5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside treatment ameliorates hyperglycaemia and hyperinsulinaemia but not dyslipidaemia in KKA^y-CETP mice

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Abstract

Aim/hypothesis. 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside increases 5'-AMP-activated kinase activity in insulin-sensitive tissues known to control glucose homeostasis. We hypothesised that 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside treatment could have a beneficial effect on glucose homeostasis in KKA^y-CETP mice, a model of Type II (non-insulin-dependent) diabetes mellitus. Our aim was to examine potential effects of acute and chronic (7-day) 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside treatment on glucose homeostasis in KKA^y-CETP diabetic mice.

Methods. Female KKA^y-CETP mice were treated with 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside by a single daily injection for 7 days (100, 300, or 500 mg · kg⁻¹ · day⁻¹).

Results. After 7 days of treatment with 500 mg · kg⁻¹ · day⁻¹ 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside, blood glucose and plasma insulin concentrations were reduced ($p < 0.01$). Body weight and food intake were also reduced after treatment ($p < 0.01$ and $p < 0.05$, respectively). Glucose and insulin tolerance were improved ($p < 0.05$), whereas

endogenous glucose production was suppressed ($p < 0.05$). The beneficial effect of 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside on hyperglycaemia and hyperinsulinaemia was due to an inhibition of endogenous glucose production, since in vivo and in vitro basal and insulin-stimulated glucose uptake in skeletal muscle was not affected by 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside. Other features of the treatment included increased plasma of free fatty acid concentration (1.9-fold, $p < 0.01$) and triglycerides (1.3-fold, $p < 0.05$).

Conclusion/interpretation. 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside treatment attenuated hyperglycaemia and hyperinsulinaemia but not dyslipidaemia in KKA^y-CETP mice, a model of Type II diabetes. The blood glucose lowering effects of 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside occurs mainly as a consequence of reduced endogenous glucose production because insulin-stimulated skeletal muscle glucose uptake has not been altered. [Diabetologia (2001) 44: 2180–2186]

Keywords Metabolic control, glucose transport, glucose production, blood lipids, skeletal muscle, therapy, animal models, glucose tolerance.

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Abbreviations: PI, 3-Kinase, Phosphatidylinositol 3-Kinase; AMPK, 5'-AMP-Activated Kinase; AICAR, 5-Aminoimidazole-4-Carboxy-Amide-1- β -D-Ribofuranoside; CEPT, cholesteryl ester transfer protein; EDL, extensor digitorum longus; KHB, krebs henseleit buffer

Type II (non-insulin-dependent) diabetes mellitus is a metabolic disorder characterised by hyperinsulinaemia, hyperglycaemia and dyslipidaemia. Other hallmarks of the metabolic syndrome include insulin resistance and pancreatic beta-cell dysfunction [1]. Insulin resistance is associated with increased hepatic glucose production and defects in glucose utilisation peripheral tissues (skeletal muscle and adipose tissue). Skeletal muscle is the major tissue responsible for glucose disposal under postprandial conditions,

implying that improvement of insulin sensitivity in skeletal muscle is important for the treatment of Type II diabetes.

Insulin and physical exercise markedly increase skeletal muscle glucose uptake by different mechanisms [2]. Insulin-stimulated glucose uptake is mediated by the insulin signalling pathway, involving tyrosine phosphorylation of the insulin receptor substrate 1, activation of phosphatidylinositol 3-kinase (PI 3-kinase) and possibly via phosphorylation of Akt [3]. The mechanism mediating muscle contraction-stimulated glucose transport seems to be different from that induced by insulin. For example, in muscle-specific insulin receptor knockout mice, glucose uptake and glycogen synthesis in skeletal muscle are increased in response to exercise but not insulin [4]. Furthermore, 5'-AMP-activated kinase (AMPK) seems to be involved in the contraction-stimulated glucose uptake, whereas PI 3-kinase is involved in the insulin-induced-pathway [2]. In addition, GLUT4, the primary glucose transporter responsible for glucose uptake, can translocate from separate pools in response to muscle contraction or insulin [5].

Insulin-stimulated glucose uptake in skeletal muscle from people with Type II diabetes is decreased [2] but skeletal muscle GLUT4 protein expression is normal [6]. In skeletal muscle from dietary-induced insulin resistant mice, agents that increase glucose transport by insulin-independent mechanisms elicit a normal response [7]. Furthermore, stimulation by insulin in combination with exercise leads to an additive effect on glucose uptake [8, 9]. These observations led us to use a pharmaceutical approach to mimic the effect of exercise by increasing the AMPK activity by 5-aminoimidazole-4-carboxy-amide-1- β -D-ribofuranoside (AICAR). AICAR activates AMPK, an enzyme implicated in exercise-induced glucose uptake [10]. In vitro studies show that AICAR increases AMPK activity in skeletal muscle [11], hepatocytes [12], and adipocytes [13]. Increased AMPK activity is reported to increase glucose uptake and NEFA oxidation in skeletal muscle [11], decrease lipogenesis and lipolysis in adipocytes [13], and decrease NEFA and cholesterol synthesis in hepatocytes [12]. In addition, an intraperitoneal injection of AICAR causes hypoglycaemia in non-diabetic mice [14] and rats [11]. Based on these observations, we hypothesised that AICAR-treatment could have a beneficial effect on diabetic hyperinsulinaemia, hyperglycaemia and dyslipidaemia. Thus, our aim was to examine the effect of AICAR treatment on several parameters associated with the metabolic syndrome in transgenic KKA^y-CETP mice. This animal model of Type II diabetes mellitus is characterised by hyperphagia, obesity, severe hyperglycaemia, hyperinsulinaemia and dyslipidaemia [15].

Materials and methods

Animals. Female KKA^y-CETP mice (4–6 months old, Pharmacia Corporation, Kalamazoo, Mich., USA) were individually housed at $21 \pm 1^\circ\text{C}$ on a 12 h light-dark cycle and had access to food and water ad libitum. This transgenic model of Type II diabetes over-expresses the simian cholesteryl ester transfer protein (CETP), resulting in increased VLDL and LDL and decreased HDL concentration, mimicking the dyslipidaemia characteristic of Type II diabetic patients [15]. The local animal ethics committee approved all study protocols. Blood glucose concentration was comparable between the treatment groups at the onset of the experimental period.

To determine the effect of an acute AICAR treatment on blood glucose, non-fasted mice received an intraperitoneal injection of either AICAR, at concentrations of 500 or 1000 mg/kg (Sigma, St. Louis, Mo., USA), or vehicle (0.9% NaCl). Blood samples were collected from the tail at 0, 30, 60, 120 and 240 min after an intraperitoneal injection to measure blood glucose by an accutrend glucometer (Roche, Indianapolis, Ind., USA).

To determine the effects of 7-day AICAR-treatment on metabolic control, diabetic mice received AICAR (100, 300, or 500 mg $\cdot$ kg⁻¹ $\cdot$ day⁻¹) or saline by intraperitoneal injection, at 09.00 h daily for 7 days. Blood glucose was monitored before dosing. Body weight and food intake was measured on the first and last day of the treatment protocol. Twenty-four hours after the final dosing, mice were anaesthetised with isoflurane, and orbital blood samples were collected for analysis of plasma glucose, insulin, non-esterified fatty acids (NEFA), triglyceride, total cholesterol, HDL, VLDL and LDL. Plasma glucose was measured spectrophotometrically (Cobas Mira, Roche, Montclair, N.J., USA) using a commercially available glucose GHD unimate 7 kit (Roche, Basel, Switzerland). Plasma triglycerides and plasma NEFA were measured spectrophotometrically (Cobas Mira) using triglyceride (Boehringer Mannheim, Indianapolis, Ind., USA) and NEFAC (WAKO, Neuss, Germany) assay kits. An ELISA method (Mercodia, Uppsala, Sweden) was used to measure plasma insulin concentrations. Lipid profiles were analysed by column chromatography using fast protein liquid chromatography on a superose-6B column (Pharmacia, Uppsala, Sweden) [15]. The 24 h urine was collected from mice after the last AICAR dosing (500 mg $\cdot$ kg⁻¹ $\cdot$ day⁻¹) or vehicle treatment using metabolic cages. Urine glucose content was measured spectrophotometrically (Cobas Mira) using a glucose GHD unimate 7 kit (Hoffmann-La Roche, Basel, Switzerland). Glucose and insulin tolerance tests were carried out after 7 days treatment with AICAR (500 mg/kg) or saline. Mice were injected with insulin (10 mU/kg i.p.) or given 30% glucose by oral gavage (3 mg/kg) 24 h after the last dosing. Blood glucose was measured at 0, 30, 60 and 120 min after insulin or glucose administration.

In vitro glucose uptake in skeletal muscle. One day after the final dosing of the 7-day AICAR treatment regime, mice were anaesthetised with 2.5% avertin (0.02 ml/g body weight). Soleus and extensor digitorum longus (EDL) muscles were dissected and transferred to vials containing 1 ml of incubation media prepared from a pre-gassed (95% O₂ / 5% CO₂) Krebs Henseleit buffer (KHB) supplemented with hydroxyethylpiperazine-ethanesulphonic acid (5 mmol/l; pH 7.2, 30°C) and bovine albumin (0.1%, RIA grade, Sigma) in a shaking water bath (30°C). Muscles were incubated for 30 min in KHB containing 5 mmol/l glucose and 15 mmol/l mannitol, with or without added insulin (20 mU/ml). Muscles were transferred to vials containing 20 mmol/l mannitol and incubated 10 min before mea-

suring 2-deoxyglucose uptake. Glucose uptake was assessed for 20 min using 1 mmol/l [^3H]2-deoxy-glucose (2.5 $\mu\text{Ci/ml}$, Am Radiolabelled Chem, St. Louis, Mo., USA) and 19 mmol/l [^{14}C]-mannitol (0.7 $\mu\text{Ci/ml}$, Am Radiolabelled Chem). Muscles were immediately frozen in liquid nitrogen and kept at -20°C . Frozen muscle samples were weighed, digested in NaOH (0.5 mol/l) and processed as described for 2-deoxyglucose uptake measurements [17].

In vivo glucose clearance and endogenous glucose production. The acute effect of AICAR on glucose clearance and endogenous glucose production was measured in KKA y -CETP mice that had not undergone AICAR-treatment, using the euglycaemic clamp. The chronic effect of AICAR was evaluated by euglycaemic-hyperinsulinaemic clamp in mice after a 7-day treatment of AICAR or vehicle. A catheter was inserted into the left jugular vein of the mouse during isoflurane anaesthesia 2 days before the clamp study. Euglycaemic-hyperinsulinaemic or euglycaemic-hyperinsulinaemic clamps [18] were carried out using conscious, unstrained mice, fasted for 6 h. After adapting to the clamp condition, KKA y -CETP mice received a constant infusion of D-[3- ^3H]-glucose (Am Radiolabelled Chem), at a dose of $0.5 \mu\text{Ci} \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}$ for 150 min. After the 60 min tracer infusion, AICAR ($0.2 \cdot 100 \text{g}^{-1} \cdot \text{min}^{-1}$) or insulin ($50 \text{mU} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) was infused intravenously for 90 min and the blood glucose concentration was maintained by adjusting the glucose infusion rate. Blood samples (50 μl) were collected from the tail at 60 and 150 min of tracer infusion to determine blood glucose, plasma insulin and D-[3- ^3H]-glucose radioactivity. Glucose turnover and endogenous glucose production were calculated [18]. Glucose clearance is reported as a ratio between total glucose metabolism and steady-state plasma glucose concentration [19].

Statistic analysis. Results are expressed as means $\pm$ SEM. Data are analysed using one-way ANOVA followed by a Neuman-Keules test when comparing more than two groups. Paired or non-paired Student's *t* test was used to distinguish differences between the two groups. Significance was accepted at a *p* value of less than 0.05.

Results

Effect of AICAR-treatment on metabolic control. The acute effect of AICAR on blood glucose in diabetic KKA y -CETP mice was measured (Fig. 1). AICAR, at a dose of 100 mg/kg, had no effect on blood glucose (data not shown). At 500 mg/kg AICAR, the blood glucose concentration was markedly decreased at 4 h ($p < 0.05$) and 6 h ($p < 0.01$), compared with saline injected mice. AICAR, at a dose of 1000 mg/kg, significantly reduced blood glucose concentration 120 min after injection ($p < 0.001$) and this effect was maintained for at least 6 h. At this dose, some mice showed severe hypoglycaemia. Thus, animals were treated with either 100, 300 or 500 mg/kg AICAR for the 7-day treatment study.

KKA y -CETP mice were treated with three different doses of AICAR (100, 300 or 500 mg/kg) or saline for 7 days. Blood glucose was monitored daily and was found to be reduced ($p < 0.05$) only when mice were treated with 500 mg/kg AICAR

Fig. 1. Acute treatment with AICAR (500 or 1000 mg/kg) (■, ●) or saline (□) on blood glucose concentration. Blood glucose was monitored for 6 h after the injection of AICAR or saline. Values are means $\pm$ SEM, $n = 12-20$. Blood glucose was reduced by AICAR treatment at 1000 mg/kg (** $p < 0.01$, *** $p < 0.001$) and 500 mg/kg ($^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$) when compared to the saline treated control mice

Fig. 2. Chronic effect of AICAR (500 mg/kg) (■) or saline (□) on blood glucose in KKA y -CETP mice. Values are given as means $\pm$ SEM, $n = 24$. All values except day 1 are lower than the corresponding control with at least $p < 0.05$

(Fig. 2). This anti-hyperglycaemic effect was most pronounced on the second day and remained decreased over time. Body weight gain and food intake were reduced ($p < 0.001$ and $p < 0.05$, respectively) in mice treated with 500 mg/kg AICAR (Table 1).

Plasma samples from AICAR- or saline-treated mice obtained 24 h after the last dosing were analysed for concentration of glucose, insulin, NEFA, triglycerides, total cholesterol and the VLDL, LDL, and HDL cholesterol (Tables 1 and 2). Seven days of repeated AICAR treatment reduced plasma insulin concentrations in a dose dependent manner, at doses of and above 300 mg/kg. However, treatment with 500 mg/kg AICAR increased the plasma NEFA ($p < 0.01$) and triglyceride ($p < 0.05$) concentration (Table 2). In contrast the total plasma cholesterol and LDL concentration did not change, HDL cholesterol was decreased ($p < 0.01$) and VLDL cholesterol was increased ($p < 0.01$). AICAR treatment im-

Table 1. Effect of 7-day AICAR treatment on animal characteristics and blood chemistry

	Body weight gain (g)	Food intake (g)	Plasma glucose (mmol/l)	Plasma insulin (ng/ml)	Urine glucose (mmol/l)	Urine volume (ml/24 h)
Saline	-1.1 ± 0.3	43.9 ± 1.3	14.6 ± 1.1	51.1 ± 4.1	145.6 ± 75.0	4.5 ± 1.0
100 mg/kg	-1.2 ± 0.6	44.6 ± 2.2	18.7 ± 2.1	54.6 ± 5.0	ND	ND
300 mg/kg	-0.5 ± 0.5	37.2 ± 4.3	16.3 ± 2.3	33.0 ± 5.0*	ND	ND
500 mg/kg	-2.2 ± 0.3***	38.3 ± 2.0*	11.0 ± 0.8**	21.2 ± 5.8***	33.2 ± 27.4	1.8 ± 0.2*

Diabetic KKA^y-CETP mice received a daily injection of AICAR or saline for 7-days. Data are given as means ± SEM, *n* = 8

**p* < 0.05

***p* < 0.01

****p* < 0.001 when compared with saline treated mice

ND not measured

Table 2. Effect of 7-day AICAR treatment on blood lipid profile

	Triglyceride (mmol/l)	NEFA (mmol/l)	VLDL (mmol/l)	LDL (mmol/l)	HDL (mmol/l)	Cholesterol (mmol/l)
Saline	5.6 ± 0.5	161.4 ± 33.8	0.6 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	1.9 ± 0.2
100 mg/kg	6.4 ± 1.3	145.4 ± 34.8	nd	ND	ND	ND
300 mg/kg	7.5 ± 1.1	187.4 ± 20.5	nd	ND	ND	ND
500 mg/kg	7.4 ± 0.6*	298.7 ± 23.8**	1.1 ± 0.2**	1.0 ± 0.4	0.4 ± 0.0***	2.6 ± 0.3

Data are given as means ± SEM, *n* = 8

**p* < 0.05

***p* < 0.01

****p* < 0.001 when compared with saline-treated mice

ND, not measured

Table 3. Glucose production and glucose clearance rate after 90 min AICAR infusion (i. v.) during the euglycaemic clamp

Parameters	Vehicle-infused	AICAR-infused
Plasma glucose (mmol/l)	13.7 ± 2.1	14.2 ± 2.3
Plasma insulin (ng/ml)	4.4 ± 0.2	4.5 ± 0.5
Glucose clearance rate (mg · kg ⁻¹ · min ⁻¹)	13.4 ± 1.2	17.3 ± 2.7
Endogenous glucose production (mg · kg ⁻¹ · min ⁻¹)	13.0 ± 4.4	6.4 ± 7.2*

Data are given as means ± SEM, *n* = 6

**p* < 0.05 when compared with the saline-infused condition in the same group of mice

proved glucose tolerance (*p* < 0.05) at 30 min (Fig. 3A). The insulin tolerance in AICAR-treated mice was also (*p* < 0.05) improved at 30 and 60 min after the insulin injection (Fig. 3B).

Effect of 7-day AICAR-treatment on skeletal muscle glucose uptake. The effect of the 7-day AICAR treatment on skeletal muscle glucose uptake was examined in vitro (Fig. 4). Although AICAR treatment resulted in a significant decrease in blood glucose, we did not observe any marked difference in glucose uptake under basal or insulin-stimulated conditions compared with those of vehicle-treated mice.

Effect of 7-day AICAR-treatment on endogenous glucose production. In vivo glucose clearance and endogenous glucose production were studied under clamp

conditions. Under euglycaemic conditions, intravenous infusion of AICAR for 90 min did not increase glucose clearance but did reduce endogenous glucose production by 80 % (Table 3). Plasma insulin concentration was not altered after 90 min of intravenous infusion of AICAR. The euglycaemic-hyperinsulinaemic clamp was utilised to evaluate the chronic effect of AICAR on insulin sensitivity. Insulin-stimulated glucose clearance was similar between AICAR-treated and vehicle-treated mice. The inhibitory effect of insulin on endogenous glucose production was more pronounced in AICAR-treated than in saline-treated mice (85 % vs 35 % inhibition of basal values, *p* < 0.05) (Table 4).

Discussion

We have examined the effects of acute and 7-day AICAR treatment on glucose homeostasis in KKA^y-CETP mice, a model of Type II diabetes mellitus, characterised by obesity and dyslipidaemia. AICAR treatment for 7 days decreased hyperglycaemia in KKA^y-CETP diabetic mice. However, a concomitant increase of plasma concentration of NEFA, triglycerides and VLDL were observed. The blood glucose-lowering effect after 7 days AICAR-treatment is likely caused by reduced endogenous glucose production, since insulin-stimulated skeletal muscle glucose uptake was not altered. Numerous in vitro studies show that AICAR stimulates AMPK activity in skeletal muscle and increases glucose transport [1]. However, this is not a universal finding because in isolated

Fig.3A, B. Glucose and insulin tolerance tests in KKA^y-CETP mice. KKA^y-CETP mice were treated for 7 days with AICAR (■) or saline (□). **A** OGTT (3 mg/kg) **B** Insulin tolerance test (ITT) (10 U/kg). Values are means $\pm$ SEM, $n = 8$, * $p < 0.05$

slow-twitch oxidative soleus muscle from rats, in vitro AICAR exposure does not increase glucose uptake or AMPK activity [20], suggesting muscle-fibre-type-specific responses. Nevertheless, in vivo infusion of AICAR in conscious Sprague-Dawley rats for 75 min increases AMPK activity, pyruvate and lactate concentration and glucose uptake in fast-twitch glycolytic skeletal muscle [11]. In our study, 7-day AICAR treatment reduced blood glucose concentration in diabetic KKA^y-CETP mice. However, AICAR-treatment was without effect on basal and insulin-stimulated glucose uptake in isolated soleus and EDL muscle from KKA^y-CETP mice, consistent with our findings in *ob/ob* mice [21].

To further analyse the possible mechanism by which AICAR treatment improves blood glucose concentration in severe diabetes, we examined the effect of AICAR on glucose disposal and endogenous glucose production in vivo using conscious, unrestrained KKA^y-CETP mice. When animals were

Fig.4A, B. Effect of 7-day treatment with AICAR (■) or saline (□) on basal and insulin-stimulated glucose uptake in isolated soleus (**A**) and EDL (**B**) skeletal muscle measured in vitro. Values are means $\pm$ SEM, $n = 6$

studied at ambient glucose concentration, a 90-min AICAR infusion did not increase glucose clearance but reduced endogenous hepatic glucose production. The 7-day AICAR treatment did not improve insulin-stimulated glucose disposal but enhanced the inhibitory effect of insulin on endogenous glucose production. Thus, the anti-hyperglycaemic effect of AICAR treatment is likely to be caused by the reduction of endogenous glucose production, rather than by increased skeletal muscle glucose uptake.

AICAR represses gene transcription of PEPCK and glucose-6-phosphatase in hepatoma cells [22]. Both enzymes play key roles in hepatic gluconeogenesis. AICAR also inhibits gluconeogenesis in isolated rat hepatocytes [23]. Furthermore, AICAR infusion in Sprague-Dawley rats substantially inhibits hepatic glucose production [11]. Our finding of an inhibitory effect of AICAR on glucose production is consistent with earlier studies [11, 23] and confirms that AICAR inhibits glucose production in a diabetic animal model. We cannot exclude the possibility that the reduc-

Table 4. Effect of 7-day AICAR treatment on glucose clearance and endogenous glucose production as assessed by the euglycaemic-hyperinsulinaemic clamp

Parameters	Vehicle		7-day AICAR treatment	
	Basal	Hyperinsulinaemia	Basal	Hyperinsulinaemia
Plasma glucose (mmol/l)	5.4 $\pm$ 0.3	6.1 $\pm$ 0.7	7.0 $\pm$ 0.8	7.5 $\pm$ 1.2
Plasma insulin (ng/ml)	12.3 $\pm$ 4.8	143.9 $\pm$ 13.5**	8.8 $\pm$ 1.0	129.8 $\pm$ 20.8**
Glucose clearance rate ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	18.7 $\pm$ 1.3	41.4 $\pm$ 4.9**	21.6 $\pm$ 3.0	46.8 $\pm$ 9.7**
Endogenous glucose Production ($\text{mg} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	17.9 $\pm$ 1.4	11.8 $\pm$ 2.3*	25.1 $\pm$ 2.6	3.7 $\pm$ 4.6*

Data are given as means $\pm$ SEM, $n = 8$

* $p < 0.05$, ** $p < 0.01$ when compared with the basal condition in the same group of mice

tion of food intake, in connection with AICAR treatment, might have influenced the effect on hepatic glucose production. In this study, food intake was reduced 13 % in diabetic KKA^y-CETP mice treated with 500 mg/kg AICAR for 7 days. However, this modest decrease in food intake is not likely to fully account for the 50 % reduction in endogenous glucose production observed during *in vivo* AICAR exposure. In addition, the AICAR effect on food intake seems to be a species-specific effect, since food intake and body weight are not altered in rats treated with a 1 g/kg AICAR for 4 weeks [24].

AICAR-treatment did not affect *in vitro* glucose uptake in skeletal muscle or *in vivo* glucose disposal. The explanation for the apparent difference between the present and published [10, 11, 25, 26] findings is not known. Possibly, higher doses of AICAR or long-term treatment regime (> 7 days) could be required to correct insulin action in skeletal muscle in diabetic mice. However, in our studies with *ob/ob* mice [21], higher doses of AICAR (1000 mg · kg⁻¹ · day⁻¹ for 7 days) did not improve *in vitro* insulin action in skeletal muscle. In both *ob/ob* [21] and KKA^y-CETP mice, AICAR treatment worsened the blood lipid profile. Since dyslipidaemia is associated with insulin resistance [27], worsening of the lipid profile could prevent improvements in insulin action in skeletal muscle. For example, increased NEFA via a protein kinase C- γ -mediated mechanism can reduce the ability of insulin receptor substrates to activate PI 3-kinase, a key signal for insulin-stimulated glucose uptake in skeletal muscle [27]. In *ob/ob* mice, AICAR treatment for 7 days did not improve insulin-stimulated PI 3-kinase activity in skeletal muscle despite a profound decrease in blood glucose [21]. This could explain, at least to some degree, why AICAR-treatment does not effect glucose disposal *in vivo*.

Neither basal nor insulin-stimulated glucose transport was altered in skeletal muscle from KKA^y-CETP diabetic mice after 7-day AICAR treatment. This finding is consistent with our previous observations for *ob/ob* mice treated with 1 g/kg AICAR for 7 days [21]. The lack of any improvement on glucose transport in either KKA^y-CETP or *ob/ob* diabetic mice after AICAR treatment is intriguing, since glucose homeostasis is improved. Thus, one could argue that AICAR did not effectively penetrate the skeletal muscle. However, AICAR treatment for 7 days in *ob/ob* mice resulted in marked increases in protein expression of GLUT 4 and Hexokinase II, as well increased MEF2 DNA binding activity [21]. Furthermore, 5 days of AICAR treatment in rats increased in GLUT4 protein expression and hexokinase activity in skeletal muscle [28]. Collectively, these studies suggest that AICAR can penetrate skeletal muscle.

Dyslipidaemia plays a crucial role in the development of Type II diabetes and associated complications [15]. Thus, reducing plasma NEFA and triglyc-

eride concentration and increasing the plasma HDL-to-VLDL ratio, are important goals in diabetes therapy. From *in vitro* studies, AICAR has been reported to decrease NEFA and triglyceride synthesis and to increase NEFA oxidation and ketogenesis in hepatocytes [1, 28]. In adipose tissue, AICAR inhibits lipogenesis and lipolysis [13, 29]. These acute effects should reduce NEFA and triglyceride concentrations in plasma. However, the opposite was observed following 7-day AICAR treatment of KKA^y-CETP diabetic mice. This led us to re-evaluate the effect of AICAR on lipid metabolism. Firstly, AICAR is a non-specific AMPK activator [30] and long-term exposure to AICAR could trigger effects other than activation of AMPK in either liver or adipose tissue and thus affect plasma lipid mobilisation. Secondly, the increased plasma concentration of NEFA and triglycerides and the decrease in HDL cholesterol, could be caused by a compensatory response induced by AICAR. AMPK is primarily regulated by a change in the intracellular AMP-to-ATP ratio. An increase in AMP, coupled with a decrease in ATP, activates the system through binding of AMP to the upstream kinase AMPK kinase and to the downstream kinase AMPK [31, 32]. Under physiological conditions, an increased ratio of AMP-to-ATP favours energy depletion and AMPK activation initiates a series of compensatory changes to maintain cellular ATP concentration and to meet energy demands [33, 34]. When diabetic mice were exposed to AICAR for 7 days, this could trigger lipid mobilisation as an energy supply and thus increase NEFA and triglyceride concentration in blood, as is often observed in the food-restricted diabetic mice [35].

Type II diabetes is a heterogeneous metabolic disorder; consequently a multitude of intervention strategies need to be applied to achieve good glucose control. We have raised important issues concerning the use of AICAR in improving metabolic control in Type II diabetes mellitus. Clearly, a more specific AMPK activator should be developed. Furthermore, a better understanding of the molecular mechanisms by which exercise improves glucose homeostasis is needed. By identifying the molecular mechanisms controlling insulin sensitivity it will be possible to develop novel pharmacological and physiological (exercise and diet) intervention strategies aimed to improve glucose homeostasis.

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