

Leptinomimetic effects of the AMP kinase activator AICAR in leptin-resistant rats: prevention of diabetes and ectopic lipid deposition

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Abstract

Aims/hypothesis. Leptin has been shown to activate AMP-activated protein kinase (AMPK), an enzyme that regulates the activities of key enzymes of lipid synthesis and metabolism. We assess here (i) whether AMPK activity is diminished in rodents deficient in leptin or the leptin receptor, and (ii) the effects of treating the diabetes-prone, leptin-receptor-deficient Zucker Diabetic Fatty (ZDF) rat with an AMPK activator.

Methods. AMPK activity and related parameters were measured in muscle and/or liver of *fa/fa* and ZDF rats and *ob/ob* mice. We also explored the effect of treatment with the AMPK activator 5-aminoimidazole 4-carboxamide 1- β -D-ribofuranoside (AICAR) (7.4 mmol/l, on Monday, Wednesday and Friday for 15 weeks, beginning at 7 weeks of age) on the phenotype of the ZDF rat.

Results. AMPK activity was diminished in muscle and/or liver of *fa/fa* (leptin-receptor-deficient, non-diabetic) and ZDF (leptin-receptor-deficient, diabetes-prone) rats and *ob/ob* mice (leptin-deficient). ZDF rats

that had free access to food became hyperglycaemic (22.2 mmol/l) and hyperphagic after 2 to 5 weeks and remained so during the remainder of the study. Treatment of ZDF rats with AICAR prevented the development of diabetes, as well as increases of triglyceride content in liver, muscle and the pancreatic islets. It also attenuated the morphological abnormalities observed in the islets of untreated rats. Rats diet-matched with the AICAR-treated animals developed diabetes of intermediate severity and showed decreases in triglyceride content in the islets, but not in liver or muscle.

Conclusions/interpretation. The results indicate that a deficiency of leptin or the leptin receptor is associated with a decrease in AMPK activity in muscle and/or liver. They also suggest that treatment with an AMPK activator prevents the development of diabetes and ectopic lipid accumulation in the ZDF rat.

Keywords Acetyl CoA carboxylase · AICAR · AMP-activated protein kinase · Leptin · Malonyl CoA · Triglycerides.

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Abbreviations: ACC, acetyl CoA carboxylase · AICAR, 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside · AMPK, AMP-activated protein kinase · CPT-1, carnitine palmitoyl transferase-1 · GPAT, glycerol 3-phosphate acyl transferase · MCD, malonyl CoA decarboxylase · ZDF, Zucker Diabetic Fatty rat

Introduction

The preferential storage of surplus calories in adipocytes in the form of triacylglycerol has been proposed to protect non-adipose tissues from the potentially toxic consequences of lipid overload [1]. When adipocytes are absent, as in generalised lipodystrophy, or when the antisteatotic adipocyte hormone leptin is deficient or inactive, the normal partitioning of surplus calories is impaired and ectopic lipid deposition occurs in non-adipose tissues [2]. In rodents, this lipid deposition is associated with a propensity to type 2 diabetes mellitus [3], lipotoxic cardiomyopathy [4], fatty liver [5], insulin resistance [6], dyslipidaemia and hypertension, a disease cluster that closely resembles that of the human metabolic syndrome [7].

Although it has not been proved that the metabolic syndrome in humans is the result of ectopic lipid deposition, there are several reasons to suspect that it has similar causes. First, obese humans have increased intramyocellular triglycerides in heart [8], skeletal muscle [9] and liver [5]; second, thiazolidinediones appear to prevent or retard the development of overt type 2 diabetes and other manifestations of the metabolic syndrome in humans [9] and obese rodents [10], as does caloric restriction [11]; and third, the beneficial effects of thiazolidinediones and caloric restriction have been associated in humans and rats with a reduction in lipid overload in non-adipose tissues.

The metabolic fate of the surplus long-chain fatty acids and the products of excess glucose metabolism are determined to a large degree by the activities of such enzymes as acetyl CoA carboxylase (ACC) [12, 13], and glycerol-3-phosphate acyltransferase (GPAT) [14]. ACC catalyses the carboxylation of cytosolic acetyl CoA to form malonyl CoA, a potent inhibitor of carnitine palmitoyl transferase (CPT-1) [15], the enzyme that controls the transfer of long-chain fatty acids into mitochondria, where they are oxidised. GPAT catalyses the first committed step in glycerolipid synthesis [14]. The enzyme AMP-activated protein kinase (AMPK) phosphorylates and inhibits ACC, it acutely diminishes the activity of GPAT and it activates malonyl CoA decarboxylase (MCD), a major enzyme that regulates malonyl CoA degradation [16, 17, 18]. In addition it diminishes amounts of ACC and GPAT by decreasing the expression of SREBP-1c. Thus, by virtue of its actions on these enzymes, AMPK activation would both increase the oxidation of fatty acids and diminish their esterification to form triglycerides and other glycerolipids. Furthermore, increases in AMPK activity caused by administering the AMPK activator, 5-aminoimidazole-4-carboxamide-1- β -D-ribofuranoside (AICAR), are associated with anti-obesity [19, 20] and insulin-sensitising effects [21, 22, 23] and genetically mediated increases in uncoupling proteins [24, 25] in rodents.

A considerable body of evidence suggests that one role of endogenous leptin is to protect the lean body mass of an overnourished organism from excessive lipid accumulation [26]. Thus, as adipocytes enlarge, they secrete proportionately more leptin. Leptin prevents overaccumulation of lipids by directly reducing triglyceride synthesis and increasing fatty acid oxidation in its target tissues [27], precisely the effects caused by AMPK activation. A recent study suggests that in skeletal muscle the effect of leptin is the result of AMPK activation and, subsequent to this, ACC inactivation, predominantly mediated by an increase in sympathetic nervous system activity [28]. However, in other tissues the role of AMPK in mediating leptin-induced lipopenia is less clear.

The lipopenic effect of leptin is not observed in the Zucker Diabetic Fatty (ZDF) rat, because of a loss-of-

function mutation in the leptin receptor, *OB-R* [29, 30]. These abnormalities in the ZDF rat are prevented by treatment with the thiazolidinedione troglitazone [10] or severe caloric restriction [11], both of which we have found to activate AMPK in rat tissues [31, 32]. In the present study we assessed whether AMPK activity is diminished in tissues of rodents deficient in either leptin or the leptin receptor. In addition, we evaluated whether AICAR treatment bypasses the receptor defect in the ZDF rat and prevents or attenuates ectopic lipid accumulation and the development of diabetes when these rats have free access to food.

Materials and methods

Animals. Obese homozygous (*fa/fa*) ZDF-drt male rats were bred in the Unger laboratory from ZDF/drt-*fa* rats originally purchased from R. Peterson (University of Indiana School of Medicine, Indianapolis, Ind., USA). They were housed in individual cages in a temperature-controlled room on a 12-hour light cycle. They received standard rat chow (Teklad FG rodent diet, Teklad, Madison, Wis., USA) containing 24% protein, 48% carbohydrate, and 6% fat (16.8 kJ/g). Food intake and body weight were measured daily at 10.00 hours on a Mettler pan balance. *Ob/ob* mice and *fa/fa* rats were obtained from Jackson laboratories (Bar Harbor, Me., USA) and were housed in the Boston University animal facility, where they had free access to Purina chow. Animal experimentation was in accordance with institutional guidelines.

Treatment of prediabetic ZDF (*fa/fa*) rats with AICAR. At the age of 6 to 7 weeks prediabetic (*fa/fa*) ZDF rats ($n=13$), matched for body weight and randomly assigned to either an AICAR-treated group ($n=5$) or a control group ($n=8$), were studied for the next 14 to 15 weeks. The control rats received sham injections. Both groups had free access to food. The AICAR-treated rats were injected subcutaneously on Monday, Wednesday and Friday at 10.00 hours with 250 mg/kg body weight of AICAR (Sigma, St. Louis, Mo., USA) dissolved in 0.9% saline and heated at 37 °C for 20 minutes. On this regimen, control rats eat approximately 20% less than treated rats on the days AICAR is administered, but eat more on the following day. In three separate studies, overall food intake compared to control rats injected with an equivalent volume of heated saline, over periods ranging from 26 to 106 days, was almost identical, indicating that AICAR did not diminish net food intake (N. Ruderman, A. Saha, unpublished results). At age 14 weeks (shortly before the development of overt diabetes), it was recognised that the ZDF rats with free access to food were eating more than the AICAR-treated group, which also had free access to food. The disparity in food intake was due to hyperphagia in the former group when these animals became overtly diabetic. However, this was only appreciated later and an additional untreated control group (previously given free access to food) was diet-matched with the AICAR-treated rats. These diet-matched animals received the same amount of food as the AICAR-treated rats, but 3 days later. In retrospect it was realised that the diet-matched rats were not suitable as controls, either for the ad-libitum-fed, or for the AICAR-treated rats; however, our findings in these animals are included for interest.

Plasma measurements. Tail vein blood was collected in capillary tubes coated with EDTA. Plasma was stored at -20 °C.

Plasma insulin and leptin were assayed using the Linco insulin and leptin assay kits (Linco Research, St. Charles, Mo., USA). Plasma glucose was measured by the glucose oxidase method with a glucose analyser (Beckman, Brea, Calif., USA). We measured plasma non-esterified fatty acids with a molecular biochemicals kit (Roche, Indianapolis, Ind., USA) and plasma triglycerides with the glycerol phosphate oxidase-Trinder triglyceride kit (Sigma).

Tissue preparation. Animals were killed subsequent to anaesthesia with sodium pentobarbital. Skeletal muscles, liver and epididymal fat were dissected and placed immediately in liquid nitrogen. Pancreatic islets were isolated according to the method of Naber and co-workers [33].

Light microscopy. A fragment of the tail of each pancreas was fixed in Bouin's solution and processed for immunohistochemistry, as described previously [34]. Sections were treated with guinea pig anti-pork insulin antibody at a dilution of 1:1000 at 20 °C for 2 h. After indirect immunofluorescence staining for insulin with FITC-conjugated IgG for 1 h at 20 °C, sections were photographed. The area of insulin-staining cells was estimated using the IMAGE J image analysis program, developed at the National Institutes of Health.

Malonyl CoA concentration, ACC, MCD and AMPK activity and phosphorylation, and tissue triglycerides and diacylglycerol. Malonyl CoA was determined radio-enzymatically by a slight modification of a previously published method [35, 36]. The activity of AMPK ($\alpha 1$ and $\alpha 2$ subunits) [16] and phosphorylation of Thr 172 on the AMPK α subunit (a measure of AMPK activation) [31] were assayed as described previously. To assay tissue triglycerides, total lipids were extracted from approximately 100 mg of tissue and dried under N_2 gas. Microdissection of muscle to remove interstitial fat was not per-

formed. Triglyceride content of tissue was measured with the GPO-Trinder triglyceride kit (Sigma). Acetyl CoA carboxylase and malonyl CoA decarboxylase activity were determined as previously described [16]. Diacylglycerol was determined as described by Saha and co-workers [37].

Statistics. Statistical analysis was performed using the ANOVA method. Results are expressed as means \pm SE. A p value of less than 0.05 was considered statistically significant.

Results

AMPK activity in rodents deficient in leptin or the leptin receptor. AMPK activity, measured after immunoprecipitation in *fa/fa* rats and $(NH_4)_2SO_4$ fractionation in *ob/ob* mice, was markedly diminished in liver and muscle of these rodents (Fig. 1). Total AMPK abundance ($\alpha 1 + \alpha 2$ isoforms) in the livers of *fa/fa* rats and *ob/ob* mice was unchanged (data not shown). In keeping with the known biological effects of AMPK, acetyl CoA carboxylase activity and tissue levels of malonyl CoA (Fig. 2) were significantly (2–3-fold) increased in these tissues in the *fa/fa* rats, and malonyl CoA decarboxylase activity was decreased by 50% (Fig. 2). Diacylglycerol content, also in keeping with these findings, was markedly increased in liver of both the *fa/fa* rat and *ob/ob* mouse (Fig. 3). Thus, a decrease in AMPK activity accompanies and presumably causes a wide variety of enzymatic and lipid abnormalities in liver and muscle of rodents deficient in leptin or with a functionally deficient leptin receptor.

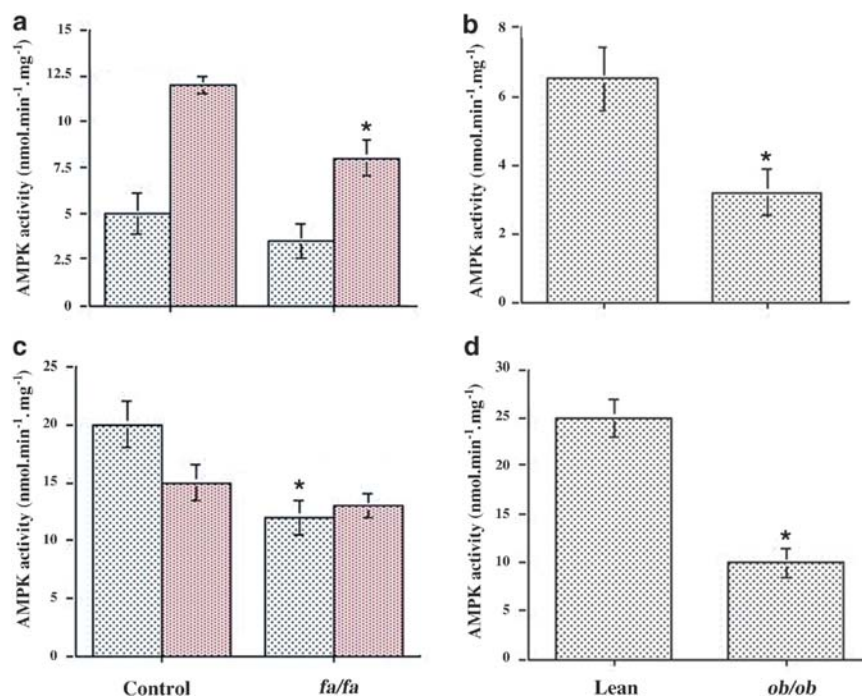


Fig. 1. AMPK activity ($\alpha 1$ and $\alpha 2$ isoforms) in muscle (a, b) and liver (c, d) of *fa/fa* rats, *ob/ob* mice and lean controls. AMPK activity was measured after immunoprecipitation using $\alpha 1$ and $\alpha 2$ antibodies in the *fa/fa* rats and $(NH_4)_2SO_4$ fraction-

ation in the *ob/ob* mice. Grey bars: $\alpha 1$ isoform; coloured bars: $\alpha 2$ isoform. Results are means \pm SE for 5 to 6 animals in each group. *Significantly different from lean control group, $p < 0.05$

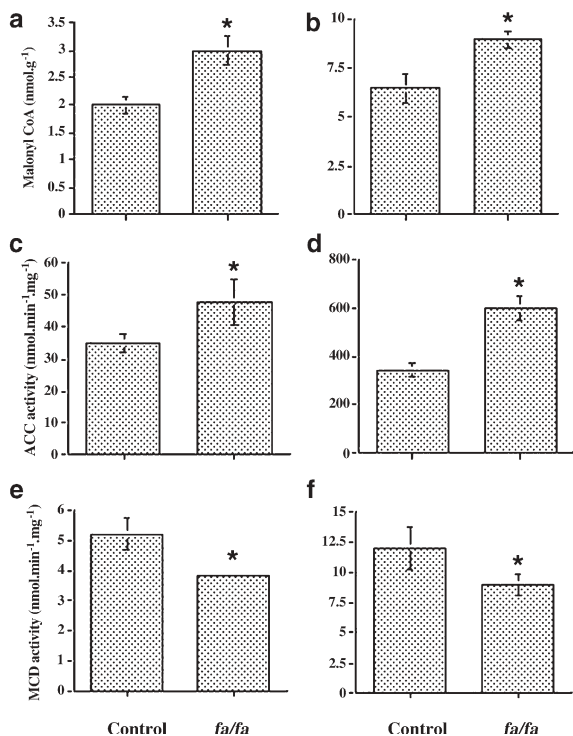


Fig. 2. Malonyl CoA content (a, b) and acetyl CoA carboxylase (ACC) (c, d) and malonyl CoA decarboxylase (MCD) (e, f) activity in muscle (a, c, e) and liver (b, d, f) of *fa/fa* and control rats. ACC and MCD activity were measured after $(\text{NH}_4)_2\text{SO}_4$ fractionation. Results are means \pm SE for 5 to 6 animals in each group. * Significantly different from lean control group, $p < 0.05$

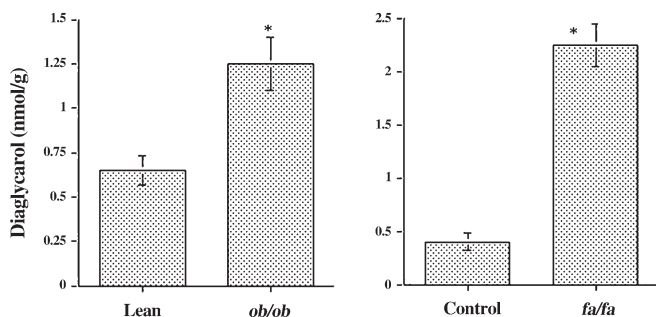


Fig. 3. Diacylglycerol content of liver in *fa/fa* rats and *ob/ob* mice. Results are means \pm SE for 5 to 6 animals in each group. *Significantly different from lean control group, $p < 0.05$

A similar decrease in AMPK activity, as reflected by a greater than 50% decrease in Thr 172 phosphorylated AMPK (23 ± 2 vs 10 ± 4 units; $p < 0.02$; $n = 4$), was observed in liver of the ZDF rat.

Effect of AICAR on food intake and body weight. To examine the effects of pharmacological activation of AMPK in a rodent with functionally deficient leptin receptors, ZDF rats were treated with AICAR. All rats entered the study at the age of 6–7 weeks except for a diet-matched group which entered at age 13–14

weeks. Rats fed ad libitum ate approximately the same amount of food as rats on the every other day AICAR regimen for the first 1.5 weeks of the study; after that, their food intake was 25% greater, and the differences were significant at week 14 and beyond (Fig. 4a). Despite this, their body weight was identical to that of the AICAR-treated rats, and slightly less than that of rats matched for food intake with the AICAR group (Fig. 4b). We attribute the disparity between food intake and weight gain in the rats fed ad libitum to the fact that with the onset of diabetes they became markedly hyperglycaemic (Fig. 4c) and polyuric. Thus, they almost certainly lost considerable calories as glucose in their urine that were replaced by increased food consumption.

The effect of AICAR on plasma glucose, lipids, insulin and leptin. Obese male ZDF (*fa/fa*) rats given free access to food typically develop severe type 2 diabetes by the age of 14 weeks [11]. Accordingly, in the present study, hyperglycaemia (20 mmol/l) developed in these rats between 3 to 5 weeks of the study (age 10–12 weeks), whereas the AICAR-treated rats remained normoglycaemic (Fig. 4c). The diet-matched control group was selected from rats that had had free access to food and were not initially used in the study. These rats were normoglycaemic (ca 7 mmol/l) at the time of entrance. They became moderately hyperglycaemic (15 mmol/l) by week 9 (age 16 weeks) and remained so thereafter. Their plasma glucose levels were less than those of the controls with free access to food ($p < 0.05$), and greater than those of the AICAR-treated rats ($p < 0.05$). Because of their method of selection, these rats were not a true control group either for the AICAR or the groups with free access to food (see Discussion). Instead, they provide a comparison with an earlier study, in which the effect of more severe calorie restriction was assessed in the ZDF rat [11]. Non-esterified fatty acid levels were not significantly different in the three groups (data not shown). Plasma triacylglycerol (triglyceride) levels were reduced to a similar degree in the AICAR-treated and diet-matched rats compared to control rats given free access to food, although because of variability the differences at the individual time points were not statistically significant (Fig. 4d).

Plasma insulin levels increased dramatically in all three groups to 15–20 ng/ml at the 7-week time point (Fig. 4e). However, at 13 weeks plasma insulin levels had declined by over 50% in both the ad-libitum-fed controls and the diet-matched rats, consistent with a defect in their beta cells. In contrast, in the AICAR-treated group, the decrease in insulin did not occur, evidence that beta cells had escaped the damage observed in the control rats. These differences were statistically significant ($p < 0.05$).

Plasma leptin levels rose initially to a similar degree in AICAR-treated rats and in rats with free ac-

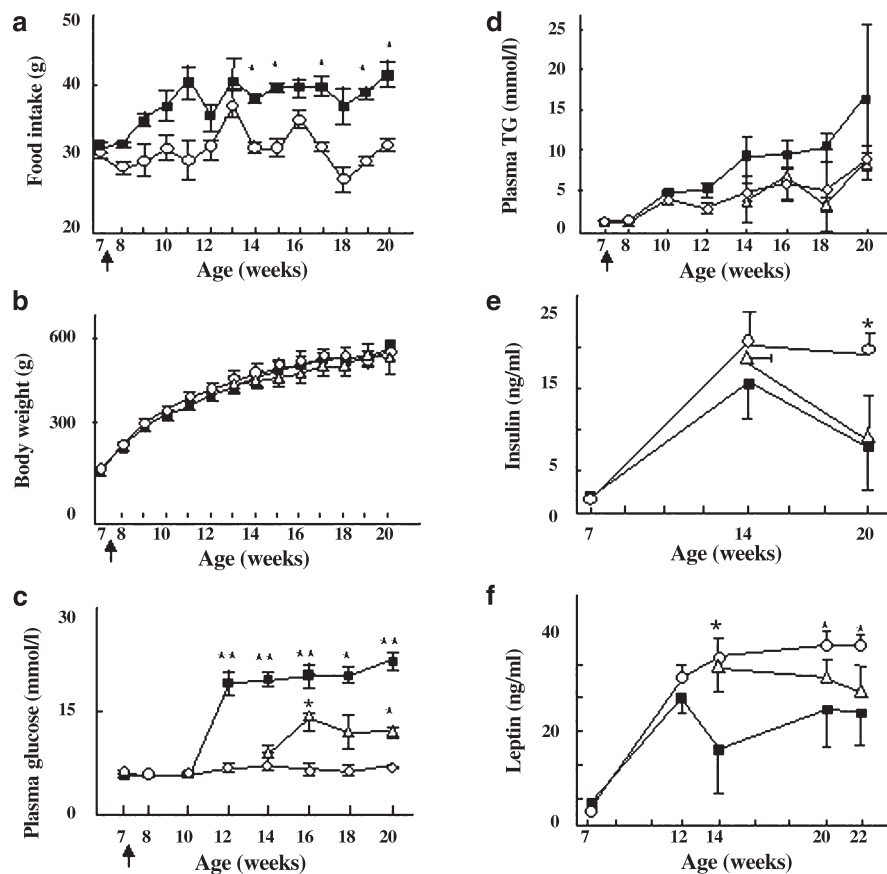


Fig. 4. Effect of AICAR treatment on food intake (a), body weight (b), plasma glucose (c), triglycerides (TG) (d), insulin (e) and leptin (f) in ZDF rats. The AICAR-treated (open circles) and ad-libitum-fed control rats (filled squares) were 6 to 7 weeks old when they entered the study. The rats diet-matched with the AICAR-treated group (open triangles) entered the study at age 14 weeks. They had previously been given free access to food. Panels a and c: * $p < 0.05$, ** $p < 0.01$ vs AICAR-treated group. Panels e and f: * $p < 0.05$ vs group fed ad libitum. Results are means \pm SE for 5 AICAR-treated rats and 4 rats in the two other groups

Effect of AICAR on non-adipose tissue triglycerides. In obese ZDF rats, triglycerides accumulate in adipose and non-adipose tissues to grossly abnormal levels, and this is associated with lipotoxicity and lipo-apoptosis in the latter [1]. The triglyceride accumulation is the result of a loss-of-function mutation in the leptin receptor [29, 30], which deprives all tissues of the antisteatotic action of leptin. If the antisteatotic action of leptin is mediated by activated AMP kinase, then AICAR therapy should mimic the action of leptin and reduce the triglyceride content in tissues of leptin-resistant animals. We therefore measured triglycerides in liver, skeletal muscle and pancreatic islets. Compared to control rats given free access to food, a significant or near-significant lowering of triglycerides was observed in all three tissues in the AICAR-treated rats ($p < 0.05$) (Fig. 5). In liver and skeletal muscle little, if any, decrease in triglycerides was observed in the diet-matched controls. In contrast, in the isolated islets a dramatic reduction in triglyceride content occurred in both the diet-matched and AICAR-treated groups.

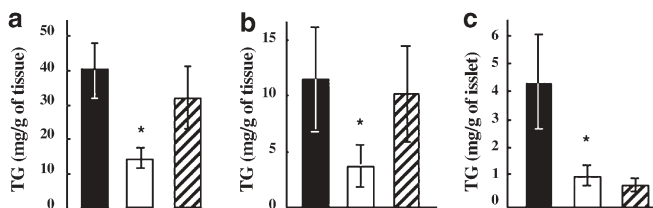


Fig. 5. Effect of AICAR treatment on tissue triglycerides (TG) content of liver (a), skeletal muscle (b), and pancreatic islets (c). Filled bars: ad-libitum-fed control; open bars: AICAR-treated; hatched bars: diet-matched control * $p < 0.05$ vs control rats fed ad libitum

cess to food (Fig. 4f), but at 7 weeks and later they were significantly lower in the latter group ($p < 0.05$). Leptin levels were relatively similar in the diet-matched and AICAR-treated rats by the end of the study (age 20 weeks).

In contrast to these findings for ectopic lipids, no differences in the weight of the epididymal fat pad were observed between the three groups. In the AICAR-treated rats the mean fat pad weight was 13 ± 0.8 g whereas in ad libitum fed and diet-matched groups it was 13 ± 1.2 g and 13 ± 0.7 g respectively.

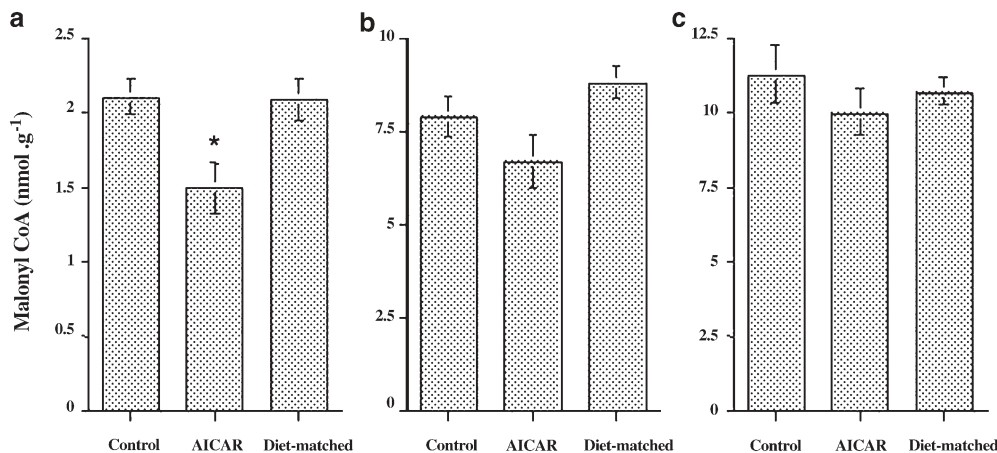


Fig. 6. Effect of AICAR treatment on the concentration of malonyl CoA in skeletal muscle (a), liver (b) and adipose tissue (c) of ZDF rats. Values are mean \pm SE ($n=4-5$). * $p<0.05$ vs control rats fed ad libitum and rats with the AICAR group diet-matched

shown). In general, values for malonyl CoA in tissues of the ZDF control rats that had free access to food and the rats diet-matched with the AICAR group were the same.

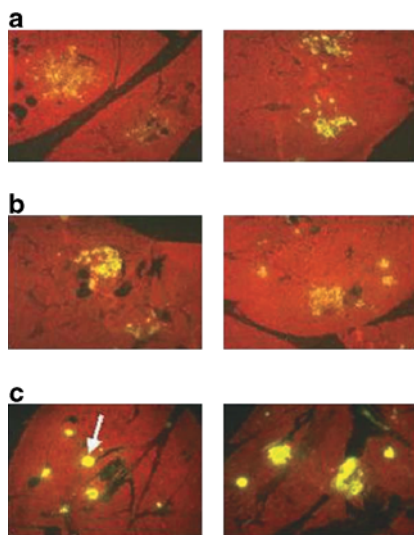


Fig. 7. Immunocytochemical staining for insulin of pancreas sections from ad-libitum-fed ZDF control rats (a), rats diet-matched with the AICAR group (b) and AICAR-treated ZDF rats (c) at 22 weeks of age (after 15 weeks of study). See text for details

Effect of AICAR on beta cell morphology. The beta cell mass of obese prediabetic ZDF (*fa/fa*) rats fed ad libitum increases ~4-fold between 5 and 10 weeks of age. After this, the hyperplastic beta cells gradually disappear, presumably through ceramide-mediated lipo-apoptosis [39]. By 12 weeks of age, up to 82% of the hyperplastic beta cells are lost [10] and the residual beta cell mass is unable to meet the increased demand for insulin imposed by obesity-associated insulin resistance. Consequently, hyperglycaemia appears.

To determine if AICAR treatment, like troglitazone therapy [10], prevents the secondary loss of beta cells, we analysed sections from the AICAR-treated and untreated rats. Striking differences in beta cell morphology were observed. In the ad-libitum-fed control rats, islets were misshapen with weak insulin staining of disorganised beta cells (Fig. 7). New islets were not observed. The beta cell area of the diet-matched control group was, if anything, somewhat less than that of the rats fed ad libitum (3.2% vs 3.9%). However, in them the insulin stain was somewhat more intense, suggesting better granulation, and occasional small islets were present, suggesting neogenesis. On the other hand, fibrous tissue and distortion of the islets were as evident as in the group fed ad libitum. By contrast, in the AICAR-treated rats, the area of insulin-staining was greater (5.8%; $p<0.05$ vs ad-libitum-fed control) and the staining more intense than in either of the other groups. Also, profusions of small, intensely stained, apparently young islets were observed. Such islets were not seen at all in the ad-libitum-fed control group and they were less common in the diet-matched group. Some deformity of the islets was evident in the AICAR-treated rats, probably reflecting beta cell replacement by fibrous tissues; however, it was far less than in the ad-libitum-fed controls.

Effect of AICAR on malonyl CoA concentration. Two hours after the administration of AICAR, the concentration of malonyl CoA is diminished in muscle, liver and adipose tissue of Sprague-Dawley control rats, and ACC and AMPK phosphorylation are increased, resulting, respectively, in decreases and increases in their activity [20]. In keeping with previous studies in other rodents [38], we found that the concentration of malonyl CoA was diminished in muscle of ZDF rats 24 hours after the last injection of AICAR (Fig. 6), although alterations in AMPK and ACC phosphorylation were no longer evident (data not

Discussion

The central findings of this study are: (i) that AMPK activity is diminished in tissues of rats that lack leptin or have a functionally deficient leptin receptor; (ii) that chronic intermittent AICAR administration (AMPK activation), beginning at 7 weeks of age, diminishes the accumulation of ectopic lipid in liver, muscle and the pancreatic islets and protects the ZDF rat from developing hyperglycaemia; and, in keeping with the above, (iii) that AICAR treatment both prevented the secondary decrease in plasma insulin levels observed in the other groups and diminished the distortion and decrease in area of the pancreatic islets and degranulation of the beta cells.

Decreased AMPK activity has been found by us previously in liver and muscle of rats infused with glucose for upwards of 5 h, in association with an increase in the concentrations of malonyl CoA and diacylglycerol, and insulin resistance [40]. Likewise, AMPK activity is diminished and the concentration of malonyl CoA increased in livers of the Dahlsalt-sensitive rat, a rodent with endogenous hypertriglyceridaemia and mild insulin resistance [31]. Previously, decreased AMPK activity has been observed in skeletal muscle of the *falfa* rat by one group [41], but not by another [42]. In the present study, similar findings were obtained in functionally leptin-receptor-deficient *falfa* rats and leptin-deficient *ob/ob* mice, both of which are obese and have ectopic lipid deposition in liver and muscle. The effect of thiazolidinedione therapy was not examined here; however, one of the investigators of this study (R.H. Unger) has previously shown that treatment with troglitazone decreases ectopic lipid accumulation in multiple tissues and prevents the development of diabetes in the ZDF rat [10, 11]. In addition, we have recently demonstrated that pioglitazone treatment increases AMPK activity and lowers malonyl CoA levels in livers both of the Dahlsalt-sensitive rat and of rats infused with glucose and insulin [31]. Collectively, these findings, together with the effects of AICAR in the present study, suggest that (i) AMPK plays a major role in the regulation of cellular lipid metabolism in vivo, and (ii) that in the absence of functional leptin receptors, treatment with AICAR restores cellular triglyceride content to normal levels.

A number of properties of AMPK suggest that its activation could account for the protective action of AICAR on beta cells, which was observed in the present study. First, AMPK enhances fatty acid oxidation and diminishes glycerolipid synthesis, both of which would diminish the accumulation of lipids [16]. Second, pancreatic beta cell damage in the ZDF rat has been related to ceramide-induced apoptosis [39], and AICAR, by activating AMPK, has been shown to inhibit apoptosis in human umbilical vein endothelial cells [43] and astrocytes [44] incubated with palmitate

or a high concentration of glucose. Furthermore, in some of these studies AICAR was shown to inhibit de novo ceramide synthesis and decrease the activity of serine palmitoyl transferase, the first committed enzyme in the de novo ceramide synthesis pathway [44, 45]. Third, in endothelial cells incubated with an elevated concentration of glucose or NEFA, AICAR, and, where studied, expression of a constitutively active AMPK, inhibited oxidative stress, mitochondrial damage and the activation of NF κ B and caspase-3 [44, 45]. All of these effects could have contributed to the observed actions of AICAR administration on the islets.

Every other day, low-dose AICAR regimen was used in the present study because it has minimal, if any, effects on overall food intake in control rats over periods comparable to those used here (15 weeks) [46]. Despite this, food intake was approximately 20% less in the AICAR-treated rats than in the rats given free access to food. As already mentioned, this difference was almost certainly due to diabetic hyperphagia in the latter group, since it was statistically significant only at age 14 weeks and beyond, when the blood glucose levels of these rats were in excess of 20 mmol/l. The diet-matched group was added in the middle of the study before the reason for the difference in food intake between the ad-libitum-fed and AICAR groups had become clear. In retrospect, it was neither necessary nor was it a proper control group for either the AICAR-treated or ad-libitum-fed rats. Indeed, the diet mediated-group was more comparable to rats previously reported by one of us (R.H. Unger), in which a 50% decrease in food intake was shown to prevent the development of diabetes and to substantially reverse it (in ZDF rats, aged 15 weeks, that had recently become diabetic) [11]. In the present study, we found that a 20 to 25% decrease in food intake in rats that had previously been fed ad libitum and not yet become diabetic (i.e. the diet-matched group), failed to prevent lipid accumulation in liver and muscle, disturbances in beta cell morphology or the development of diabetes in the ZDF rat, although it did attenuate the increase in beta cell lipid. This contrasts with the effects of a more marked decrease in food intake (50%), which both prevented the development of hyperglycaemia and loss of beta cells in the ZDF rat and restored normoglycaemia when the diabetes was already established [11]. AMPK activity was not assayed in this earlier study; however, it is noteworthy that AMPK activity in liver is diminished by 50% when a rat which has been starved for 48 h is refed [32].

An additional mechanism by which AICAR could have preserved beta cell function was by diminishing hyperglycaemia and secondary to this cellular glucotoxicity. Thus AICAR via an AMPK-independent mechanism suppresses hepatic glucose production by inhibiting fructose 1–6, biphosphatase [47]. Also, AMPK activation by itself has been shown to inhibit

hepatic glucose production [48] and gluconeogenesis [18, 49], and to increase glucose utilisation [50], and insulin sensitivity in liver and muscle [21, 38]. In keeping with the notion that AICAR diminished insulin resistance, it decreased triglyceride accumulation (a marker of insulin resistance) in both liver and muscle, whereas this did not occur in the diet-matched rats, which became diabetic. Robertson and co-workers have suggested that hyperglycaemia may be necessary for the development of lipotoxicity in the ZDF rat [51, 52]. Effects of hyperglycaemia on the concentration of malonyl CoA and subsequently fatty acid metabolism in islets and/or on the hexosamine pathway and protein o-glycosylation, could hypothetically account for this. Somewhat against this notion is the finding that, even before the onset of hyperglycaemia, lipid formation from glucose is 84% higher in ZDF rat islets than in those of control animals [53].

From the findings presented here it can be inferred that the effects of leptin on lipid metabolism in peripheral tissues may be mediated by AMPK, either by a direct action of leptin on tissue or secondary to a central effect that leads to increased sympathetic nervous system activity. Other interpretations of the data are possible, however. Thus, it has been reported that corticosterone levels are elevated and that adrenalectomy diminishes both the hyperglycaemia and hyperinsulinemia in the *fa/fa* rat [54]. Likewise hypothalamic signalling in response to insulin [55] is impaired in this rodent. Whether effects on the adrenal or hypothalamus contributed to the AICAR (AMPK)-induced changes observed in the ZDF rat study remains to be determined.

In conclusion, the results suggest that the lipid abnormalities and development of diabetes in the ZDF rat are associated with a decrease in AMPK activity and that both are corrected by an agent that activates AMPK. In this context it is noteworthy that metformin [56, 57, 58] and troglitazone [31, 58], both of which can activate AMPK, like diet and exercise, have been shown to delay or prevent the onset of type 2 diabetes in humans [9, 59]. Likewise, these agents have been reported to prevent the development of diabetes in the ZDF rat [60, 61]. Thus, strategies targeting AMPK may merit consideration for the prevention and treatment of type 2 diabetes and possibly other disorders associated with the metabolic syndrome [62].

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