Effect of 5-Aminoimidazole-4-Carboxamide-1-β-D-Ribofuranoside Infusion on In Vivo Glucose and Lipid Metabolism in Lean and Obese Zucker Rats

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Activation of AMP-activated protein kinase (AMPK) with 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) increases glucose transport in skeletal muscle via an insulin-independent pathway. To examine the effects of AMPK activation on skeletal muscle glucose transport activity and whole-body carbohydrate and lipid metabolism in an insulin-resistant rat model, awake obese Zucker *fa/fa* rats ($n = 26$) and their lean ($n = 23$) littermates were infused for 90 min with AICAR, insulin, or saline. The insulin infusion rate ($4\ mU \cdot kg^{-1} \cdot min^{-1}$) was selected to match the glucose requirements during AICAR (bolus, 100 mg/kg; constant, $10\ mg \cdot kg^{-1} \cdot min^{-1}$) isoglycemic clamps in the lean rats. The effects of these identical AICAR and insulin infusion rates were then examined in the obese Zucker rats. AICAR infusion increased muscle AMPK activity more than fivefold ($P < 0.01$ vs. control and insulin) in both lean and obese rats. Plasma triglycerides, fatty acid concentrations, and glycerol turnover, as assessed by [2-^{13}C]glycerol, were all decreased in both lean and obese rats infused with AICAR ($P < 0.05$ vs. basal), whereas insulin had no effect on these parameters in the obese rats. Endogenous glucose production rates, measured by [U-^{13}C]glucose, were suppressed by >50% during AICAR and insulin infusions in both lean and obese rats ($P < 0.05$ vs. basal). In lean rats, rates of whole-body glucose disposal increased by more than twofold ($P < 0.05$ vs. basal) during both AICAR and insulin infusion; [^{3}H]2-deoxy-D-glucose transport activity increased to a similar extent, by >2.2-fold (both $P < 0.05$ vs. control), in both soleus and red gastrocnemius muscles of lean rats infused with either AICAR or insulin. In the obese Zucker rats, neither AICAR nor insulin stimulated whole-body glucose disposal or soleus muscle glucose transport activity. However, AICAR increased glucose transport activity by ~2.4-fold ($P < 0.05$ vs. control) in the red gastrocnemius from obese rats, whereas insulin had no effect. In summary, acute infusion of AICAR in an insulin-resistant rat model activates skeletal muscle AMPK and increases glucose transport activity in red gastrocnemius muscle while suppressing endogenous glucose production and lipolysis. Because type 2 diabetes is characterized by diminished rates of insulin-stimulated glucose uptake as well as increased basal rates of endogenous glucose production and lipolysis, these results suggest that AICAR-related compounds may represent a new class of antidiabetic agents. *Diabetes* 50:1076–1082, 2001

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AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; ANOVA, analysis of variance; 2-DG, 2-deoxy-D-glucose; EGP, endogenous glucose production; GIR, glucose infusion rate; ZMP, monophosphorylated AICAR.

Insulin resistance is a major factor contributing to the pathogenesis of type 2 diabetes. It is largely attributable to reduced insulin-stimulated muscle glucose uptake (1–3) and muscle glycogen synthesis (4), which in turn can be attributed to reduced muscle glucose transport activity (5,6). In contrast, contraction-stimulated muscle glucose transport, which is thought to be insulin independent, is normal in obese Zucker rats (7,8). In addition, exercise increases GLUT4 plasma membrane content to a similar extent in type 2 diabetic patients and normal subjects (9). Furthermore, the insulin-independent phase of glycogen resynthesis immediately after exercise is normal in the muscles of insulin-resistant offspring of type 2 diabetic patients (10). Therefore, the exercise-mediated non–insulin-dependent pathway for the stimulation of muscle glucose uptake may provide an important target for the treatment of type 2 diabetes. Recent evidence indicates that pharmacological activation of AMP-activated protein kinase (AMPK) by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) stimulates glucose transport activity in skeletal muscle (11–13) and heart (14) in the absence of phosphoinositide 3-kinase activation, suggesting that this action is independent of the insulin-signaling pathway. Furthermore, muscle AMPK is activated during exercise (15), and may play an important role in contraction-induced stimulation of glucose transport (12,13). However, the effect of AMPK activation on muscle glucose uptake in an insulin-resistant state has not been studied.

Increased basal rates of endogenous glucose production (EGP) and diminished insulin-induced suppression of EGP are two other characteristics of type 2 diabetes (16–18). In vitro incubation of rat hepatocytes with AICAR has been shown to decrease hepatic gluconeogenesis by inhibiting fructose-1,6-bisphosphatase (19). In addition, intraperitoneal injection of AICAR causes hypoglycemia in mice (20).

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In vivo, AICAR inhibits EGP under euglycemic clamp conditions in normal rats (13). However, the effect of in vivo AICAR infusion on EGP in an insulin-resistant state is unknown.

Increased fasting plasma fatty acid concentrations are common among patients with type 2 diabetes (21,22) because of impaired insulin-induced suppression of lipolysis (23), and may contribute to skeletal muscle (24–26) and liver (27,28) insulin resistance. In vivo infusion of AICAR leads to a 50% reduction in fasting plasma fatty acid concentrations in normal rats (13). Activation of AMPK stimulates the oxidation of fat in skeletal muscle (11) through the inhibition of acetyl CoA carboxylase and subsequent activation of carnitine palmitoyl-CoA transferase-1. Furthermore, isoprenaline-induced lipolysis from rat adipocytes is inhibited by AICAR, suggesting that AMPK activation exerts an antilipolytic effect by preventing the activation of hormone-sensitive lipase in rat adipocytes (29,30). Therefore, AMPK also plays an important role in the regulation of fat metabolism, a factor that has not been studied in an insulin-resistant state.

Thus, this study was performed to determine the effects of AICAR infusion on in vivo skeletal muscle glucose transport activity and whole-body glucose and glycerol turnover rates in insulin-resistant obese Zucker rats.

RESEARCH DESIGN AND METHODS

Infusion protocols. We studied genetically obese Zucker *fa/fa* male rats ages 11–12 weeks (Charles River, Raleigh, NC) and their age-matched lean littermates. The rats were maintained on standard Rat Chow (Ralston Purina, St. Louis, MO) and housed in an environmentally controlled room with a 12:12-h light-dark cycle. Rats were chronically catheterized via the right jugular vein and carotid artery (31) and allowed to recover (5–8 days) until they regained their preoperative weight. The 12-h fasted animals were infused with [U-^{13}C]glucose (0.4 μmol · kg^{-1} · min^{-1}) and [2-^{13}C]glycerol (1 μmol · kg^{-1} · min^{-1}) (Cambridge Isotopes, Andover, MA; both 99% ^{13}C atom percent excess) for 120 min to determine rates of basal EGP and glycerol turnover, and infusions were continued for the 90-min experimental infusions. Following this baseline period, rats were randomly infused with isotonic saline (control), AICAR (bolus, 100 mg/kg; constant, 10 mg · kg^{-1} · min^{-1}; Sigma, St. Louis, MO), or insulin (4 mU · kg^{-1} · min^{-1}; Humulin Regular; Eli Lilly, Indianapolis, IN). During the AICAR and insulin studies, plasma glucose concentrations were maintained at basal fasting concentrations (~7 and ~8 mmol/l in lean and obese rats, respectively) using a 50% (wt/vol) glucose solution enriched with [U-^{13}C]glucose (~0.8% isotopic enrichment). Blood was sampled every 5 min for glucose measurements and immediately before and at the end of the clamp to determine plasma insulin, fatty acid, glycerol, triglyceride, lactate, and AICAR concentrations, and every 15 min during the last 30 min before and at the end of the clamp to determine plasma isotopic enrichment of [U-^{13}C]glucose and [2-^{13}C]glycerol. The protocol was approved by the Yale Animal Care and Use Committee.

Glucose uptake. Skeletal muscle glucose uptake was measured according to a previously described method (32). Briefly, 30 min into the clamp, a 37-μCi bolus of 2-deoxy-D-[1,2-^{3}H]glucose (2-DG) was injected intravenously. Plasma samples were obtained at 0.5, 1.0, 1.5, 2, 3, 5, 7.5, 10, 15, 20, 30, 45, and 60 min after the bolus infusion to estimate the plasma tracer activity. Glucose uptake rate calculations were based on the mean plasma glucose concentration, the radiolabeled phosphorylated 2-DG tissue concentration, and the area under the plasma 2-DG curve as described by Kraegen et al. (32).

Tissue analysis. At the end of the experiment, the rats were anesthetized intravenously with pentobarbital (50 mg/kg). The calf muscle group from the right hindlimb was quickly freeze-clamped in situ for determination of AMPK activity and nucleotide and metabolite analyses. Muscle samples were kept in liquid nitrogen until analyzed. Soleus and red gastrocnemius were dissected from the left hindlimb and individually freeze clamped for determination of glucose uptake rates.

AICAR and EGP during high plasma fatty acid concentrations. Lean Zucker rats were infused for 5 h either with a triglyceride emulsion (8.5 mg · kg^{-1} · min^{-1} triglycerides; Intralipid, 20%; Abbott Laboratories, Abbott Park, IL) combined with heparin (210 mU · kg^{-1} · min^{-1}; lipid group, n = 6) or glycerol (1.25 mg of glycerol · kg^{-1} · min^{-1}; glycerol group, n = 5) as a control. Then 3 h into the lipid or glycerol infusion, all rats were infused with [U-^{13}C]glucose for a period of 120 min to assess whole-body glucose metabolism. Rats then underwent a 90-min AICAR euglycemic clamp, as described above. Blood was sampled before the lipid or glycerol infusions were started, then immediately before the AICAR infusion, and finally at the end of the experiment.

Analytical procedures. Plasma glucose and lactate concentrations were determined using an automated analyzer (YSI Instruments, Yellow Springs, OH). Immunoreactive insulin was assayed using a double-antibody immunoassay kit (Linco Research, St. Louis, MO). Plasma fatty acid (Wako, Osaka, Japan), glycerol, and triglyceride (Sigma) concentrations were assayed using colorimetric kits. AICAR plasma concentrations were determined spectrophotometrically (33). Enrichment of [U-^{13}C]glucose and [2-^{13}C]glycerol in plasma was determined by gas chromatography−mass spectrometry, as previously described (34). Phosphorylated muscle [^{3}H]2-DG was separated from a perchloric acid extract using ion exchange chromatography (35). Muscle glycogen was assayed using the amyloglucosidase method (36). Muscle nucleotides and monophosphorylated AICAR (ZMP) were separated and quantified by high-performance anion-exchange liquid chromatography (37). Skeletal muscle AMPK activity was determined by following the incorporation of [^{32}P]ATP into a synthetic peptide (15) containing the following 15–amino acid sequence: AMARAASAAALARRR (38).

Calculations. Rates of glucose and glycerol turnover were measured under steady-state conditions and were calculated using the following formula: basal glucose or glycerol turnover = $f \times ([IE_{infusate}/IE_{plasma}] - 1)$ where f is the infusion rate of either the [U-^{13}C]glucose or the [2-^{13}C]glycerol tracer (μmol · kg^{-1} · min^{-1}), and IE is the isotopic enrichment (39). Clamp EGP was calculated as follows: clamp EGP = GIR × $([E_{infusate}/E_{plasma}] - 1)$, where GIR is the mean glucose infusion rate for the last 30 min of the clamp. The clamp glucose disposal rate (R_d) was calculated as follows: R_d = clamp EGP + GIR.

Statistical analyses. All data are reported as means ± SE. Data from blood sampling and substrate turnover measurements were analyzed by a two-way analysis of variance (ANOVA) for repeated measures, and data obtained from tissue samples were analyzed with a two-way ANOVA. These analyses were followed by Tukey's test for post hoc comparisons. Differences were considered statistically significant at $P < 0.05$.

RESULTS

The mean body weight of the obese rats was significantly greater when compared with their age-matched lean littermates (355 ± 10 vs. 275 ± 9 g, respectively; $P < 0.01$). Basal plasma concentrations of glucose, insulin, fatty acids, glycerol, and triglycerides were all significantly higher in the obese as compared with the lean rats ($P < 0.01$) (Table 1). The infusion of AICAR resulted in similar plasma AICAR concentrations of 2.26 ± 0.56 and 2.51 ± 0.39 mmol/l in the obese and lean rats, respectively.

During the infusion of AICAR or insulin, plasma glucose concentrations remained stable and did not differ from their respective basal concentrations. Plasma insulin concentrations were significantly increased during insulin infusion in the lean ($P < 0.01$) but not in the obese rats as compared with basal levels (Table 1). AICAR infusion was associated with a significant reduction in plasma insulin concentrations in the obese rats ($P < 0.01$), whereas concentrations did not change in the lean rats. During the AICAR and insulin clamps, plasma fatty acid and triglyceride concentrations were significantly decreased by AICAR in both lean and obese rats, whereas insulin decreased only plasma fatty acids concentrations in the lean rats (Table 1). This decrease in plasma fatty acid concentrations was greater in the AICAR-infused compared with the insulin-infused lean rats ($P < 0.05$). Plasma glycerol concentrations during AICAR infusion decreased in the obese rats but increased in the lean rats (both $P <$ 0.05) (Table 1). AICAR-infused rats showed substantial increases in plasma lactate concentrations ($P < 0.01$) compared with insulin-infused rats; in the latter, there was

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TABLE 1
Plasma metabolite arterial concentrations obtained under basal state or during an isoglycemic clamp from lean and obese Zucker rats

		Lean			Obese		
		Control	AICAR	Insulin	Control	AICAR	Insulin
Glucose (mmol/l)	Basal	7.0 ± 0.2	6.8 ± 0.1	6.9 ± 0.2	8.1 ± 0.2*	8.2 ± 0.2*	8.6 ± 0.2*
	Clamp	7.0 ± 0.1	6.9 ± 0.1	6.7 ± 0.1	8.3 ± 0.2*	7.6 ± 0.1*	8.4 ± 0.2*
Insulin (pmol/l)	Basal	238 ± 57	156 ± 24	167 ± 35	1189 ± 166*	895 ± 11*	1008 ± 127*
	Clamp	290 ± 48	136 ± 48‡	380 ± 60‖	1032 ± 158*	167 ± 74‡‖	1224 ± 165*
Fatty acids (mmol/l)	Basal	0.46 ± 0.07	0.50 ± 0.08	0.59 ± 0.12	1.72 ± 0.47*	1.59 ± 0.37*	1.84 ± 0.34*
	Clamp	0.38 ± 0.07	0.11 ± 0.02‖‡	0.34 ± 0.08‖	1.71 ± 0.26*	0.28 ± 0.09‖‡	1.06 ± 0.19*
Glycerol (mmol/l)	Basal	0.21 ± 0.02	0.24 ± 0.02	0.22 ± 0.03	0.56 ± 0.08*	0.55 ± 0.07*	0.65 ± 0.09*
	Clamp	0.23 ± 0.03	0.38 ± 0.05§	0.21 ± 0.04	0.61 ± 0.06*	0.34 ± 0.02§†	0.69 ± 0.12*
Triglycerides (mg/dl)	Basal	43.8 ± 7.3	32.7 ± 5.2	25.1 ± 4.5	97.7 ± 14.8*	117.1 ± 20.2*	89.3 ± 15.8*
	Clamp	33.5 ± 9.1	21.1 ± 4.2‖	24.7 ± 3.8	96.1 ± 24.2	20.8 ± 3.8‖†	64.6 ± 17.6
Lactate (mmol/l)	Basal	0.55 ± 0.04	0.57 ± 0.03	0.63 ± 0.04	1.10 ± 0.16*	1.21 ± 0.21*	1.30 ± 0.14*
	Clamp	0.60 ± 0.03	13.20 ± 0.38‖‡	0.90 ± 0.06‖	1.03 ± 0.05	12.00 ± 0.53‖‡	1.51 ± 0.13‖

Data are means ± SE; n = 7–10 in each group. Rats were infused either with isotonic saline (control), AICAR (bolus, 100 mg/kg body wt; constant, 10 mg · kg^{-1} body wt · min^{-1}) or insulin (4 mU · kg^{-1} min^{-1}). *Significantly different from lean littermates undergoing the same treatment ($P < 0.01$); ‡significantly different from control and insulin-infused groups at same time interval ($P < 0.01$); †significantly different from control infused group at same time interval ($P < 0.05$); §clamp value is significantly different from basal ($P < 0.05$); ‖clamp value significantly different from basal ($P < 0.01$).

just a modest increase in plasma lactate concentrations ($P < 0.05$) compared with the saline-infused rats (Table 1).

Tissues analyses. Muscle glycogen concentrations were similar in lean and obese rats infused with saline (21.6 ± 2.1 vs. 21.6 ± 2.3 μmol/g wet wt, respectively), and were not altered by AICAR or insulin infusion (data not shown). Although skeletal muscle nucleotide concentrations were not significantly different between the lean and obese rats (data not shown), the infusion of AICAR significantly increased ZMP concentrations in the calf muscle group of both lean and obese rats compared with the levels in the control and insulin-infused groups ($P < 0.01$). Muscle ZMP content was higher in the lean rats than in the obese rats infused with AICAR (1.70 ± 0.37 vs. 0.55 ± 0.02 μmol/g wet wt, respectively; $P < 0.05$), although AMPK activation was similar (6.6- and 5.3-fold, respectively) when compared with that of the lean rats ($P < 0.01$ vs. control) (Fig. 1). Insulin infusion had no significant effect on AMPK activity.

Skeletal muscle glucose uptake. In lean rats, both AICAR and insulin infusion increased the uptake of [^{3}H]2-deoxy-D-glucose in the red gastrocnemius and soleus muscle of lean rats by more than twofold when compared with saline infusion ($P < 0.01$) (Fig. 2*A* and *B*). In obese rats, AICAR infusion stimulated glucose uptake in the red gastrocnemius by 2.4-fold ($P < 0.02$), but did not increase glucose uptake in the soleus muscle. In contrast, insulin failed to increase glucose uptake in either the soleus or the red gastrocnemius muscle of obese rats.

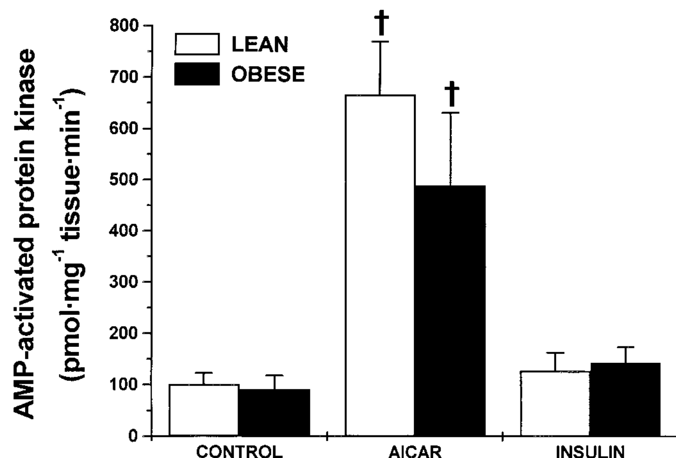

FIG. 1. AMPK activity in calf muscle group from lean or obese Zucker rats after in vivo AICAR (bolus, 100 mg/kg body wt; constant, 10 mg · kg^{-1} body wt · min^{-1}), insulin (4 mU · kg^{-1} body wt · min^{-1}) or a volumetric equivalent infusion of isotonic saline (CONTROL). Values are reported as means ± SE; n = 7–8 each group. †Significantly different from both corresponding control and insulin-infused groups ($P < 0.01$).

Whole-body glucose turnover. By design, the glucose infusion rates necessary to maintain isoglycemia during the AICAR and insulin infusions were similar in the lean rats (Table 2). In the obese rats, the mean glucose infusion rates during the insulin and AICAR clamps were lower (71 and 72%, respectively) than in the lean rats ($P < 0.01$). During the AICAR and insulin clamps, glucose disposal rates increased two- to threefold in the lean rats compared with the control rats (both $P < 0.05$) (Table 2). As expected, insulin-stimulated glucose disposal was blunted in the obese rats, reflecting their insulin-resistant state ($P < 0.01$). Somewhat surprisingly, AICAR infusion did not increase the rate of whole-body glucose disposal in the obese rats.

Under basal conditions, EGP was 33% higher in the obese rats ($P < 0.01$) (Table 2). Both AICAR and insulin infusions suppressed EGP in lean and obese rats ($P < 0.01$). In the lean group, AICAR suppressed EGP to a greater extent than did insulin (82 ± 6 vs. 53 ± 12%, respectively; $P < 0.05$). However, in the obese rats, suppression of EGP during AICAR and insulin infusions was similar (52 ± 8 vs. 55 ± 11%, respectively). Although the relative suppression of EGP (expressed in percent of the basal rate) during AICAR infusion was greater in the lean rats ($P < 0.05$), the absolute changes in EGP were similar in lean and obese rats (29.1 ± 3.9 vs. 27.3 ± 3.9 μmol · kg^{-1} · min^{-1}, respectively) (Table 2).

Whole-body glycerol turnover. The basal rates of glycerol turnover were similar in the obese and lean rats (Table 2). During the AICAR infusion, glycerol turnover decreased in both obese and lean rats ($P < 0.05$). In

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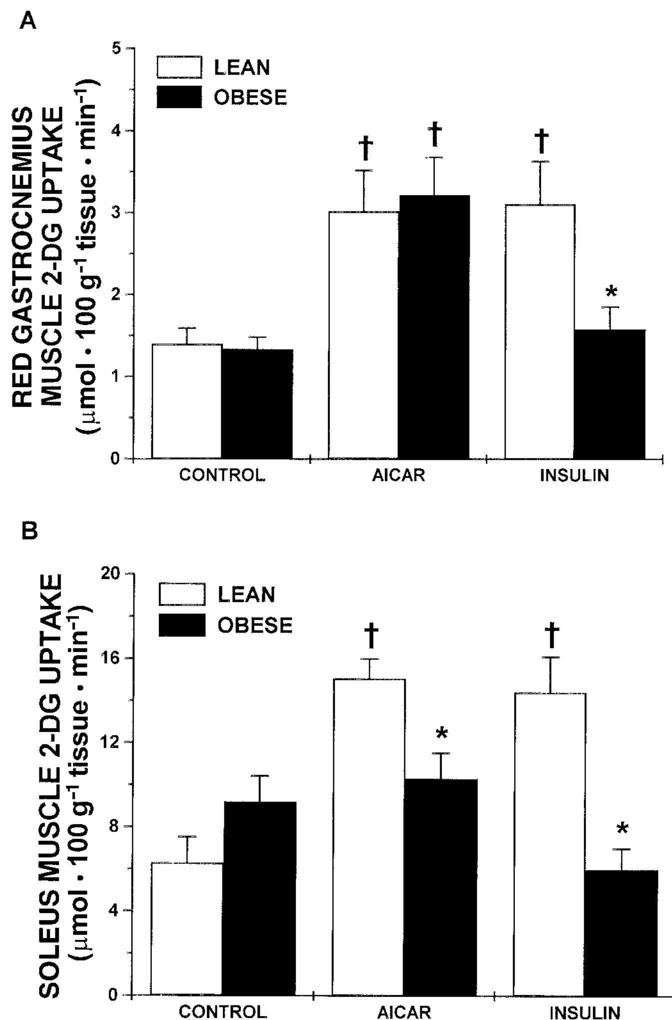

FIG. 2. Effect of an infusion of AICAR (bolus, 100 mg/kg body wt; constant, 10 mg · kg^{-1} body wt · min^{-1}), insulin (4 mU · kg^{-1} body wt · min^{-1}), or isotonic saline (CONTROL) in awake lean or obese Zucker rats on red gastrocnemius (*A*) and soleus 2-DG uptake (*B*). Data are reported as means ± SE; n = 7–10 each group. *Significantly different from lean littermates undergoing the same treatment (P < 0.05); †significantly different from corresponding control group (P < 0.01).

contrast, insulin infusion decreased glycerol turnover in the lean, but not in the obese, rats (Table 2).

AICAR and EGP during high plasma fatty acid concentrations. Because plasma fatty acids are potential modulators of EGP, we infused a triglyceride/heparin emulsion to eliminate the confounding effects of AICAR-induced decreases in plasma fatty acid concentrations on EGP. The infusion of lipid increased the plasma concentrations of fatty acids from 0.54 ± 0.04 to 2.36 ± 0.23 mmol/l (P < 0.005 vs. basal), whereas fatty acid concentrations remained stable (~0.50 mmol/l) in the glycerol-infused group. AICAR infusion decreased plasma fatty acid concentrations in the glycerol-infused rats to 0.11 ± 0.01 mmol/l (P < 0.01 vs. basal), whereas plasma fatty acid concentrations remained elevated (3.08 ± 0.53 mmol/l) in the lipid group. The mean plasma insulin concentrations measured after the 5 h of lipid infusion were significantly higher than in the glycerol-infused rats (228 ± 39 vs. 90 ± 16 pmol/l, respectively; P < 0.05) and remained stable during the AICAR infusion in the lipid group, whereas plasma insulin concentrations decreased in the glycerol group to 18 ± 4 pmol/l. Glucose concentrations were clamped at similar concentrations during the AICAR infusion in both the lipid and glycerol groups (6.1 ± 0.3 and 5.8 ± 0.3 mmol/l, respectively). Glucose infusion rates during AICAR infusion were similar in both lipid and glycerol groups (89 ± 7 and 78 ± 7 μmol · kg^{-1} · min^{-1}, respectively). AICAR infusion similarly suppressed EGP in both the lipid- and glycerol-infused groups (both P < 0.01) when compared with their respective basal rates of EGP. Thus, AICAR was able to suppress EGP independent of any changes in plasma fatty acid concentrations (Fig. 3).

DISCUSSION

In this study, the metabolic effects of AICAR, a known activator of AMPK, were compared with the effects of an equipotent dose of insulin in awake lean and obese Zucker rats. The AICAR infusion rate that was used in the present study was selected to produce a plasma AICAR concentration of ~2 mmol/l, a concentration known to induce maximal AICAR stimulation of muscle glucose uptake in vitro (40). The insulin infusion rate of 4 mU · kg^{-1} · min^{-1} was chosen based on pilot studies that have shown this dose to be equally potent to AICAR with respect to glucose infusion rates required to maintain isoglycemia.

The present data extend our previous in vivo findings in normal SD rats (13) and demonstrate that under similar rates of glucose flux in lean Zucker rats, AICAR has a greater effect in suppressing EGP than insulin, indicating a potent effect of AICAR on the liver. Because plasma fatty acid concentrations can also modulate EGP (28,41), it is possible that the AICAR-induced decrease in plasma fatty acid concentrations could have indirectly decreased the rates of EGP (27). However, this possibility is not likely, as our data show that AICAR was able to suppress EGP when plasma fatty acid concentrations were maintained above 2 mmol/l by a concomitant intralipid/heparin infusion. This finding suggests that AICAR directly suppresses EGP independent of its effect on plasma fatty acid concentrations. The effect of AICAR in suppressing EGP may be mediated by activation of AMPK or by the allosteric inhibition of fructose-1,6-biphosphatase by ZMP, thereby suppressing hepatic gluconeogenesis (19). The observed increase in plasma lactate and glycerol concentrations in the lean rats infused with AICAR is consistent with the latter possibility.

AICAR significantly suppressed EGP in obese insulin-resistant Zucker rats, which have elevated fasting EGP when compared with their lean littermates, as previously reported (42). In obese Zucker rats, insulin-induced suppression of EGP was blunted during a 3.3 mU · kg^{-1} · min^{-1} hyperinsulinemic-euglycemic clamp that increased plasma insulin concentrations twofold (43). However, in the present study, insulin suppressed EGP to a similar extent in obese rats and lean littermates. This observation may be partly explained by the higher glucose concentration in the obese rats, as hyperglycemia per se has been shown to suppress hepatic glucose production (44,45).

Whole-body glucose disposal was increased by both insulin and AICAR infusions in the lean rats. This observation is likely attributable to the stimulation of skeletal muscle glucose uptake by both AICAR and insulin, as reflected by the soleus and red gastrocnemius muscles of

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TABLE 2
Glucose infusion rate, endogenous glucose production, glucose disposal, and glycerol turnover data under basal state or during an isoglycemic clamp from lean and obese Zucker rats

		Lean			Obese		
		Control	AICAR	Insulin	Control	AICAR	Insulin
Glucose infusion rates (μmol · kg^{-1} · min^{-1})	Clamp	NA	74.3 ± 10.7	82.4 ± 3.5	NA	21.0 ± 2.2*	24.0 ± 4.4*
Glucose disposal (μmol · kg^{-1} · min^{-1})	Clamp	40.5 ± 3.8	80.5 ± 5.7†	103.5 ± 15.8†	52.4 ± 4.2	46.4 ± 4.1*	45.7 ± 4.8*
Endogenous glucose production (μmol · kg^{-1} · min^{-1})	Basal	37.3 ± 3.2	35.3 ± 2.7	45.1 ± 3.2	49.4 ± 4.4*	52.7 ± 2.8*	48.4 ± 5.2*
	Clamp	40.5 ± 3.8	6.2 ± 1.9§†	21.1 ± 5.3§	53.3 ± 4.2	25.4 ± 2.3§†	21.7 ± 6.1§†
Glycerol turnover (μmol · kg^{-1} · min^{-1})	Basal	20.6 ± 4.6	16.1 ± 2.0	17.8 ± 5.0	15.2 ± 2.2	23.1 ± 4.5	19.7 ± 2.3
	Clamp	16.4 ± 4.0	10.5 ± 1.9§	14.0 ± 4.6§	14.5 ± 1.4	13.2 ± 3.5§	18.2 ± 2.0

Data are means ± SE, n = 7–10 in each group. Rats were infused with isotonic saline (control), AICAR (bolus, 100 mg/kg body wt · constant, 10 mg · kg^{-1} body wt · min^{-1}), or insulin (4 mU · kg^{-1} · min^{-1}). *Significantly different from lean littermates undergoing the same treatment ($P < 0.01$); †significantly different from control group at same time interval ($P < 0.01$): §clamp value significantly different from basal ($P <$ 0.05); NA, not applicable.

lean rats. As expected, insulin-stimulated glucose disposal was impaired in the obese Zucker rats. However, somewhat surprisingly, AICAR failed to increase whole-body glucose disposal in the obese rats. Glucose uptake in the soleus was not stimulated by AICAR in the obese Zucker rats. However, AICAR increased glucose uptake in the red gastrocnemius by more than twofold. The soleus muscle in the rat is primarily composed of type I muscle fibers (I, >80%; IIa, 10%; IId/x, 0%; and IIb, 0%), whereas the red gastrocnemius is composed of mixed muscle fibers (I, 5–20%; IIa, 5–19%; IId/x, 20–40%; IIb, 1–8%) (46–48). The lack of an effect of AICAR on whole-body glucose disposal in obese Zucker rats might be partly explained by a high proportion (>70%) of type IIb muscle fibers, rather than type IId/x and IIa muscle fiber types, which are abundant in the red gastrocnemius (48). Although whole-body glucose disposal was not stimulated by AICAR in the obese Zucker rat, the observation that muscle glucose uptake is increased by more than twofold in the red gastrocnemius is important from a clinical standpoint, as human skeletal muscle mass is predominantly constituted of similar muscle fibers (49).

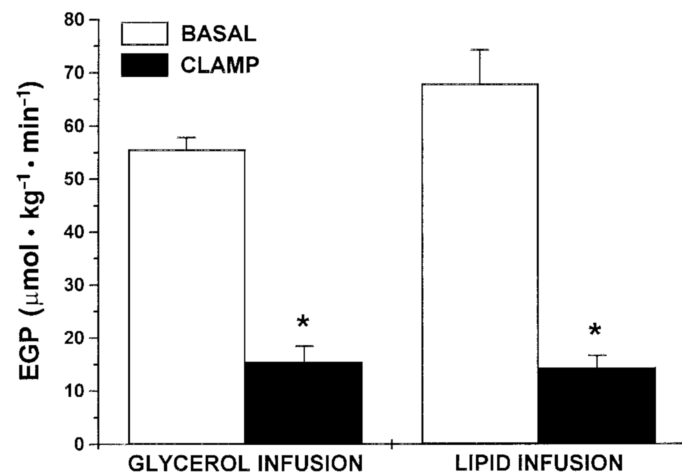

FIG. 3. EGP during a 90-min AICAR clamp (bolus, 100 mg/kg body wt; constant, 10 mg · kg^{-1} body wt · min^{-1}), estimated with the use of [U-^{13}C]glucose in awake lean rats infused either with a triglyceride emulsion in combination with heparin (lipid) or glycerol. Data are reported as means ± SE; n = 5–6 each group. *Significantly different from lean littermates undergoing the same treatment ($P < 0.05$).

Because soleus muscle GLUT4 content appears to be similar in obese and lean rats (50,51), it is unclear why AICAR did not increase glucose uptake in the soleus muscle of obese Zucker rats. The content of monophosphorylated AICAR (ZMP) was lower in the calf muscle of obese rats compared with lean rats. The total AMPK activity also tended to be lower, although this trend was not statistically significant and AMPK activity was substantially activated in the muscle of obese rats. The lack of AICAR-induced stimulation of whole-body glucose disposal and glucose uptake in the soleus raises the possibility of impaired downstream signaling of AMPK on GLUT4 translocation in this model of insulin resistance.

Infusion of AICAR caused a decrease in lipolysis, as reflected by decreases in plasma fatty acid concentrations and glycerol turnover rates (52) in both lean and obese rats. As expected, insulin also decreased glycerol turnover in lean rats, whereas insulin failed to suppress glycerol turnover rates in the obese Zucker rats. The greater suppressive effects of AICAR infusion, as compared with the effects of insulin infusion on plasma fatty acid concentrations and glycerol turnover rates in the obese Zucker rats, suggest that AICAR has a potent antilipolytic effect in this insulin resistant rat model. In addition, AICAR infusion resulted in a decrease in plasma triglyceride concentrations in both lean and obese rats, which can likely be attributed to an inhibition of hepatic lipogenesis. The results are consistent with previous in vitro findings demonstrating AICAR-induced inhibition of mitochondrial glycerol-3-phosphate acyltransferase activity and subsequent inhibition of triacylglycerol synthesis (53).

In summary, these are the first studies to examine the effects of an acute infusion of AICAR in an insulin resistant rat model. We found that an infusion of AICAR in the awake obese Zucker rat *1*) activated skeletal muscle AMPK more than fivefold, *2*) increased glucose transport activity in the red gastrocnemius by ~240%, *3*) reduced EGP by ~50%, and *4*) suppressed lipolysis by ~45%. Because type 2 diabetes is characterized by diminished rates of insulin-stimulated glucose uptake as well as increased basal rates of EGP and lipolysis, these results suggest that AICAR-related compounds may represent a new class of antidiabetic agents.

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