Long-Term AICAR Administration and Exercise Prevents Diabetes in ZDF Rats

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Lifestyle interventions including exercise programs are cornerstones in the prevention of obesity-related diabetes. The AMP-activated protein kinase (AMPK) has been proposed to be responsible for many of the beneficial effects of exercise on glucose and lipid metabolism. The effects of long-term exercise training or 5-aminoimidazole-4-carboxamide-1-β-D-riboruranoside (AICAR) treatment, both known AMPK activators, on the development of diabetes in male Zucker diabetic fatty (ZDF) rats were examined. Five-week-old, prediabetic ZDF rats underwent daily treadmill running or AICAR treatment over an 8-week period and were compared with an untreated group. In contrast to the untreated, both the exercised and AICAR-treated rats did not develop hyperglycemia during the intervention period. Whole-body insulin sensitivity, as assessed by a hyperinsulinemic-euglycemic clamp at the end of the intervention period, was markedly increased in the exercised and AICAR-treated animals compared with the untreated ZDF rats ($P < 0.01$). In addition, pancreatic β-cell morphology was almost normal in the exercised and AICAR-treated animals, indicating that chronic AMPK activation in vivo might preserve β-cell function. Our results suggest that activation of AMPK may represent a therapeutic approach to improve insulin action and prevent a decrease in β-cell function associated with type 2 diabetes. *Diabetes* 54:928–934, 2005

The incidence of type 2 diabetes is increasing dramatically throughout the world (1). This increase is due to lifestyle factors such as excessive food intake and a lack of physical activity (2). Recent epidemiological studies demonstrate that lifestyle intervention programs can prevent or delay the onset of type 2 diabetes (3–5).

One of the main features in the pathogenesis of obesity-related diabetes is the presence of insulin resistance (6). It is well established that regular skeletal muscle contraction enhances insulin action in healthy as well as in insulin-resistant individuals (7,8). It is likely that exercise can prevent the progression from the pre-diabetic insulin-resistant condition to overt diabetes by diminishing peripheral insulin resistance and consequently reducing the work load on the β-cells in the pancreas. Although a vast amount of research has been conducted, the underlying mechanisms by which exercise alters the development of diabetes are not fully clarified.

The AMP-activated protein kinase (AMPK) is an energy preserving enzyme sensitive to changes in the AMP-to-ATP ratio. AMPK is thought to be an important regulator of glucose and fat metabolism in skeletal muscle during metabolic stress (9) and has been shown to be activated during muscle contraction in both rat (10) and human (11) skeletal muscle. 5-Aminoimidazole-4-carboxamide-1-β-D-riboruranoside (AICAR) is a known activator of AMPK and can be used as an experimental tool to activate AMPK in vivo. Chronic AICAR administration in rats can result in marked changes in skeletal muscle including increases in glycogen stores, GLUT4, and the activity of hexokinase and mitochondria oxidative enzymes (12–14). AICAR can also lead to an increase in maximal insulin-stimulated glucose transport and GLUT4 translocation (15). Thus, it is conceivable that repetitive activation of AMPK may be part of the mechanism leading to improved insulin action after exercise.

A commonly used animal model for the study of diabetes, Zucker diabetic fatty (ZDF) rats, are characterized by a progressive β-cell dysfunction and a leptin receptor defect, the latter resulting in hyperphagia and obesity. After an initial period of compensatory hyperinsulinemia, the animals develop diabetes at ~10 weeks of age due to gradually impaired β-cell function (16). The present study was performed in order to investigate whether repetitive AMPK activation induced by long-term exercise training or AICAR treatment would be capable of preventing the development of diabetes in ZDF rats.

RESEARCH DESIGN AND METHODS

All procedures were approved by The Danish Animal Experiments Inspectorate and complied with European Convention for the Protection of Vertebrate Animals Used for Experiments and Other Scientific Purposes. Male ZDF rats (ZDF/Gmi-*fa/fa*) and their heterozygous (ZDF/Gmi-+/fa) lean littermates were purchased from Charles River Laboratories (Wilmington, MA) at 5 weeks of age and housed at a constant temperature (22–23°C) on a 12/12-h light/dark cycle with free access to food and water. Typically ZDF rats are fed a diet

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AICAR, 5-aminoimidazole-4-carboxamide-1-β-D-riboruranoside; AMPK, AMP-activated protein kinase; ZMP, 5-amino-4-imidazole carboxamide riboside 5′-monophosphate.

containing 16.7% fat (Purina 5008), on which they can develop overt diabetes at 6–8 weeks of age. To initiate the intervention before the ZDF rats developed diabetes, they were fed a lower-fat laboratory chow (Altromin 1324, fat 10.1%) (Chr. Petersen, Ringsted, Denmark).

Acute study. A single injection of AICAR or treadmill running was examined in separate groups of rats to see if AMPK activity would increase in response to these acute treatments. Male 5-week-old ZDF rats were either subcutaneously injected with a single dose of AICAR (0.5 mg/g body wt) or underwent a single bout of treadmill (Columbus Instrument; Columbus, OH) running (60 min, speed of 25 m/min at a 5% incline). Untreated ZDF rats served as controls ($n = 5$ in each group). One hour after the subcutaneous AICAR injection or immediately after treadmill running, rats were killed by cervical dislocation. To avoid any effect of muscle spasm and hypoxia, red and white gastrocnemius muscles were removed within seconds and immediately freeze clamped for later determination of AMPK activity.

Intervention study. The following four groups were studied ($n = 12$ per group): ZDF AICAR-treated group (AICAR group), ZDF exercise-trained group (exercise group), ZDF untreated control group (untreated group), and lean untreated control group (lean group). The AICAR group was injected with AICAR (0.5 mg/kg s.c.; Toronto Research Chemicals, Toronto, CA) every morning (between 8:00 A.M. and 10:00 A.M.) as previously described (17). The exercise group was subjected to treadmill running for 60 min (speed of 25 m/min at a 5% incline) 5 days a week (between 3:00 P.M. and 6:00 P.M.). The two control groups (untreated and lean) were left untreated. The intervention study was initiated when the rats were 5 weeks of age and lasted for 8 weeks, until 13 weeks of age. Fasting plasma glucose and insulin as well as body weight and food and water consumption were measured weekly. At the end of the 8-week intervention period, a subgroup of rats from the three ZDF groups (AICAR, exercise, and untreated groups) was subjected to hyperinsulinemic-euglycemic clamp studies. Clamped as well as fasted nonclamped rats were finally killed by cervical dislocation, and various tissues were removed, weighed, frozen in liquid nitrogen, and stored for further biochemical or histological examination.

Hyperinsulinemic-euglycemic glucose clamp studies. To recover before the clamp, a subgroup of rats was instrumented with chronic catheters in the carotid artery (blood sampling) and jugular vein (infusions) 7–10 days before the clamp study in week 8 of the intervention period. Antibiotic (Tribessen, 24%, 0.2 ml s.c. per rat) and analgesic (Anorphin 0.06 mg s.c. per rat and Rimadyl 2.0–2.5 mg/kg s.c. per rat) treatments were employed for 3 days after surgery. Diabetic untreated rats were given insulin (Actrapid 2–5 units/kg s.c.; Novo Nordisk) on the day of surgery and the following day in order to improve their postsurgical recovery. Exercised rats were allowed to rest the day after surgery. During the following days we observed no change in their exercise capacity due to the operation. The clamp studies were done 20–24 h after the last AICAR injection or treadmill run, and rats were fasted overnight for 12 h before the clamp. After catheters had been connected to the infusion system, the rats were placed in clamp cages allowing unrestricted behavior. A hyperinsulinemic-euglycemic clamp (60 min tracer equilibration, 30 min basal, and 180 min clamp) was performed in conscious, unrestricted rats as previously described (18). Insulin was infused at a rate of 7.5 mU · kg^{-1} · min^{-1}, and plasma glucose levels were clamped close to 7 mmol/l by adjusting an exogenous glucose infusion at 10-min intervals. During the 1st hour of the clamp, plasma glucose in the untreated rats was gradually lowered to the same level as in the two intervention groups. Endogenous glucose appearance and disappearance rates were measured using constant and variable infusions of [3-^{3}H]glucose as described previously (18).

Analytical procedures. Plasma glucose and insulin were determined using plasma obtained by tail-vein bleeding from rats fasted overnight (10 h). Blood sampling took place 20–24 h after the last AICAR injection or treadmill run. Plasma glucose was measured in duplicate immediately after sampling on a Beckmann Glucose Analyzer II (Beckman Instruments, Palo Alto, CA). Insulin levels were determined using an ultrasensitive rat insulin enzyme-linked immunosorbent assay kit (DRG Diagnostics, Marburg, Germany). Glucose concentrations during the clamp experiments were analyzed by the glucose oxidase method using a YSI 2500 STAT (Yellow Springs Instruments, Yellow Springs, OH). Insulin during the clamp and ^{3}H counts in plasma samples were measured as described by Brand et al. (18).

Plasma cholesterol, HDL cholesterol, and triglycerides were determined in a subgroup ($n = 5$) of fasted nonclamped rats from the three ZDF groups as previously described (17). To minimize stress in rats used in the clamp study, no blood samples were drawn from these rats before they were clamped.

Total crude membrane GLUT4 contents and AMPK subunit isoform and activities. Twenty micrograms of protein from cardiac and red and white gastrocnemius muscles was used for determination of total GLUT4 content, and AMPK subunit isoform expression was determined by Western blotting as previously described (15,19). Isoform-specific AMPK-α1 and -α2 activities were measured in red and white gastrocnemius muscles according to Jessen et al. (20).

Islets histology. After the animals were killed, the pancreas was removed en bloc, fixed in 4% paraformaldehyde for 4–8 days, dissected free of surrounding tissue, weighed, and fractionated by the smooth fractionator method (21,22). Each capsule contained 8–11 randomly picked pancreas cubes, systematically, uniformly representing one-fourth of the total pancreas. These were postfixed, dehydrated, and embedded in paraffin, and 3-μm–thick sections were cut from five different levels 600 μm apart, with the depth of the first level selected from a table of random numbers (23). The deparaffinized sections were stained for insulin and a mixture of antibodies to glucagon, somatostatin, and pancreatic polypeptide to visualize β and non-β endocrine cells as described (23,24). Furthermore, sections were counterstained with Mayer's hematoxyline. Endocrine cell mass (β and non-β) was evaluated stereologically in five sections with the origin of the sections blinded to the observer. Area-weighted mean values were calculated from the five sections. The mass of endocrine cells is expressed as milligrams per kilogram of body weight.

Statistics. Data are presented as the mean ± SE. Statistical significance was assessed by group comparison with the use of one-way ANOVA followed by Tukey's post hoc test. Significance was accepted at $P < 0.05$.

RESULTS

Acute study

AMPK activity measurement. Acute AMPK activity was assessed to evaluate whether the AMPK system was activated by the interventions. AMPK-α2 activity in white gastrocnemius muscles was significantly increased in both exercised and AICAR-injected rats when compared with untreated control ZDF rats (487 ± 129, 829 ± 241, and 100 ± 43 in the exercise, AICAR, and untreated groups, respectively; data are expressed as the percentage of the untreated group, $P < 0.05$ for the exercise vs. untreated group and $P < 0.01$ for the AICAR vs. untreated group). In contrast, only exercised rats had elevated AMPK-α2 activity in red gastrocnemius muscles (447 ± 120, 83 ± 25, and 100 ± 19% in the exercise, AICAR, and untreated groups, respectively, and $P < 0.05$ for the exercise vs. untreated group). Neither exercise nor AICAR injections changed the AMPK-α1 activity (data not shown).

Intervention study

Plasma glucose, insulin levels, and food intake during the intervention period. Preintervention fasting glucose levels were similar among the three pre-diabetic ZDF groups but were elevated as compared with the lean ZDF rats (Fig. 1). At week 5 of the intervention period, fasting plasma glucose increased sharply in the untreated group and remained high throughout the study period. In contrast, plasma glucose was almost unchanged in the AICAR and exercise groups. Before initiation of the intervention study, the pre-diabetic ZDF rats were hyperinsulinemic compared with the lean ZDF rats. In the untreated group, insulinemia increased gradually when compared with the two intervention groups and was already significantly elevated after 3 weeks of the study. Hyperinsulinemia increased until week 5 of the intervention period, after which a decline was observed. As expected, the latter occurred concomitantly with the marked increase in plasma glucose. In contrast, plasma insulin in the AICAR and exercise groups exhibited a sluggish and almost superimposable increase. During the first 3 weeks, rats in the untreated group consumed ~3 and 9% more chow than the exercise and AICAR groups, respectively. This difference increased even more during the last 5 weeks of the intervention study, when rats in the untreated group had glucosuria and hyperglycemia. During these weeks, rats in the untreated group consumed 9 and 28% more chow than rats in the exercise and AICAR, respectively.

Body and organ weight. Body weight was identical in the

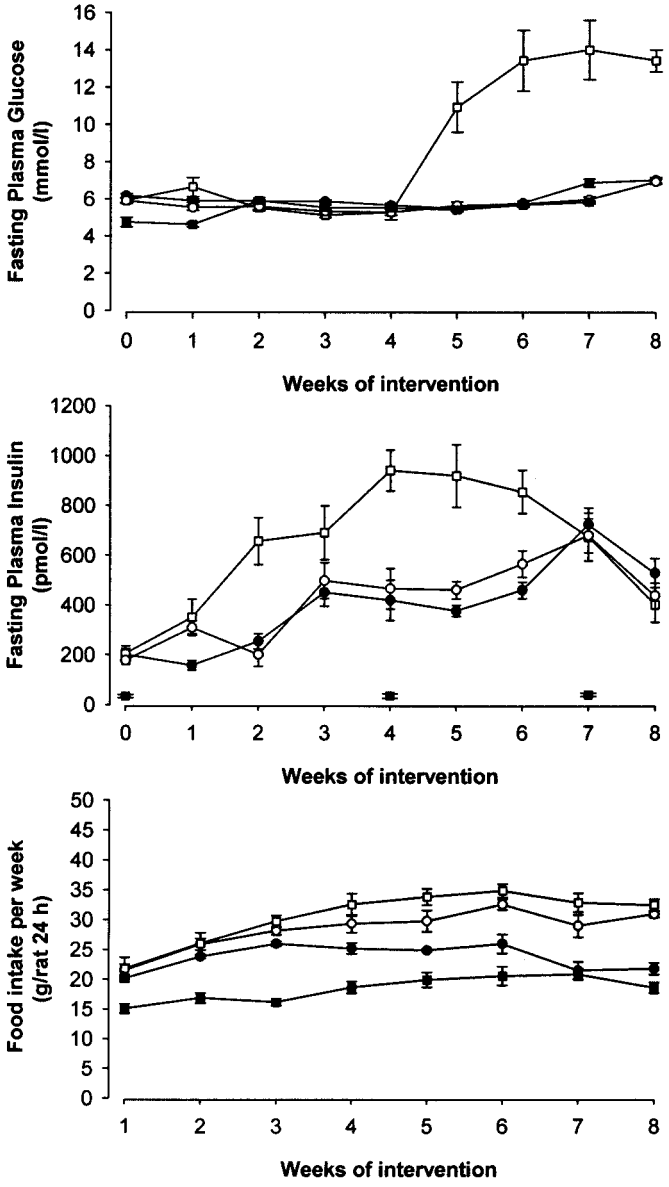

FIG. 1. Fasting plasma glucose and insulin were measured weekly in overnight-fasted rats. To avoid any acute effect of AICAR or exercise, blood sampling took place at least 24 h after the last AICAR injection or treadmill running. Average food intake per rat per day is presented for the weeks during the intervention period. ○, exercise group; ●, AICAR group; □, untreated group; ■, lean group. Data are presented as means ± SEM. n = 12 rats/group.

three different ZDF groups before initiation of the study (Table 1). At the end of the study, body weight in the exercise group was 8% higher than in the two other pre-diabetic ZDF groups. The kidney weight was significantly higher in the untreated group when compared with both the exercise and AICAR groups. The heart weight was higher in the exercise group than in the other groups, whereas the liver weight was significantly higher in the AICAR-treated group when compared with the exercise and untreated groups. There was no difference in weight of peritesticular or retroperitonal fat content between the pre-diabetic ZDF groups; neither was there any difference between skeletal muscle mass of soleus and extensor digitorum longus muscles among these groups. However, the muscle weight was lower when compared with the lean ZDF rats.

Triglycerides and cholesterol. Triglycerides, total cholesterol, and HDL cholesterol were measured in fasted, nonclamped rats (Table 2). No significant difference was observed between the rats in the three groups examined.

Hyperinsulinemic-euglycemic clamp. Plasma glucose was comparable in all three groups during the last 30 min of the clamp, and there was no difference in the incremental plasma insulin values during the clamp (Table 3). The glucose infusion rates required to maintain euglycemia during the clamp were 42 and 81% higher in the exercise and AICAR groups, respectively, as compared with the untreated group, although this was only statistically significant in the latter group. The ability of insulin to suppress endogenous glucose release was most pronounced in the AICAR-treated animals. Although the absolute rates of insulin-stimulated glucose disposal (R_d) were similar in all groups, the increment from basal was higher in the two intervention groups in which insulin induced a 173 ± 8 and 160 ± 15% increase over basal in the R_d in the exercise and AICAR groups, respectively, compared with a 103 ± 11% increase in the untreated group ($P < 0.05$).

Total GLUT-4 content. Rats in the untreated group had a marked decreased GLUT4 expression in both white and red gastrocnemius muscles when compared with lean controls; this decrease was especially pronounced in white muscles (Fig. 2). AICAR administration led to a more than twofold elevation in GLUT4 content in white gastrocnemius muscles but had no significant effect on GLUT4 content in red muscles. In contrast, exercise training displayed a different fiber type–specific change; the GLUT4 content was the most increased in red gastrocnemius muscles, while the effect on GLUT4 content in white muscles was only very moderate and significantly lower than the effect seen after AICAR treatment. In cardiac muscles, both exercise training and AICAR treatment resulted in a significant increase in GLUT4 protein expression when compared with rats in the untreated group.

AMPK subunit protein expression. To determine whether protein levels of the different AMPK subunits were altered after 8 weeks of treatment, protein levels of the different subunit isoforms were measured in white and red gastrocnemius (α1, α2, β1, β2, γ1, and γ3) and cardiac muscles (α1, α2, β1, and β2) (Table 4). Only the protein content of the AMPK-α1 subunit in the red gastrocnemius muscle was significantly upregulated in exercised animals. Protein levels of the other subunits were similar in the three groups.

Morphology of pancreatic islet. A representative islet from each of the four groups is shown in Fig. 3. Islets from rats in the untreated group contained less insulin compared with the two other groups and were much more disorganized in structure. Islets from the exercise group were clearly enlarged and stained normally for insulin. In contrast, islets from AICAR-treated animals maintained a normal rounded appearance, and the staining for insulin was much like the morphology of an age-matched lean ZDF rat. β-Cell mass was clearly increased in the exercise group compared with both the AICAR and untreated group (11.25 ± 1.95, 6.97 ± 0.87, and 5.16 ± 0.67 mg β-cell for the

TABLE 1
Body weight at start and end of the study and organ weights after 8 weeks of intervention

	Exercise group	AICAR group	Untreated group	Lean group
n	12	12	12	10
Body weight (g)				
Start	179.8 ± 3.5	179.8 ± 3.2	180.7 ± 2.4	137.4 ± 2.4
After 8 weeks	395.5 ± 9.8*	366.7 ± 8.2	368.8 ± 6.6	318.3 ± 3.6
Fat content (g)				
Peritesticular	8.12 ± 0.31	8.26 ± 0.32	7.46 ± 0.62	2.36 ± 0.08
Retroperitonal	9.71 ± 0.55	8.97 ± 0.40	9.34 ± 0.43	2.06 ± 0.10
Muscles (g)				
Soleus	0.24 ± 0.01	0.24 ± 0.01	0.24 ± 0.01	0.27 ± 0.01†
Extensor digitorum longus	0.24 ± 0.01	0.24 ± 0.01	0.25 ± 0.01	0.29 ± 0.02†
Kidneys (g)	1.22 ± 0.03	1.13 ± 0.02	1.41 ± 0.02‡	1.09 ± 0.01
Heart (g)	1.23 ± 0.04*	1.14 ± 0.02	1.13 ± 0.03	0.96 ± 0.01
Liver (g)	15.32 ± 0.79	18.44 ± 0.34§	16.49 ± 0.67	10.68 ± 0.17
Pancreas (g)	0.97 ± 0.03	0.99 ± 0.04	0.86 ± 0.06	1.23 ± 0.05

Data are means ± SE. *$P < 0.05$ for the exercise vs. AICAR and untreated groups; †$P < 0.01$ for the lean vs. exercise, AICAR, and untreated groups; ‡$P < 0.05$ for the untreated vs. AICAR and exercise groups; §$P < 0.05$ for the AICAR vs. untreated and exercise groups.

exercise, AICAR, and untreated groups, respectively; $P < 0.01$ for the exercise vs. AICAR and untreated groups). Furthermore, β-cell mass in the AICAR group tended to be higher than in the untreated group ($P = 0.13$).

DISCUSSION

Our data clearly demonstrate that daily subcutaneous AICAR injections can prevent, or at least postpone, the development of hyperglycemia in a diabetic rodent model. These effects of AICAR are similar to the effects of exercise training, and the dramatic effects in glucose homeostasis were evident by several measurements.

Circulating insulin during the intervention period in the untreated group exhibited a bell-shaped form with initially increasing insulin values until the rats were 9–10 weeks of age. After the age of 9–10 weeks, fasting plasma glucose increased sharply with a concomitant decrease in insulin secretion, emphasizing a progressive β-cell dysfunction in the untreated group. In contrast, in the treated groups, plasma glucose was almost unchanged and circulating insulin was only gradually increased during the entire intervention period. The improved glucose homeostasis of the two intervention groups was further underlined by the hyperinsulinemic-euglycemic clamp data, demonstrating an increase in glucose infusion rate in these groups and especially in the AICAR group. The incremental insulin-stimulated increase in glucose disposal from basal was found to be higher in the two intervention groups when compared with the untreated group. Insulin sensitivity of the liver was apparently also improved in both intervention groups but was predominantly pronounced in the AICAR group. However, the latter should be interpreted cautiously, as the glucose rate of appearance was not reduced in all groups.

Through its conversion to 5-amino-4-imidazole carboxamide riboside 5-monophosphate (ZMP) and probably also the triphosphorylated 5-amino-4-imidazole carboxamide riboside 5′-triphosphate (ZTP), AICAR injection might result in stimulation of other enzyme systems in the cells other than AMPK (25,26). One of these is fructose 1-6-bisphosphatase, which is involved in gluconeogenesis in the liver. Inhibition of this enzyme by ZMP will result in decreased hepatic glucose release. As the present study was undertaken 20–24 h after the last AICAR injection, and as no detectable amount of ZMP was found in the liver at this time (data not shown), the insulin-mediated suppression of endogenous glucose release was not likely due to a direct effect of ZMP. Instead, decreased hepatic glucose production with chronic AICAR treatment may be due to the downregulation of several of the key enzymes in the gluconeogenic pathways, a finding observed with AICAR treatment of cultured hepatoma cells (27).

In the current study, both AICAR administration and exercise training augmented peripheral insulin action, and the changes exhibited by either of the treatment groups were nearly identical. Previous studies have shown that both long-term AICAR treatment and exercise training are capable of enhancing GLUT4 protein expression in skeletal muscle (12,28). The present experiment shows a rise in GLUT4 expression in skeletal muscle tissue as a consequence of both AICAR treatment and exercise training. Therefore, one can speculate that the increased whole-body insulin sensitivity demonstrated in the present study, at least partly, might be due to an increased GLUT4 protein level in skeletal muscle tissue.

Rats in the untreated group had a slight but significant increase in kidney weight when compared with those in the exercise and AICAR groups. Increase in kidney weight is often seen as the initial sign of an early diabetic kidney disease in animals (29), and it seems that both exercise

TABLE 2
Triglycerides, total cholesterol, and HDL cholesterol in fasted nonclamped rats at the end of 8 weeks of intervention

	Exercise group	AICAR group	Untreated group
n	5	5	5
Triglycerides (mmol/l)	3.71 ± 0.83	3.99 ± 0.53	4.86 ± 0.87
Total cholesterol (mmol/l)	2.98 ± 0.48	3.45 ± 0.37	3.11 ± 0.35
HDL cholesterol (mmol/l)	1.43 ± 0.14	1.68 ± 0.12	1.48 ± 0.17

Data are means ± SE.

TABLE 3
Data related to the hyperinsulinemic-euglycemic clamp

	Exercise group	AICAR group	Untreated group
n	5	5	7
Basal $PG_{30–0\ min}$ (mmol/l)	6.9 ± 0.1*	7.3 ± 0.2†	14.3 ± 0.6
Clamp $PG_{150–180\ min}$ (mmol/l)	7.6 ± 0.2	7.4 ± 0.1	7.8 ± 0.2
Basal $PI_{0\ min}$ (pmol/l)	243 ± 23	314 ± 50†	160 ± 26
Clamp $PI_{180\ min}$ (pmol/l)	723 ± 22*	817 ± 49†	588 ± 32
ΔPI (pmol/l)	480 ± 43	503 ± 74	428 ± 25
$GIR_{150–180\ min}$ (mg · kg^{-1} · min^{-1})	9.0 ± 0.5	11.4 ± 0.4†	6.3 ± 1.2
Basal R_a (mg · kg^{-1} · min^{-1})	6.0 ± 0.2*	6.7 ± 0.3†	7.7 ± 0.3
Clamp R_a (mg · kg^{-1} · min^{-1})	7.1 ± 0.3*	6.0 ± 0.5†	9.5 ± 0.7
ΔR_a (mg · kg^{-1} · min^{-1})	1.1 ± 0.3	−0.6 ± 0.6	1.8 ± 0.8
Basal R_d (mg · kg^{-1} · min^{-1})	5.9 ± 0.2*	6.7 ± 0.3†	7.9 ± 0.2
Clamp R_d (mg · kg^{-1} · min^{-1})	16.2 ± 0.4	17.3 ± 0.6	15.9 ± 0.7
ΔR_d (mg · kg^{-1} · min^{-1})	10.3 ± 0.4*	10.6 ± 0.7†	8.0 ± 0.7

Data are means ± SE. *$P < 0.05$ for the exercise vs. untreated groups; †$P < 0.05$ for the AICAR vs. untreated groups. GIR, glucose infusion rate; R_a, rate of glucose appearance (endogenous glucose release); R_d, rate of glucose disappearance.

training and AICAR administration ameliorate this increase. In the present study, no significant difference in triglycerides and cholesterol was found between rats in the exercise, AICAR, and untreated groups. We have previously reported that in obese Zucker rats, AICAR treatment was associated with a lower level of triglycerides and an increase in HDL cholesterol (17). However, these animals were more obese than in the present study and exhibited considerably higher levels of triglycerides.

Rats in the AICAR group had a slight decrease in food intake during the 1st week of treatment when compared with those in the untreated group. The excessive food intake by the rats in the untreated group increased quite dramatically during the last 5 weeks of the study, when the rats first had glucosuria and then later hyperglycemia. This difference in food intake could be attributed to the fact that 25–30% of the intake of calories by a ZDF rat is excreted due to glucosuria (C.L.B., unpublished data). The difference in food intake in the exercise and AICAR groups is explained by the increased calorie consumption due to the daily exercise training and also to the fact that the exercised rats had a larger increase in body weight at the end of the intervention period.

A recent study has indicated that the γ3 subunits might be several-fold increased after several weeks of very intense exercise training in rats (30). This increase was found in red quadriceps muscles but not in soleus or white quadriceps muscles. In the present study, we used a more moderate form of exercise training and did not find a change in the γ3 subunit protein expression, neither in red nor in white gastrocnemius muscles after the 8-week training period. Nevertheless, we demonstrated markedly improved peripheral insulin sensitivity in these trained rats. This might indicate that the observed increase in γ3 subunit expression is only seen after very intense exercise training or is restricted to very specific muscles, e.g., red quadriceps muscles, but may also indicate that an increased γ3 subunit expression is not a major contributor to increased insulin sensitivity, as seen after exercise training. Indeed, it should be noticed that a recent report in human muscles demonstrates that training leads to a decrease in the content of the regulatory γ3 subunits (31).

In the present study, only the AMPK-α1 subunit protein level was found to be increased after exercise training in red muscles when compared with sedentary untreated and AICAR-treated rats.

It has been shown that prolonged, sustained activation of AMPK by AICAR in β-cell lines induces apoptosis in insulin-producing cells (32,33). This implies that future pharmaceutical approaches to activate AMPK might lead

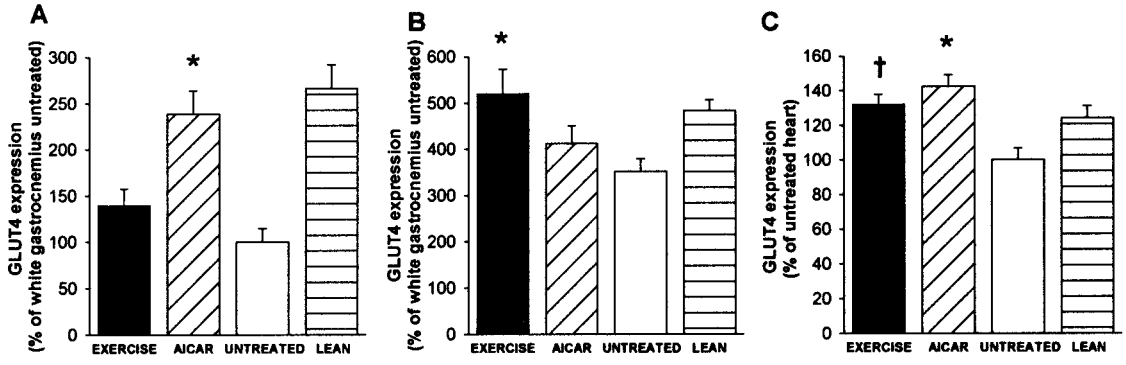

FIG. 2. GLUT4 content in white gastrocnemius muscles (*A*), red gastrocnemius muscles (*B*), and cardiac muscles (*C*) determined at the end of the intervention period. *$P < 0.01$ for the AICAR vs. untreated ZDF groups and the exercise vs. untreated ZDF groups. †$P < 0.01$ for the exercise vs. AICAR groups. GLUT4 data in both white and red gastrocnemius muscles are expressed as percentage of the GLUT4 content in white gastrocnemius muscles from the untreated group. GLUT4 levels in cardiac muscles are given as percentage of the GLUT4 content in cardiac muscles from the untreated group. Mean value of the untreated group was set at 100%, and the individual data were then normalized to the 100% level and SE calculated after the normalization. Data are presented as means ± SEM. n = 10–12 rats/group.

TABLE 4
AMPK isoform protein levels in red gastrocnemius, white gastrocnemius, and heart muscle fiber

	Exercise group	AICAR group	Untreated group	Lean group
n	7–10	7–10	7–10	7–10
Red gastrocnemius				
α1	141.0 ± 9.0*	108.4 ± 2.9	100.0 ± 10.8	87.3 ± 9.6
α2	89.0 ± 11.7	92.3 ± 9.3	100.0 ± 7.8	85.4 ± 9.8
β1	126.5 ± 64.3	105.1 ± 62.6	100.0 ± 42.3	133.1 ± 62.8
β2	103.9 ± 18.9	87.6 ± 13.6	100.0 ± 11.3	94.4 ± 13.6
γ1	119.5 ± 1.9	117.0 ± 4.9	100.0 ± 10.2	96.6 ± 8.0
γ3	119.7 ± 33.9	93.9 ± 16.7	100.0 ± 24.6	103.4 ± 29.1
White gastrocnemius				
α1	160.7 ± 67.1	133.2 ± 56.0	100.0 ± 29.1	92.3 ± 39.0
α2	104.7 ± 2.0	97.3 ± 3.8	100.0 ± 1.9	99.1 ± 8.0
β1	127.8 ± 73.3	129.6 ± 67.9	100.0 ± 54.6	223.8 ± 122.7
β2	127.3 ± 9.1	102.0 ± 9.7	100.0 ± 13.5	131.6 ± 9.2
γ1	97.7 ± 41.4	109.3 ± 44.8	100.0 ± 40.2	105.9 ± 43.4
γ3	90.1 ± 24.5	89.3 ± 27.9	100.0 ± 23.2	120.1 ± 35.0
Cardiac muscles				
α1	97.6 ± 1.2	88.7 ± 3.6	100.0 ± 3.2	94.4 ± 2.2
α2	94.8 ± 3.2	95.6 ± 3.7	100.0 ± 3.0	94.6 ± 2.6
β1	100.6 ± 11.9	116.7 ± 16.1	100.0 ± 8.9	89.1 ± 10.3
β2	87.3 ± 16.2	96.8 ± 27.4	100.0 ± 21.1	79.8 ± 14.8

Data are means ± SE. Protein levels of the different AMPK isoforms are expressed as percentage of control animals. *$P < 0.05$ for the exercise vs. untreated and AICAR groups.

to a devastating damage of pancreatic β-cells. When AICAR was injected daily subcutaneously as a single dose, β-cell mass was preserved in the AICAR-treated animals, and morphologically the islet cells in the AICAR-treated group had the most similar appearance as compared with islet cells of lean ZDF rats. This clearly indicates that no serious β-cell damage had occurred in these rats. In this study, which to our knowledge is the first in vivo study to examine the effect of AICAR on β-cells, it seems that AICAR treatment preserved β-cell function close to nearly normal. This could be secondary, of course, to the improved insulin sensitivity observed in these rats and, consequently, less stress on the β-cell. However, a possible direct protective effect of daily AICAR injection, and therefore repetitive activation of AMPK in the β-cells, on the function and survival of pancreatic β-cells cannot be excluded. Further studies in animal models that are predisposed to developing diabetes, but characterized by a developing β-cell dysfunction rather than by peripheral insulin resistance like the ZDF rats, are needed to explore whether repetitive activation of AMPK in β-cells in contrast to prolonged sustained activation might directly have a positive effect on β-cell function and survival. The β-cell mass in ZDF rats initially increases in size due to the peripheral insulin resistance when the rats grow older. At this point, the rats are able to maintain a slightly elevated plasma glucose level as compared with lean ZDF rats. When the rats grow older, the structure of the islet cells in the ZDF rats starts to degenerate with increasing amounts of connective tissue and the β-cell mass dramatically decreases as seen in the untreated group in the present study. The enhanced β-cell mass in the exercised rats compared with the AICAR-treated rats might be due to the fact that the β-cells in the exercised animals were more

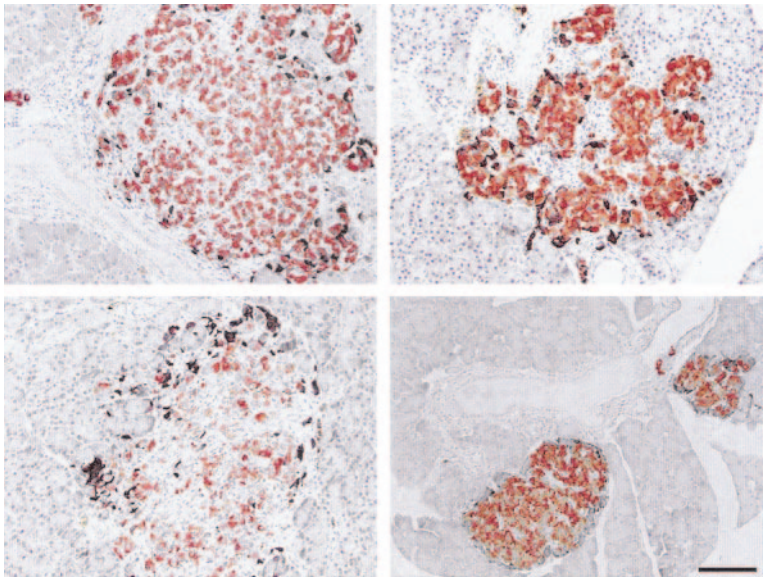

FIG. 3. Representative section of pancreas islet from ZDF rats after the intervention period. Immunohistochemical staining for β-cells appears reddish brown and for non–β-cells appears black. *Upper left*: exercise group; *upper right*: AICAR group; and *lower left*: untreated group. *Lower right*: pancreas islet from lean ZDF rats was not examined in the previous study, so for comparison reasons a vehicle-treated lean ZDF rat of similar age from Sturis et al. (24) is shown. Black bar = 200 μm. The AICAR group demonstrated higher and more regular insulin staining intensity than the untreated group. β-Cell volume was higher in the exercise group than in the AICAR and untreated groups.

stressed, as they were found to be less insulin sensitive than the AICAR-treated animals. It is possible that a more vigorous exercise program would have improved insulin sensitivity more than the exercise protocol used in the present study, therefore preventing the expansion in β-cell mass.

The present study is the first to show that long-term AICAR administration, like exercise, can prevent the development of hyperglycemia in an animal model predisposed to developing diabetes. This is partly due to an improved peripheral insulin sensitivity in skeletal muscles, apparently also due to an increase in insulin-mediated suppression of endogenous glucose release and partly due to the fact that long-term AICAR administration and exercise training maintain β-cell function. To our knowledge, this is the first in vivo study to demonstrate how long-term AICAR administration can preserve nearly normal islet morphology in the pancreas of animals predisposed to developing diabetes. As AMPK has been suggested to play an important role in muscle metabolism during exercise (26), and as both exercise training and AICAR administration activate AMPK, it is obvious to speculate that at least part of the beneficial effects of the two stimuli in preventing the development of hyperglycemia in the treated rats might be mediated through this kinase.

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