# Effect of long-term treatment with a small-molecule glucokinase activator on glucose metabolism, lipid profiles and hepatic function

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## ABSTRACT

We investigated the long-term effect of a glucokinase (GK) activator (GKA) on the changes in hepatic gene expression, glucose metabolism, lipid profiles and hepatic function in wild-type mice and the haploinsufficiency of β-cell-specific GK mice on a high-fat (HF) diet. Twenty weeks of GKA treatment had no effect on hepatic GK activity or expression of genes related to glucose or lipid metabolism, suggesting that chronic GK activation by GKA showed a sustained reduction of ambient blood glucose levels without causing significant impact on hepatic lipid and glucose metabolisms. Furthermore, GKA exerted glucose-lowering efficacy lasted for up to 40 weeks without increasing bodyweight or exerting adverse effects on lipid metabolism or hepatic function in either genotype on the HF diet. The present results show that GKA is capable of chronically improving glucose metabolism in mice on the HF diet without exerting a harmful influence on their lipid profile or hepatic function. (J Diabetes Invest, doi: 10.1111/j.2040-1124.2011.00104.x, 2011)

## INTRODUCTION

The predominant role of glucokinase (GK) in glucose sensing and its dual role in β-cells and hepatocytes have made glucokinase a promising drug target for diabetes therapy[1]. Since the first report by Grimsby *et al.* in 2003[2], several GK activators (GKAs) have been developed and have been shown to lower blood glucose in several animal models of type 2 diabetes and in initial trials in humans with type 2 diabetes[3].

We recently showed that a GKA improved glucose tolerance in wild-type mice and mice that were haploinsufficient for β-cell-specific GK (*Gck*^{+/−}) after 20 weeks on a high-fat (HF) diet, and we confirmed that both insulin secretion by pancreatic β-cells and glucose utilization in the liver were increased, and that the GKA had no adverse effects on lipid metabolism, hepatic function or steatosis in these mice[4]. However, because overexpression of GK increases hepatic lipogenesis and circulating lipids in animals[5], GKA might exert a harmful influence on lipid profiles and hepatic function after administration for more than 20 weeks. Furthermore, because the improvement in glycemic control when type 2 diabetes is treated with oral antihyperglycemic agents is often lost over time[6], the long-term efficacy of GKA should also be investigated. We therefore investigated the changes in the expression of genes related to glucose and lipid metabolism in the liver as a result of treatment with GKA in wild-type and *Gck*^{+/−} mice for 20 weeks and the effect of GKA on the glucose metabolism, lipid profiles and hepatic function of these mice after 40 weeks on the HF diet.

## MATERIALS AND METHODS

### Chemicals

A GKA (3-[(1S)-2-hydroxy-1-methylethoxy]-5-[4-(methylsulfo-nyl)phenoxy]-N-1,3-thiazol-2-ylbenzamide) was prepared by Tsukuba Research Institute, Banyu Pharmaceutical Co., Ltd. (Tsukuba, Japan) as described previously[7].

### Animals

*Gck*^{+/−} mice were generated as described previously[4,8]. Both wild-type and *Gck*^{+/−} mice were fed standard chow until 8 weeks-of-age, and then, given free access to either a HF diet or a HF diet containing GKA, which resulted in the formation of four groups: wild-type mice fed the HF diet (WT group), wild-type mice treated with 0.04% GKA mixed in the HF diet (WT + GKA group), *Gck*^{+/−} mice fed the HF diet (Gck group) and *Gck*^{+/−} treated with 0.04% GKA mixed in the HF diet (Gck + GKA group). All experiments were carried out on male littermates. The mice were maintained by means of standard animal care procedures based on the institutional guideline.

### Diet Protocol

The composition of the HF diet (High Fat Diet 32; Clea Japan, Tokyo, Japan) has been described previously[4,8]. GKA was added to the HF diet at 0.04% (wt/wt), as described previously[4].

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Figure 1 | Impact of long-term administration of glucokinase (GK) activator (GKA) on glucose and lipid metabolism in the liver. (a) GK activity after 20 weeks in the wild-type (WT) group (filled bar), WT + GKA group (striped bar), haploinsufficient for β-cell-specific GK (Gck) group (open bar) and Gck + GKA group (dotted bar), ($n$ = 9–11). Expression of genes associated with (b) glucose metabolism, (c) fatty acid synthesis and cholesterol metabolism, (d) and fatty acid oxidation and others in the four groups. The mRNA levels were measured by real-time quantitative polymerase chain reaction. Data were normalized to rRNA expression and are expressed as fold changes relative to the levels in the WT for 20 weeks ($n$ = 5–11). Values are means ± SE. ACC1, 2, acetyl-CoA carboxylase-1,2; ACO, acyl-CoA oxidase; AMPK, AMP activated kinase; CPT1, carnitine palmitoyltransferase 1; FAS, fatty acid synthase; G6Pase, glucose-6-phosphatase; GP, glycogen phosphorylase; GS, glycogen synthase; HMGCoA, hydroxymethylglutaryl-CoA; MCD, malonyl-CoA decarboxylase; PEPCK, phosphoenolpyruvate carboxykinase; PK, pyruvate kinase; SREBP1c, sterol regulatory element-binding protein-1c; UCP2, uncoupling protein 2.

## Measurement of Glucokinase Activity

GK activity in the livers of the mice in the WT group, WT + GKA group, Gck group and Gck + GKA group was measured under fed conditions by continuous spectrometric assay, as described previously[9], except that an excess amount of GKA (10 μmol/L) was added in the assay to avoid the potential for remaining GKA in the liver extract. Therefore, the Vmax value obtained from the assay can interpret into the protein amount of GK in the liver extract.

## RNA Preparation and Real-time Quantitative Polymerase Chain Reaction

Under fed conditions, total liver RNA was extracted with an RNeasy Mini kit (Qiagen, Hilden, Germany) and cDNA was generated by using TaqMan Reverse Transcription Reagents (Applied Biosystems, Foster City, CA, USA). Real-time quantitative polymerase chain reaction was carried out by using Applied Biosystems 7900HT (Applied Biosystems).

## Measurement of Biochemical Parameters

Blood glucose was measured with a portable glucose meter and Glutest Neo (Sanwa Chemical Co., Nagoya, Japan). Plasma alanine aminotransferase, free fatty acids, total cholesterol and triglycerides were assayed by enzymatic methods (Wako Pure Chemical Industries Ltd., Osaka, Japan).

## Statistical Analysis

Individual comparisons among the four groups were made by using the post-hoc Fisher's PLSD test, and $P$-values <0.05 were considered statistically significant. Because the numbers of killed mice were variable among the groups and the amount of cDNA obtained from liver tissues was small in certain mice, some analyses or comparisons among groups have different numbers.
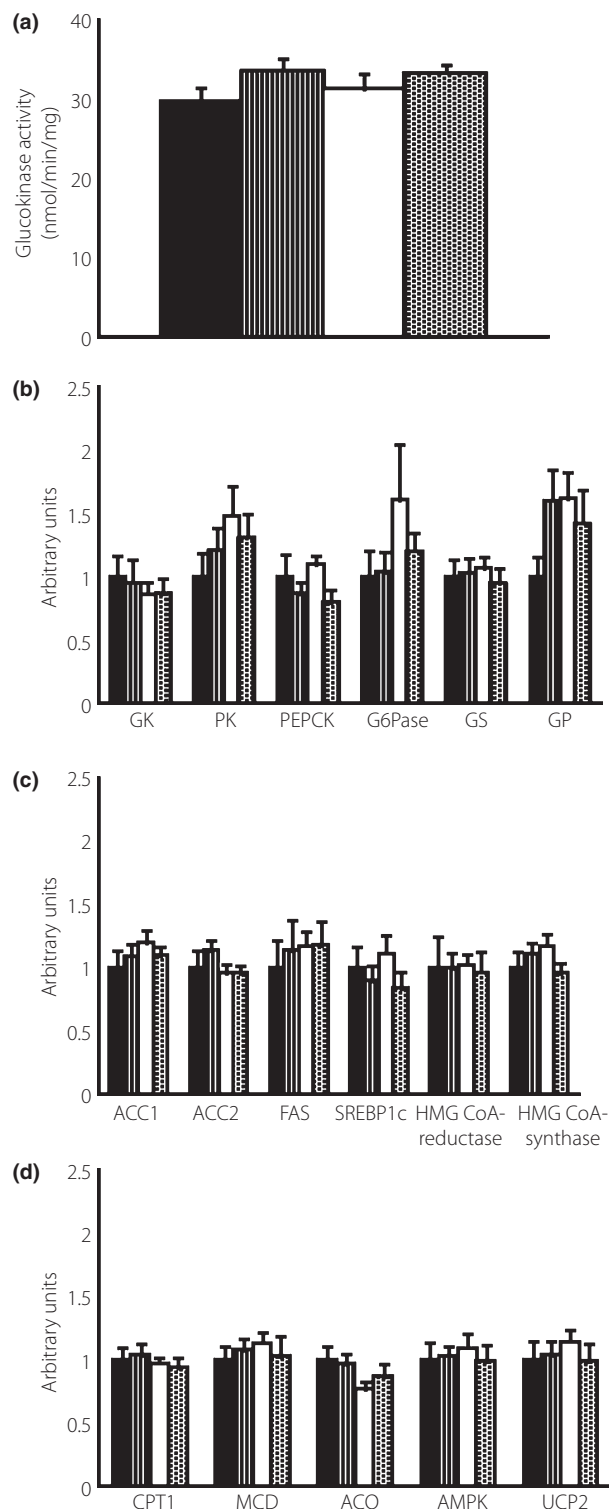
## RESULTS

First, we evaluated hepatic GK activity after 20 weeks of either treatment. The measurements of GK activity in the present study reflected the amount of GK protein, because they were made in the presence of 50 mmol/L glucose, which activated

**Table 1** | Impact of long-term (40 weeks) administration of glucokinase activator on bodyweight, blood glucose, lipid profiles and hepatic function among the four groups of mice

|  | WT | WT + GKA | Gck | Gck + GKA |
|---|---|---|---|---|
| Bodyweight (g) | 59.0 ± 3.3 | 56.1 ± 0.9 | 53.9 ± 1.8 | 59.9 ± 1.9 |
| Fed blood glucose (mg/dL) | 120.0 ± 3.6 | 70.3 ± 6.2* | 238.5 ± 13.3* | 112.0 ± 12.1# |
| Fasting blood glucose (mg/dL) | 55.7 ± 3.4 | 49.8 ± 4.7 | 94.6 ± 4.7* | 111.3 ± 20.1 |
| Alanine aminotransferase (IU/L) | 90.0 ± 18.4 | 88.3 ± 20.3 | 122.4 ± 30.6 | 104.1 ± 11.0 |
| Free fatty acid (mEq/L) | 1.63 ± 0.12 | 1.63 ± 0.15 | 1.85 ± 0.06 | 2.12 ± 0.32 |
| Total cholesterol (mg/dL) | 283.3 ± 44.0 | 302.3 ± 39.8 | 340.6 ± 30.5 | 333.1 ± 21.0 |
| Triglyceride (mg/dL) | 69.3 ± 12.2 | 58.1 ± 13.6 | 67.7 ± 10.3 | 90.9 ± 11.7 |

Values are means ± SE. *$P < 0.01$ vs wild-type (WT) and #$P < 0.01$ vs haploinsufficient for β-cell-specific glucokinase (Gck). GKA, glucokinase activator.

GK maximally. There were no differences in hepatic GK activity among the four groups (Figure 1a). Next, we investigated changes in gene expression levels in the liver. As shown in Figure 1b–d, the levels of mRNA expression by genes related to glucose metabolism, fatty acid synthesis, cholesterol metabolism, fatty acid oxidation and others were indistinguishable among the four groups.

After 40 weeks on the HF diet or HF diet containing 0.04% GKA, there were no differences in bodyweight among the four groups (Table 1). Fed blood glucose level in the WT + GKA group was significantly lower than in the WT group, and fed blood glucose level in the Gck + GKA group was significantly lower than in the Gck group, but a significant decrease in fasting glucose level was not observed by administration of GKA in both genotypes of mice. Next, we compared the plasma alanine aminotransferase, free fatty acid, total cholesterol and triglyceride levels (Table 1). There were no differences among the four groups.

## DISCUSSION

There has been concern about the possibility of long-term GK activation by small-molecule GKA having a harmful influence on lipid profiles and hepatic function, because long-term over-expression of hepatic GK by two- to five-fold had been found to increase hepatic lipogenesis and circulating lipids[5]. In contrast, overexpression of the GK transgene by as little as 50% can correct hyperglycemia without affecting plasma free fatty acid and hepatic triglyceride content, even under chronic HF diet fed conditions[10]. Thus, we have assessed the long-term effect of GKA treatment on lipid profiles and hepatic function in WT and *Gck*^{+/−} mice. As a result, long-term administration of GKA had no effect on these in our previous study[4] or in the present study (Table 1). To investigate the mechanism responsible for the difference between GK overexpression and GK activation by GKA, in the present study we examined hepatic GK activity and changes in gene expression after chronic treatment with GKA, but the results showed no effect on these parameters. Because GKA treatment should increase hepatic GK activity compared with non-treated animals during the treatment period, our data strongly suggest that chronic GK activation in the liver does not significantly alter hepatic function. Thus, GKA treatment had a beneficial effect without significant effects on the lipid profiles or hepatic function. Nevertheless, further studies are needed to clarify the changes in hepatic gene expression evoked by short-term administration of GKA.

GKA was able to improve glucose metabolism in mice even after 40 weeks on the HF diet. It has been reported that chronic treatment with glimepiride resulted in a loss of its glucose-lowering efficacy, whereas GKA retained its efficacy in Sprague–Dawley rats[11]. Also, when type 2 diabetes patients are treated with oral antihyperglycemic agents, improvements in glycemic control are often lost over time[6]. Because the attainment and maintenance of near-normal glycemia reduces the risk of long-term complications of diabetes, the results of the present study suggest that GKA has outstanding potential for the treatment of diabetes.

In conclusion, the results of the present study show that GKA was able to improve glucose metabolism in the mice on the HF diet in the long term without having any harmful effect on lipid profiles or hepatic function. GKA shows potential for improving the current drug treatment of type 2 diabetes.

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