin-stimulated glucose uptake observed in these cells is not consistent with a direct competition between glucose and β-OHB as energy sources. However, the regulation of glucose uptake could potentially result from the intracellular metabolism of β-OHB. It has been shown that ketone bodies modulate the production of a number of metabolites in the heart. Perfusion of the isolated heart with the ketone body acetacetate increases the concentration of acetyl-CoA, acetoacetyl-CoA, and citrate (31–33, 36). Randle and coworkers (33) have proposed that an increase in citrate and acetoacetyl-CoA levels could inhibit phosphofructokinase and pyruvate dehydrogenase activity, respectively. This would lead to an increase in glucose 6-phosphate concentrations that could then inhibit hexokinase activity and reduce glucose uptake in the heart. An increase in glucose 6-phosphate has been observed in hearts perfused with acetacetate and could play a role in the decreased glucose uptake seen in β-OHB-treated cardiomyocytes (36). A second possibility is that ketone bodies increase glyco
gen and/or triglyceride content in cardiomyocytes and could reduce glucose transport by a feedback mechanism. An increase in glycogen accumulation could be caused either by the stimulation of glycogen synthase activity by glucose 6-phosphate or by an increase in β-OHB utilization during hyperketonemia, which could divert some of the glucose taken up by the cardiomyocytes toward either glycogen or lipid synthesis. This would also explain the 8-h period that is necessary to restore insulin action in β-OHB-treated cardiomyocytes. In skeletal muscle, an increase in glycogen or triglycerides levels has been associated with reduced insulin-stimulated glucose uptake (16, 20, 21, 35). Similar mechanisms could also operate in the heart and induce insulin resistance in this tissue. We are currently examining these various possibilities.

In addition to its effect on insulin action, β-OHB induces a nonsignificant decrease in basal glucose uptake compared with control cells. Because β-OHB induces a similar decrease in basal glucose transport at both 10^{-7} and 10^{-11} M insulin, we believe that this reduction is not the result of incomplete removal of the hormone during the washes. Reductions in basal and insulin-stimulated glucose uptake have been observed in cardiomyocytes exposed to extracellular ATP because of a redistribution of glucose transporters from the plasma membrane to their intracellular site (11). Potentially, ketone bodies could promote a similar internalization of these transporters and thus lower basal glucose transport in these cells.

Our results demonstrated that neither GLUT-4 nor GLUT-1 protein content was altered by ketone body treatment. Thus a change in glucose transporter expression cannot explain the reduction in agonist-stimulated glucose uptake observed in cardiomyocytes. In the heart, glucose uptake results from glucose transport into the cells and its subsequent phosphorylation by the enzyme hexokinase. It has been shown that, during insulin stimulation, the rate-limiting step for this process switches from glucose transport to glucose phosphorylation (29). Recent studies suggest that, in the presence of β-OHB, glucose transport may not be the rate-limiting step for glucose uptake (5). Thus ketone body-induced insulin resistance could be the result of a decrease in GLUT-4 translocation, a decrease in hexokinase activity, or a combination of both. Ketone bodies could also impair glucose transporter trafficking or their fusion/insertion in the plasma membrane independently of the insulin-signaling cascade. A defect at either of these steps would diminish the effect of glucose transport agonists in β-OHB-pretreated cardiomyocytes.

Our results show that, in addition to insulin, ketone bodies also impair the activation of glucose uptake and response to both phorbol esters and pervanadate. This suggests that more than one signaling pathway is affected by ketone body treatment or alternatively that they regulate a common step of the three glucose uptake agonists or a combination of both. Recent data support a role for PKB in the regulation of insulin-stimulated glucose uptake. Impaired PKB activation in response to insulin has been observed in skeletal muscle from type 2 diabetic subjects (24) and diabetic Goto-Kakizaki rats (39). Furthermore, improvement in glucose uptake in these rats was associated with normalization of PKB activation in response to insulin treatment (23). For these reasons, we investigated the effect of β-OHB on both insulin and pervanadate activation of PKB. Our results demonstrated that β-OHB impairs PKB phosphorylation by both agonists in adult cardiomyocytes. It has been shown that PKB lies downstream of the insulin receptor substrates (IRS)-phosphatidylinositol (PI)-3-kinase signaling pathway. The induction of insulin resistance caused by ketone bodies could therefore arise from impaired activation of this signaling cascade, resulting in decreased PKB activation. Alternatively, the block in insulin action after
KETONE BODIES INDUCE INSULIN RESISTANCE IN CARDIOMYOCYTES

ketone body treatment could directly target PKB. In skeletal muscle, both mechanisms have been implicated in the development of insulin resistance by different fatty acids. Infusion of a mixture of fatty acids reduced both IRS-1 tyrosine phosphorylation and PI 3-kinase activity in human skeletal muscle (8). On the other hand, Schmitz-Peiffer et al. (38) have shown that the fatty acid palmitate inhibits PKB activation by insulin without altering IRS-1 phosphorylation and PI 3-kinase activation in response to the hormone. Although we have established that ketone bodies act at the level of PKB, it remains to be determined whether this effect involves alteration in PI 3-kinase activation and/or direct inhibition of PKB. Potentially, ketone bodies could activate a phosphatase, resulting in enhanced PKB dephosphorylation and thus inactivation of the enzyme. Selective dephosphorylation and inhibition of PKB by protein phosphatase 2A has been observed after osmotic shock (30). Interestingly, hypomotic stress also prevents PKB activation in response to pervanadate (30). It is therefore possible that ketone bodies antagonize both insulin and pervanadate action in cardiomyocytes by promoting PKB dephosphorylation.

In conclusion, our results demonstrate that chronic exposure to ketone bodies inhibits insulin-stimulated glucose uptake in primary cultures of adult cardiomyocytes. This effect is both time and concentration dependent. Ketone bodies also alter the ability of both phorbol esters and pervanadate to stimulate glucose uptake in these cells. Our data also indicate that ketone body pretreatment is associated with impaired PKB activation in response to insulin and pervanadate.

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KETONE BODIES INDUCE INSULIN RESISTANCE IN CARDIOMYOCYTES


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