AICAR Administration Causes an Apparent Enhancement of Muscle and Liver Insulin Action in Insulin-Resistant High-Fat–Fed Rats

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Exercise improves insulin sensitivity. As AMP-activated protein kinase (AMPK) plays an important role in muscle metabolism during exercise, we investigated the effects of the AMPK activator 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR) on insulin action in insulin-resistant high-fat–fed (HF) rats. Rats received a subcutaneous injection of 250 mg/kg AICAR (HF-AIC) or saline (HF-Con). The next day, euglycemic-hyperinsulinemic clamp studies were performed. Glucose infusion rate during the clamp was enhanced (50%) in HF-AIC compared with HF-Con rats. Insulin-stimulated glucose uptake was improved in white but not in red quadriceps, whereas glycogen synthesis was improved in both red and white quadriceps of HF-AIC rats. HF-AIC rats also showed increased insulin suppressibility of hepatic glucose output (HGO). AICAR-induced responses in both liver and muscle were accompanied by reduced malonyl-CoA content. Clamp HGO correlated closely with hepatic triglyceride content ($r = 0.67$, $P < 0.01$). Thus, a single dose of AICAR leads to an apparent enhancement in whole-body, muscle, and liver insulin action in HF rats that extends beyond the expected time of AMPK activation. Whether altered tissue lipid metabolism mediates AICAR effects on insulin action remains to be determined. Follow-up studies suggest that at least some of the post-AICAR insulin-enhancing effects also occur in normal rats. Independent of this, the results suggest that pharmacological activation of AMPK may have potential in treating insulin-resistant states and type 2 diabetes. *Diabetes* 51:2886–2894, 2002

AMP-activated protein kinase (AMPK), which is present in most mammalian tissues, acts as a fuel gauge for the cell (1). Its activity is stimulated by increases in the AMP/ATP ratio in response to stresses such as exercise, hypoxia, or prolonged starvation (2). In muscle, AMPK mediates glucose uptake (3,4) by increasing glucose transporter (GLUT)4 translocation to the cell membrane (5). It also increases muscle free fatty acid (FFA) oxidation by inhibiting acetyl-CoA carboxylase (ACC) and activating malonyl-CoA decarboxylase (6,7). The net effect of these actions is to decrease the concentration of malonyl-CoA, removing its inhibition of carnitine-palmitoyl transferase-1, which facilitates FFA entry to mitochondria for oxidation. In liver, AMPK activation has also been shown to inhibit gluconeogenesis, glycolysis, lipogenesis, and cholesterol formation (8–10). As the AMPK signaling system is relatively intact in insulin-resistant states, there is considerable interest in targeting it for the treatment of diabetes (11). AMPK can be pharmacologically activated by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR). While not ideal in that it may possibly affect other AMP-sensitive enzymes (such as fructose-1,6-bisphosphatase (8), AICAR is commonly used to examine consequences of AMPK activation. In hind limbmuscle of normal rats, AICAR stimulates both glucose uptake and fat oxidation (12). Recent studies in obese Zucker rats show that AICAR increases muscle glucose uptake and suppresses endogenous glucose production (13). While these latter effects resemble those mediated by insulin, AMPK activation is known to directly stimulate muscle glucose uptake (4,14) and inhibit hepatic gluconeogenesis (8,9) independent of the insulin signaling pathway. Whether AICAR activation of AMPK can acutely or subacutely lead to an improvement of insulin sensitivity in muscle or liver was not examined in these studies.

Exercise training is well known to increase insulin sensitivity under normal conditions (15), and to ameliorate impaired insulin action in insulin-resistant humans (16) and experimental animals (17). A previous study from our laboratory demonstrated that a single bout of exercise enhanced insulin sensitivity 24 h later in insulin-resistant high-fat–fed (HF) rats (18). Interestingly, the improved insulin action in these rats was associated with reductions in the concentration of muscle malonyl-CoA and long-chain acyl-CoA (LCACoA). Since the AMPK pathway regulates glucose and lipid metabolism during exercise (11), we speculated that the improvement in insulin action after exercise may result from the acute activation of AMPK. To test this hypothesis, the present study investigated whether a single injection of AICAR 24 h earlier has effects

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ACC, acetyl-CoA carboxylase; AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside; AMPK, AMP-activated protein kinase; FFA, free fatty acid; GIR, glucose infusion rate; GLUT, glucose transporter; HF, high-fat fed; HGO, hepatic glucose output; LCACoA, long-chain acyl-CoA; R_d, glucose disappearance rate; R_g', glucose uptake.

similar to those of exercise on whole-body and tissue-specific insulin action in HF rats.

RESEARCH DESIGN AND METHODS

All experimental procedures were approved by the Animal Experimentation Ethics Committee (Garvan Institute/St. Vincent's Hospital) in accordance with the National Health and Medical Research Council of Australia Guidelines on Animal Experimentation. Male Wistar rats, supplied from the Animal Resources Center (Perth, Australia), were communally housed at 22 ± 0.5°C with a 12/12-h day/night cycle (lights on 0600) for 1 week and were fed ad libitum a standard diet (Rat maintenance diet, Gordons Specialty Feeds, Sydney, Australia) containing 5% fat, 69% carbohydrate, and 21% protein plus fiber, vitamins, and minerals. After this acclimatization, rats (~300 g) were fed the standard diet (CH-Con) or an isocaloric high-fat diet (HF; 350 kJ/d given at 1,600 h) for 3 weeks. The energy percentage composition of the fat diet was 59% fat, 20% carbohydrate, and 21% protein, with equal quantities of fiber, vitamins, and minerals to the standard diet (19). One week before the study, the right jugular vein and left carotid artery were cannulated under anesthesia (halothane) and aseptic conditions. Catheters were exteriorized at the back of the neck. Rats recovered for 1 week and were handled daily to minimize stress. Only those that reached presurgery weight or above were used for experiments.

Experiment 1: acute responses after AICAR injection. The experiment was performed on conscious HF rats in the postabsorptive period (~5 h fasted). After catheters were connected to sampling syringes, the rats were allowed to rest for 40–50 min. Two blood samples were taken 20 min apart between 0900 and 1000 h, constituting the basal samples (time 0). The animals were then randomly assigned to receive a subcutaneous injection of either AICAR (HF-AIC; 250 mg/kg in saline, Sigma) or an equivalent volume of normal saline (HF-Con). Blood samples were collected at 30, 60, 90, and 120 min after the injection. After the last blood sample, rats were killed with an overdose of pentobarbital. Tissues were immediately freeze-clamped with aluminum tongs precooled in liquid nitrogen and stored at −80°C for subsequent determinations.

Experiment 2: studies 24 h after AICAR injection. HF rats were assigned to receive an injection of AICAR (HF-AIC) or normal saline (HF-Con) as described above. They were then returned to their cages and given their normal high-fat meal. The injection of AICAR caused a minor reduction in the food intake overnight (15%) but was without any significant impact on body weight. However, for avoiding other possible effects of a reduced caloric intake, HF-Con rats were pair-fed with the HF-AIC group. Twenty-four hours after the injection (and 5 h fasted), rats were subjected to a euglycemic-hyperinsulinemic clamp as described previously (20) to assess insulin sensitivity. Briefly, after two basal samples were taken, insulin was infused intravenously at a constant rate (0.25 units · kg^{-1} · h^{-1}). Blood samples (50 μl) were taken every 10 min to determine blood glucose and adjust the glucose infusion. Once the glucose infusion rate was stabilized, a blood sample was taken to determine other plasma parameters at steady state. The rats then received a bolus of 2-deoxy-D-(2,6-^{3}H)-glucose and ^{14}C-glucose, followed by collection of plasma samples (100 μl) at 2, 5, 10, 15, 20, 30, and 45 min (21). The animals were then killed, and tissues were collected as described above. The euglycemic-hyperinsulinemic clamp was performed in a control group of untreated rats that were fed a standard diet (CH-Con). Glucose disappearance rate (R_d) and hepatic glucose output rate were calculated from the disappearance of ^{14}C-glucose. Insulin-stimulated tissue glucose uptake (R_g'), net glycogen synthesis, and ^{14}C-glucose incorporation into lipids were calculated as described previously (21). Plasma levels of insulin and triglycerides during the clamp were expressed as the mean of samples taken before the tracer injection and at the end of the experiment.

All blood samples were collected in vials containing K-EDTA as anticoagulant. The plasma was immediately separated, collected, and frozen for subsequent determinations. The blood cells were resuspended in normal saline and returned to the animal.

Experiment 3: follow-up studies in normal rats fed a standard diet 24 h after AICAR injection. After experiment 2 was completed, follow-up studies were performed to examine whether any insulin-enhancing effects after AICAR injection could be demonstrated in normal rats that were fed a standard diet. Euglycemic clamp studies were performed in two groups of rats that were fed a standard diet and received an injection of either AICAR or saline (control) 24 h earlier. Experimental details are as for experiment 2 except for the different diet.

Metabolite measurements. Plasma glucose was determined with a glucose analyzer (YSI 2300, Yellow Springs, OH). Plasma free fatty acids (FFAs) were determined spectrophotometrically with a commercial kit (NEFA-C; WAKO Pure Chemical Industries, Osaka, Japan). Plasma triglycerides and glycerol were measured using enzymatic colorimetric methods (Triglyceride INT, procedure 336 and GPO Trinder, Sigma, St. Louis, MO) and plasma insulin by commercial radioimmunoassay kit (Linco, St. Louis, MO). Tissue triglycerides and LCACoAs were extracted and measured as previously described (22).

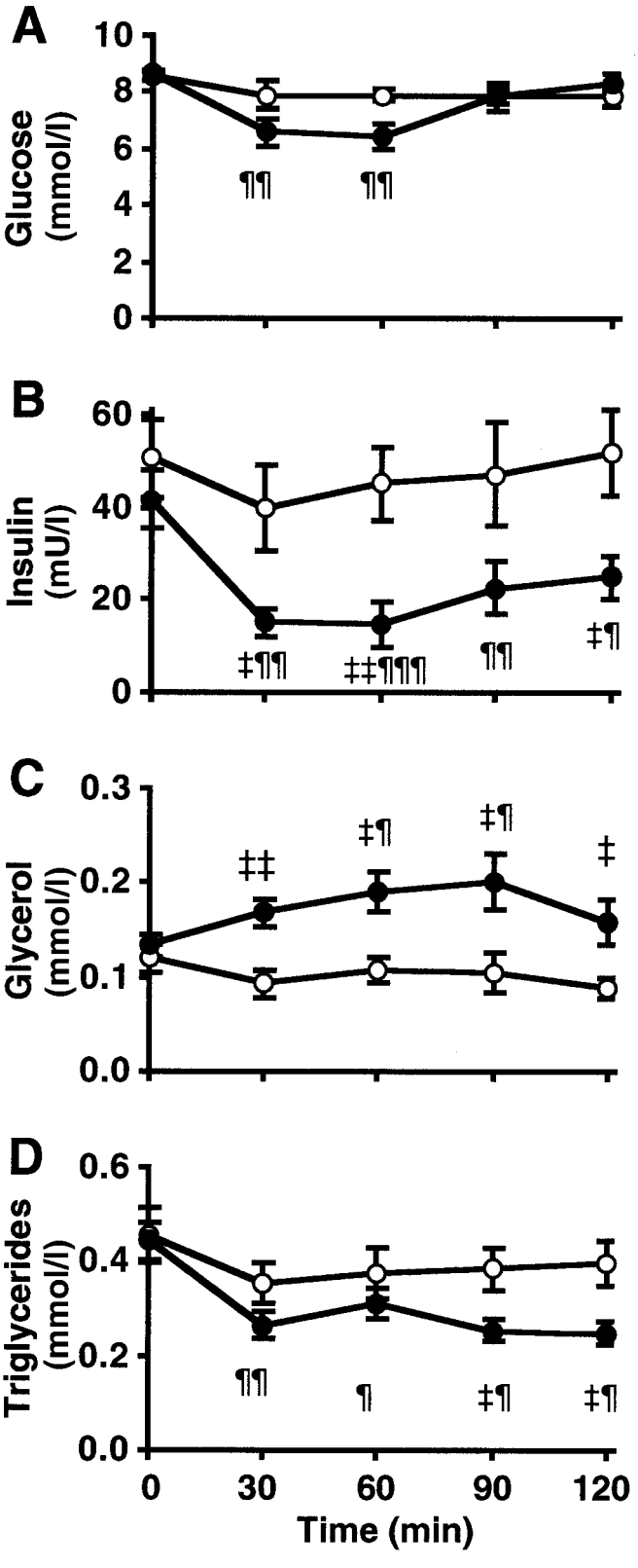

FIG. 1. Plasma glucose (*A*), insulin (*B*), glycerol (*C*), and triglycerides (*D*) over 2 h after AICAR (HF-AIC, ●) or saline (HF-Con, ○) injection in HF rats. ‡*P* < 0.05, ‡‡*P* < 0.01 vs. HF-Con; ¶*P* < 0.05, ¶¶*P* < 0.01; ¶¶¶*P* < 0.001 vs. HF-AIC time 0.

TABLE 1
Tissue glycogen, triglyceride, and LCACoA content 2 h after AICAR (HF-AIC) or saline (HF-Con) injection in high-fat–fed rats

	HF-Con	HF-AIC
White quadriceps		
Glycogen content (μmol/g)	34.9 ± 2.0	40.6 ± 0.9*
Triglyceride content (μmol/g)	5.8 ± 0.8	6.7 ± 1.1
Total LCACoAs (nmol/g)	0.83 ± 0.1	0.54 ± 0.1
Red quadriceps		
Glycogen content (μmol/g)	34.4 ± 1.8	40.9 ± 2.7
Triglyceride content (μmol/g)	3.3 ± 0.2	3.0 ± 0.2
Total LCACoAs (nmol/g)	2.54 ± 0.5	3.21 ± 0.6
Liver		
Glycogen content (μmol/g)	109.4 ± 16.0	52.1 ± 11.1*
Triglyceride content (μmol/g)	17.0 ± 1.2	16.2 ± 0.7

Data are means ± SE. Total LCACoAs represent the sum of the three major species: Linoleoyl-CoA, Palmitoyl-CoA, and Oleoyl-CoA; $n = 6$ per group. *$P < 0.05$ vs. HF-Con.

Glycogen was measured as described by Chan and Exton (23). Malonyl-CoA was assayed radioenzymatically in neutralized perchloric acid filtrates by the method of McGarry et al. (24) as described previously (25). Acetyl-CoA carboxylase (ACC) activity was assayed as described by Vavvas et al. (26), and glucose transporter (GLUT4) content was assayed by Western blotting (27).
Statistical analyses. Results are presented as means ± SE. One-way analysis of variance followed by post hoc (Fisher's protected least significant difference) tests or a Student's t test was used to assess statistical significance between groups.

RESULTS

Experiment 1: acute responses after AICAR injection. Body weight (HF-AIC: 368 ± 4 g; HF-Con: 364 ± 7 g) and plasma parameters were similar between HF-AIC and HF-Con groups (Fig. 1, time 0). AICAR caused a decrease in plasma glucose between 30 and 60 min (Fig. 1*A*) that was accompanied by a decrease in plasma insulin (Fig. 1*B*). Plasma FFA levels were not affected by AICAR (HF-AIC: 0.46 ± 0.05, 0.40 ± 0.04, 0.53 ± 0.04, 0.46 ± 0.06, and 0.44 ± 0.06 mmol/l at 0, 30, 60, 90, and 120 min after AICAR injection, respectively); however, AICAR administration increased plasma glycerol and decreased plasma triglycerides (Fig. 1*C* and *D*).

Glycogen and lipid content, 2 h after AICAR injection, were determined in two types of skeletal muscle and in liver (Table 1). HF-AIC rats showed a 16% increase in glycogen content in white quadriceps with a similar trend in the red quadriceps ($P = 0.08$ vs. HF-Con). In contrast, liver glycogen was reduced by 52% after the AICAR injection. No significant changes were found in triglyceride in muscle and liver or LCACoA content in muscle.

Experiment 2: studies 24 h after AICAR injection in HF rats

Whole-body insulin sensitivity, glucose disappearance, and hepatic glucose output. Figure 2 shows the parameters of whole-body insulin sensitivity determined 24 h after AICAR or saline injection to HF rats and in rats fed a standard diet. As expected, high-fat diet caused a decrease in the clamp glucose infusion rate (GIR) required to maintain euglycemia (Fig. 2*A*). Previous AICAR administration increased GIR by 50%. R_d was significantly reduced in both groups of HF rats compared with the CH-Con group (Fig. 2*B*) but was not significantly different between HF-AIC and HF-Con rats. Hepatic glucose output (HGO) was completely suppressed during the clamp in

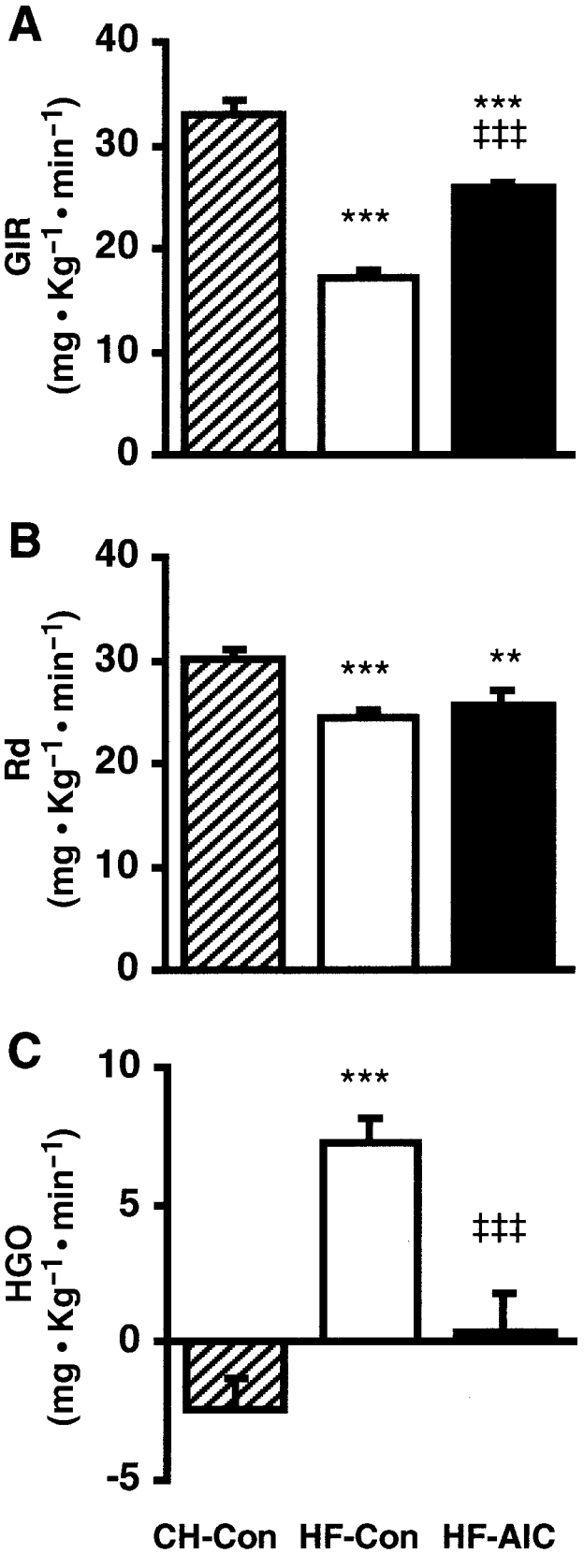

FIG. 2. Parameters of whole-body and liver insulin sensitivity determined during the euglycemic-hyperinsulinemic clamp performed on standard diet–fed (CH-Con, ▨) or HF rats 24 h after saline (HF-Con, □) or AICAR (HF-AIC, ■) injection. GIR (*A*), R_d (*B*), and HGO (*C*). ***$P < 0.001$ vs. CH-Con; ‡‡‡$P < 0.001$ vs. HF-Con.

TABLE 2
Plasma parameters immediately before (Preclamp) and during insulin stimulation (Clamp) in the euglycemic-hyperinsulinemic clamp performed on standard diet–fed (CH-Con) or high-fat–fed rats 24 h after AICAR (HF-AIC) or saline (HF-Con) injection

	CH-Con		HF-Con		HF-AIC	
	Preclamp	Clamp	Preclamp	Clamp	Preclamp	Clamp
Glucose (mmol/l)	7.9 ± 0.3	7.9 ± 0.1	9.5 ± 0.3†	7.6 ± 0.2	8.4 ± 0.2§	7.5 ± 0.2
Insulin (mU/l)	38 ± 5	108 ± 9	63 ± 7*	131 ± 14	53 ± 5	127 ± 8
Fatty acids (mmol/l)	0.60 ± 0.06	0.43 ± 0.06	0.36 ± 0.04*	0.37 ± 0.05	0.48 ± 0.05	0.32 ± 0.03
Triglycerides (mmol/l)	0.97 ± 0.16	0.40 ± 0.11	0.49 ± 0.05‡	0.15 ± 0.04‖	0.41 ± 0.04‡	0.14 ± 0.03‖
Glycerol (mmol/l)	0.15 ± 0.04	0.24 ± 0.04	0.10 ± 0.02	0.18 ± 0.04	0.14 ± 0.01	0.15 ± 0.01

Data are means ± SE. n = 6–12 per group. *$P < 0.05$, †$P < 0.01$, ‡$P < 0.001$ vs. CH-Con preclamp; §$P < 0.05$ vs. HF-Con pre-clamp; ‖$P < 0.05$ vs. CH-Con clamp.

CH-Con and HF-AIC rats (Fig. 2*C*), but its suppression was impaired in the HF-Con rats.

Plasma and tissue metabolic parameters. In the basal state (preclamp as shown in Table 2), HF rats had higher plasma glucose and insulin levels than the CH-Con rats but slightly lower plasma concentrations of FFA and triglycerides. Previous administration of AICAR decreased the hyperglycemia in HF rats but did not alter the other parameters. We also determined key tissue parameters in the basal state 24 h after saline or AICAR injection in subsets of both HF-Con and HF-AIC rats (n = 6/group). LCACoA content was similar in the two groups in both red (HF-AIC: 2.1 ± 0.4 nmol/g; HF-Con: 2.0 ± 0.2 nmol/g) and white (HF-AIC: 3.1 ± 0.4 nmol/g; HF-Con: 2.2 ± 0.4 nmol/g) quadriceps muscles. Compared with HF-Con, liver triglyceride content was reduced in HF-AIC rats (HF-AIC: 22 ± 2 μmol/g; HF-Con: 27 ± 1 μmol/g; $P < 0.05$). Although liver glycogen content was acutely reduced by AICAR as shown in Table 1, its level had returned to similar levels to HF-Con rats (HF-AIC: 172 ± 16 μmol/g; HF-Con: 148 ± 19 μmol/g; $P > 0.05$) 24 h after AICAR injection. During the clamp, both groups of HF rats showed decreased plasma triglycerides when compared with CH-Con, but no differences were observed in the levels of glycerol and FFA among groups (Table 2).

Effects of previous AICAR administration on the response of skeletal muscle during the clamp. Previous AICAR injection increased insulin-mediated glucose uptake (R_g') in white muscle to a value 58% above that in HF-Con rats but had no effect in red quadriceps (Fig. 3*A* and *B*). Insulin-stimulated glucose incorporation into glycogen was significantly increased in both white (224%) and red (74%) muscles of HF-AIC rats (Fig. 3*C* and *D*). Similar trends were also found for insulin-stimulated glucose incorporation into lipids (0.04 ± 0.01 vs. 0.09 ± 0.02 μmol · 100 g^{-1} · min^{-1} in white muscle and 0.24 ± 0.01 vs. 0.37 ± 0.08 μmol · 100 g^{-1} · min^{-1} in red muscle for HF-Con and HF-AIC, respectively). Consistent with the increase in net glycogen synthesis, glycogen content was greater in both muscles of the HF-AIC compared with the HF-Con group at the end of the clamp (Table 3). No significant differences in muscle triglycerides or LCACoAs were observed ($P > 0.05$).

After the clamp, malonyl-CoA was reduced in both red (by 44%) and white (by 23%) gastrocnemius of HF-AIC rats compared with HF-Con, to a value similar to that in CH-Con rats (Table 3). Malonyl-CoA of red gastrocnemius was also determined in the basal state, i.e., in a similar set of rats killed 24 h after the injection of AICAR or saline, without infusion of insulin (n = 6/group). There was a similar pattern of response observed in these groups with suppression of malonyl CoA in the AICAR-treated group. The results were as follows: CH-Con 0.9 ± 0.1, HF-Con 1.45 ± 0.1 ($P < 0.05$) vs. CH-Con; HF-AIC 1.0 ± 0.1 nmol/g ($P < 0.05$) vs. HF-Con.

Values for GLUT4 content are shown in Fig. 4. The levels of GLUT4 in white gastrocnemius were similar in the standard diet–fed and HF rats, and in the latter they were not altered by AICAR (Fig. 4*A*). In contrast, in red gastrocnemius, GLUT4 levels were significantly increased both by fat feeding and by AICAR (Fig. 4*B*).

Effect of previous AICAR administration on the response of liver during the clamp. Compared with CH-Con rats, liver triglycerides were elevated threefold in

TABLE 3
Muscle glycogen, triglyceride, and total LCACoA content after hyperinsulinemic-euglycemic clamp

	CH-Con	HF-Con	HF-AIC
White muscle			
Glycogen content (μmol/g)#	49.6 ± 3.9	40.7 ± 2.1*	53.7 ± 1.1‖
Triglyceride content (μmol/g)#	4.1 ± 1.8	5.6 ± 0.9	3.3 ± 1.0
Total LCACoAs (nmol/g)#	ND	1.15 ± 0.13	1.19 ± 0.22
Malonyl-CoA (nmol/g)¶	0.9 ± 0.1	1.5 ± 0.1†	1.1 ± 0.1§
Red muscle			
Glycogen content (μmol/g)#	55.1 ± 1.8	51.3 ± 1.0	65.7 ± 4.1*‖
Triglyceride content (μmol/g)#	2.6 ± 0.2	3.4 ± 0.1*	2.8 ± 0.5
Total LCACoAs (nmol/g)#	ND	4.87 ± 0.61	4.57 ± 0.95
Malonyl-CoA (nmol/g)¶	1.1 ± 0.1	1.8 ± 0.1†	1.0 ± 0.1‖

Data are means ± SE. Clamps were performed on standard diet–fed (CH-Con) or high-fat–fed rats 24h after AICAR (HF-AIC) or saline (HF-Con) injection. Total LCACoAs represent the sum of the three major species: linoleoyl-CoA, palmitoyl-CoA, and oleoyl-CoA. ND, not determined. *$P < 0.05$, †$P < 0.01$, §$P < 0.05$, ‖$P < 0.01$ vs HF-Con; #quadriceps; ¶gastrocnemius.

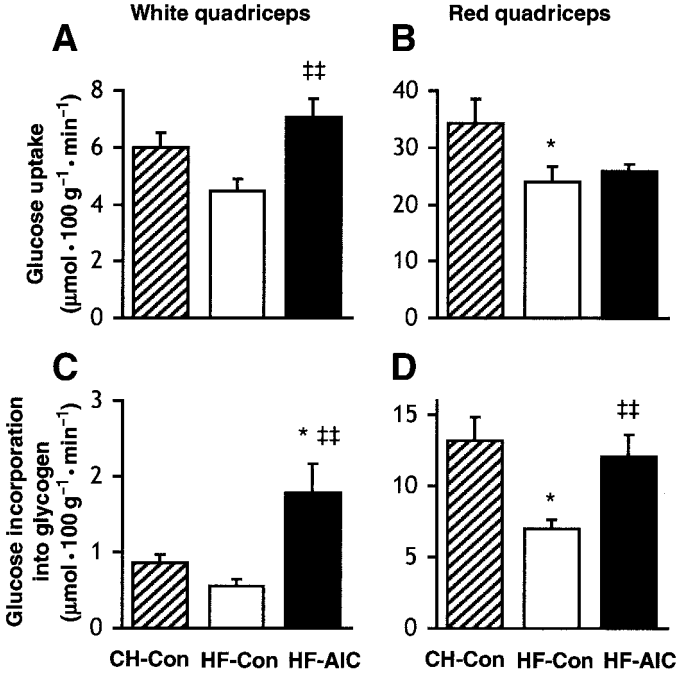

FIG. 3. Parameters of muscle insulin sensitivity determined after the euglycemic-hyperinsulinemic clamp performed on standard diet–fed (CH-Con) or HF rats 24 h after AICAR (HF-AIC) or saline (HF-Con) injection. Insulin-stimulated glucose uptake (*A*, *B*) and insulin-stimulated glycogen synthesis (*C*, *D*) in white (*A*, *C*) and red (*B*, *D*) quadriceps. *$P < 0.05$ vs. CH-Con; ‡‡$P < 0.01$ vs. HF-Con.

HF-Con. Previous AICAR administration reduced triglyceride accumulation by 33% (Fig. 5*A*) and also malonyl-CoA by 21% (Fig. 5*B*). It is interesting that the latter changes occurred despite the absence of a decrease in ACC activity (Fig. 5*C*). Hepatic triglyceride content in the three groups was directly correlated with HGO ($r = 0.67$, $P < 0.01$) and inversely correlated with GIR ($r = -0.78$, $P < 0.001$) during the clamp. Compared with the CH-Con rats, glucose incorporation into hepatic glycogen (4.4 ± 0.4 to 3.0 ± 0.3 μmol · 100 g^{-1} · min^{-1}; $P < 0.05$) and triglycerides (3.8 ± 0.5 vs. 1.4 ± 0.1 μmol · 100 g^{-1} · min^{-1}; $P < 0.01$) were reduced in both HF groups. However, previous AICAR injection did not improve hepatic glucose incorporation into glycogen in the liver (2.7 ± 0.1 μmol · 100 g^{-1} · min^{-1}) and it diminished its incorporation into triglycerides (0.8 ± 0.1 μmol · 100 g^{-1} · min^{-1}).

Experiment 3: follow-up studies in normal rats that were fed a standard diet 24 h after AICAR injection. In a separate follow-up study, we examined whether there was evidence of enhanced insulin action 24 h after AICAR injection in normal rats that were fed a standard diet. Table 4 shows hyperinsulinemic-euglycemic clamp responses in normal rats that were fed a standard diet and received an injection of AICAR (CH-AIC) or saline (CH-Con2) 24 h previously. No significant differences were found in GIR, HGO, or red muscle R_g' between CH-Con2 and CH-AIC groups. There was, however, evidence of enhanced peripheral (muscle) insulin action after AICAR injection as peripheral glucose disposal R_d was significantly increased in the CH-AIC versus the CH-Con2 group. In addition, a significant increase in white muscle R_g' in the CH-AIC group indicated a qualitatively similar pattern of response in normal rats as had occurred in the insulin-resistant HF rats.

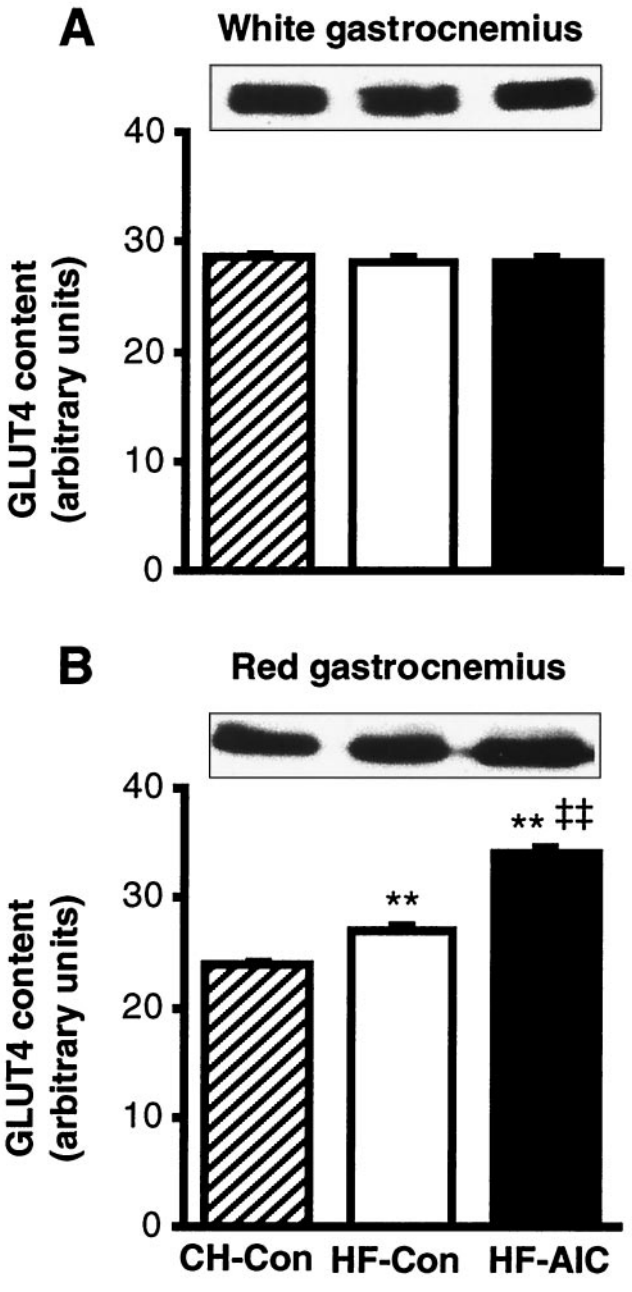

FIG. 4. Total GLUT4 content of white (*A*) and red (*B*) gastrocnemius muscle after the euglycemic-hyperinsulinemic clamp performed on standard diet–fed (CH-Con) or HF rats 24 h after AICAR (HF-AIC) or saline (HF-Con) injection. **$P < 0.01$ vs. CH-Con; ‡‡$P < 0.01$ vs. HF-Con.

DISCUSSION

The present study demonstrates an apparent insulin-sensitizing effect of the AMPK activator AICAR 24 h after its administration in the insulin-resistant HF rat. A major contributor to this was an improvement in insulin-mediated suppression of HGO. In addition, insulin action was enhanced in muscle, with a greater relative enhancement in white compared with red muscle. In both tissues, these effects were associated with a decrease in the concentration of malonyl CoA and in liver a decrease in the concentration of triglyceride. Although our investigation focused on AICAR effects in the insulin-resistant HF rat, follow-up studies demonstrated that at least some insulin-

sensitizing effects after AICAR injection also occur in normal rats that were fed a standard diet, notably also with significant enhancement of insulin-stimulated glucose uptake in white muscle.

AMPK plays a crucial role in mediating glucose and lipid metabolism during exercise (2,26,28). A previous study from our laboratory showed that a single bout of exercise, 24 h earlier, resulted in a 44% increase in whole-body insulin sensitivity (as indicated by clamp GIR) in HF rats (18). The present study shows a comparable degree of improvement of whole-body insulin sensitivity 24 h after a single injection of AICAR, suggesting that a transient activation of AMPK (which also occurs after exercise) can enhance insulin action in HF rats. The two studies differ in that a previous bout of exercise enhanced insulin sensitivity primarily by increasing peripheral glucose uptake (R_d) (18), whereas the improvement of insulin sensitivity caused by AICAR was substantially due to enhanced insulin suppressibility of HGO. Although the major site of AICAR action was liver, it also significantly enhanced insulin action in muscle of HF rats. Insulin-mediated glucose uptake (R_g') and glycogen synthesis in muscle both were markedly increased 24 h after AICAR injection. It is interesting that the enhancement of R_g' was relatively greater in white than in red muscle. The reason for this is not clear, but it is in keeping with the observation that the acute stimulation of glucose transport by AICAR is greater in white than in red muscles (17,18). Although white muscle (type IIb) fibers account for ~70% of the total muscle mass in the rat (31), insulin-mediated glucose uptake is quantitatively much greater in red than in white muscle (Fig. 3*A* and *B*) (32). This may explain why the increase in insulin action on muscle was not reflected in the whole-body glucose disposal measurement.

One of the major objectives of this study was to assess whether observed effects of AICAR on insulin sensitivity correlate with its effects on lipid metabolism. Excess lipid (triglyceride) accumulation in muscle and liver is associated with insulin resistance in humans (33–35) and rodents (18). In addition, the intramuscular concentration of the active form of FFA, LCACoA, is increased in various insulin-resistant states, including that produced by chronic high-fat feeding (22,36). LCACoAs can inhibit the activities of hexokinase (37), pyruvate dehydrogenase, and glycogen synthase (38). In addition, they are precursors of diacylglycerol, an endogenous activator of novel and conventional protein kinase C isoforms, some of which can inhibit insulin signaling by phosphorylating the insulin receptor and insulin receptor substrate on serine or threonine residues (39). One of the important regulators of the cytosolic concentration of LCACoAs is malonyl-CoA, which modulates LCACoA influx into mitochondria by its inhibiting action on carnitine-palmitoyl transferase-1 (40,41). Insulin action after a bout of exercise is associated with reductions in muscle LCACoA and malonyl-CoA concentration (18) and an increase in fatty acid oxidation. The improved insulin action observed after AICAR treatment in HF rats was also associated with changes in lipid metabolism. Plasma triglyceride levels were decreased in the absence of a decrease in plasma FFA, suggesting an increased oxidation of FFA at the expense of its esterification. In keeping with this conclusion, malonyl-CoA

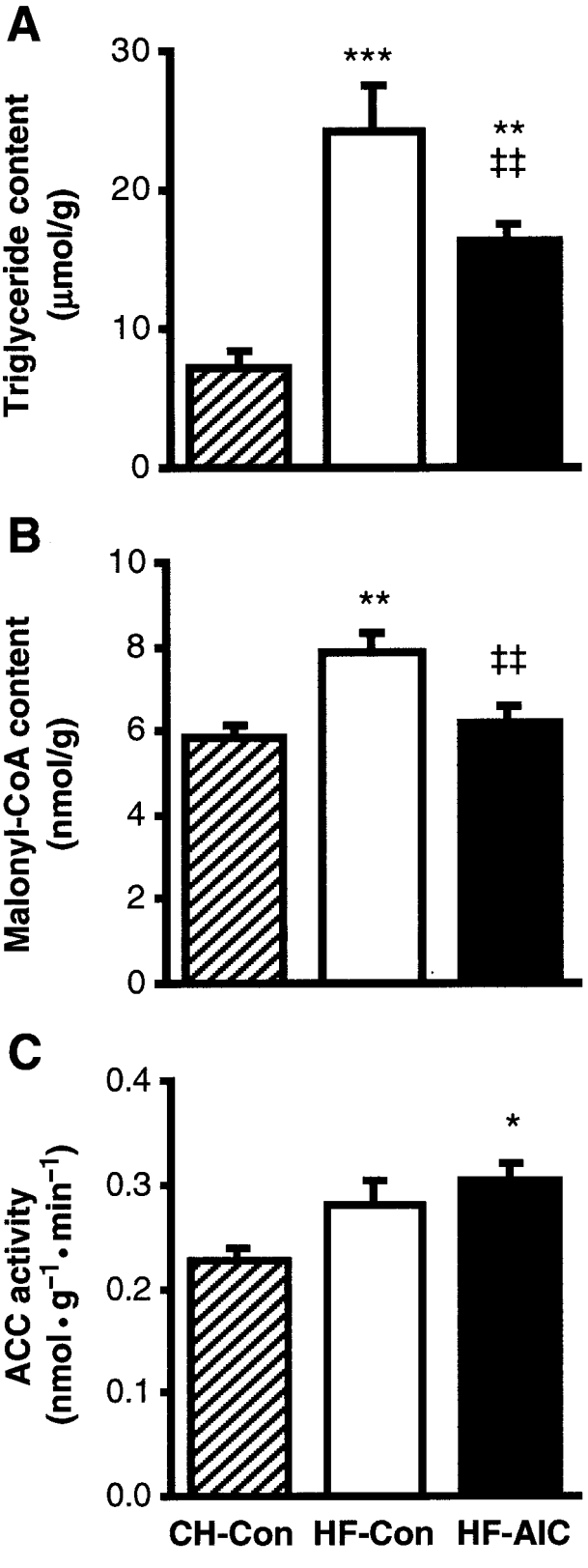

FIG. 5. Liver triglyceride content (*A*), malonyl-CoA content (*B*), and ACC activity (*C*) after the euglycemic-hyperinsulinemic clamp. *P < 0.05, **P < 0.01, ***P < 0.001 vs. CH-Con; ‡‡P < 0.01 vs. HF-Con.

levels in both muscle and liver were lower at the time of the clamp study in the AICAR-treated rats, and the enhanced insulin sensitivity of liver 24 h after AICAR admin-

TABLE 4
Effects of AICAR administration on in vivo insulin action in standard diet–fed rats

	CH-Con2	CH-AIC	*P*
GIR ($mg \cdot kg^{-1} \cdot min^{-1}$)	32.0 ± 1.4	37.6 ± 3.4	NS
R_d ($mg \cdot kg^{-1} \cdot min^{-1}$)	30.4 ± 1.4	37.6 ± 2.9	<0.05
HGO ($mg \cdot kg^{-1} \cdot min^{-1}$)	−1.6 ± 0.1	0.0 ± 1.3	NS
Red muscle R_g' ($\mu mol \cdot 100g^{-1} \cdot min^{-1}$)	33.8 ± 3.8	41.8 ± 7.3	NS
White muscle R_g' ($\mu mol \cdot 100g^{-1} \cdot min^{-1}$)	5.9 ± 0.5	13.4 ± 2.4	<0.05

Data are means ± SE. Responses determined during euglycemic-hyperinsulinemic clamps performed on standard diet–fed rats 24 h after AICAR (CH-AIC) or saline (CH-Con2) injection ($n \geq 7$ per group).

istration was associated with a marked decrease in triglyceride content. In addition, a strong correlation between liver triglyceride content and HGO during the clamp was observed, suggesting a possible important role of reduced lipid accumulation in the improvement of hepatic insulin action.

Questions remain, however, particularly in muscle, concerning the hypothesis that the AICAR-induced enhancement of insulin action is mediated via altered lipid metabolism. In muscle, triglyceride and total LCACoA contents were not altered 24 h after AICAR administration in HF rats. However, the measured LCACoA content includes both cytosolic and mitochondrial fractions, and presumably the decrease in malonyl CoA would cause repartitioning of LCACoAs from the cytosol to the mitochondrion. This should be confirmed, however, in more direct studies. The failure of muscle triglyceride to decrease could reflect a relatively slow turnover of this lipid pool, because with longer periods of treatment with AICAR, significant decreases in muscle triglycerides have been found (J.Y. and N.B.R., unpublished observations). Nevertheless, the finding of muscle effects of AICAR to enhance insulin action in normal rats argues against a principal post-AICAR muscle effect being mediated by correction of excessive lipid accumulation. Our data in experiment 2 suggest that although whole-body insulin resistance was ameliorated by AICAR in the HF rats, it was not normalized. This, combined with the demonstration of effects of AICAR in normal rats in experiment 3, suggests that the major effect of AICAR may be a compensation rather than a correction of the insulin resistance.

Another possibility is that enhancement of insulin-stimulated glucose uptake by muscle is mediated by an increase in GLUT4 content. Administration of AICAR at a higher dose (fourfold greater than that used here) consecutively for 5 days has been shown to increase muscle GLUT4 content in normal rats (30,42). Although a small increase in GLUT4 content was found in red muscle in the present study, enhanced insulin action occurred mainly in white muscle in which GLUT4 was not significantly altered. Thus, it is unlikely that the principal effect of AICAR on muscle insulin sensitivity is mediated by an increased GLUT4 content. This interpretation is supported by a recent finding that insulin-stimulated glucose transport into muscle 3 h after AMPK activation by either exercise or AICAR is enhanced even when GLUT4 synthesis is pharmacologically blocked (43).

Glycogen depletion has been implicated as a cause of the increase in glucose uptake in muscle after exercise and hypoxia (44–46), conditions in which AMPK is activated. Glycogen depletion did not seem to be a factor in enhancing insulin action after AICAR administration. However, consistent with a previous report in normal rats (47), our results show that muscle glycogen content was increased rather than decreased 24 h after AICAR administration. Furthermore, although AICAR transiently reduced liver glycogen, glycogen levels had fully recovered 24 h after the AICAR injection, when insulin sensitivity was studied. These results suggest that glycogen depletion per se was not involved in the enhancement of insulin sensitivity by AICAR.

The possibility that the enhanced insulin action observed after AICAR administration is related to residual AMPK activation is also unlikely. Insulin action was assessed 24 h after AICAR administration, long after its acute effects on plasma glucose and glycerol levels had dissipated. Also, ACC activity, which is considered a good indicator of AMPK activation (48), was similar in liver of HF-Con and HF-AIC groups during the clamp, suggesting that the effect of AICAR on AMPK activation had worn off by 24 h. Finally, in a separate study, we found no residual increase in AMPK or reduction in ACC activity in muscle or liver of control rats 24 h after injection of a similar dose of AICAR (A.S. and N.B.R., unpublished observations). Whether by activating AMPK a single dose of AICAR induced chronic changes (e.g., at the level of transcription [49]) that inhibited hepatic glucose output or enhanced glucose utilization was not studied. However, supporting that such an effect does not account for our findings is a recent study showing that basal hepatic glucose output is not altered in diabetic mice that were treated with AICAR over 7 days (50). The failure to find a large increase in GLUT4 protein or mRNA in the muscle of rats in the present study is also consistent with this view.

The final aspect of our study demonstrated that some of the effects of AICAR to enhance insulin-stimulated muscle glucose uptake also occur in normal rats. It has long been appreciated that the ability of insulin to stimulate glucose transport and glycogen synthesis may be enhanced for several hours or longer after exercise in a normal rat (44,45), and recent studies suggest that a similar increase in insulin action can occur in muscle in vitro 3.5 h after AICAR administration (43). Our in vivo findings in white muscle of normal rats are in accord with this. However, it remains to be determined whether AICAR-induced increases in insulin action in the control and HF rats occur by the same mechanism. Additional studies will also be necessary at lower clamp insulin levels to resolve whether previous AICAR could lead to enhanced insulin suppressibility of HGO in normal rats as HGO was fully suppressed by insulin in the control group. Irrespective of possible effects after AICAR administration in normal states, the

findings of the present study suggest that AMPK may be a novel target for the treatment of insulin-resistant states.

In conclusion, a single dose of the AMPK activator AICAR leads to an improvement in whole-body, muscle, and liver insulin action in HF rats well beyond the expected time range of activation of AMPK. The improvement of insulin-mediated glycogen synthesis occurs in both red and white muscle, is associated with a decrease in malonyl CoA, and is independent of previous glycogen depletion. Enhancement of insulin-stimulated suppression of hepatic glucose production is associated with decreases in both liver triglyceride content and malonyl CoA. The present study suggests that AMPK activators such as AICAR may have therapeutic potential for the treatment of insulin resistance and type 2 diabetes either by bypassing the defective insulin signaling pathway or by creating metabolic conditions that improve insulin action.

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