ATP-sensitive potassium channels participate in glucose uptake in skeletal muscle and adipose tissue

TAKASHI MIKI, KOHTARO MINAMI, LI ZHANG, MIZUO MORITA, TOHRU GONOI, TETSUYA SHIUCHI, YASUHIKO MINOKOSHI, JEAN-MARC RENAUD, and SUSUMU SEINO

1Department of Cellular and Molecular Medicine, Graduate School of Medicine, 2Research Center for Pathogenic Fungi and Microbial Toxicooses, and 3Gene Research Center, Chiba University, Chiba 260-8670; 4Department of Medical Biochemistry, Ehime University School of Medicine, Ehime 791-0295, Japan; and 5Department of Cellular and Molecular Medicine, University of Ottawa, Ottawa, Ontario K1H 8M5, Canada

Received 15 July 2002; accepted in final form 3 August 2002

KATP CHANNELS are found in many tissues, including heart, pancreatic β-cell, pituitary, skeletal and smooth muscle, and brain, where they couple the metabolic state of the cell to the membrane potential (1, 3, 35). The channel is a heterooctameric complex of two subunits. A Kir6.1 or Kir6.2 subunit, belonging to the inwardly rectifying potassium channel subfamily Kir6.x, forms the potassium-conducting pore. A SUR1 or SUR2 subunit, belonging to the ATP-binding cassette (ABC) protein superfamily, confers various drug sensitivities (1, 3, 35). Several splice variants of SUR2, such as SUR2A and SUR2B, have been identified (6, 17, 18). Different combinations of the Kir6.x subunit (Kir6.1 or Kir6.2) and the SUR subunit (SUR1 or SUR2) comprise KATP channels with distinct nucleotide and pharmacological properties in native tissues (1, 3, 35). Kir6.2 and SUR1 constitute the KATP channel in pancreatic β-cells, substantia nigra (21, 40), and the ventromedial hypothalamus (VMH) (24). Co-expression of Kir6.2 plus SUR2A (17) and Kir6.2 plus SUR2B (18) elicits KATP channel currents resembling those in cardiac muscle and smooth muscle, respectively. Kir6.1 and SUR2B also comprise the KATP channels in vascular smooth muscle, which are activated by nucleoside diphosphates and are sensitive to sulfonylureas (41). Recently, disruption of Kir6.1 in mice has been shown to cause coronary vasospasm that mimics Prinzmetal angina in humans (26).

The KATP channel in skeletal muscles was first identified by Spruce et al. (37), and its pharmacological and electrophysiological properties have been examined in native skeletal muscles isolated from several vertebrates (2, 10). The electrophysiological properties of the KATP channels recorded in native skeletal muscles vary to some extent in sensitivities to ATP, glibenclamide, and K+ channel openers (2, 5, 10). These differences could be due to differences in experimental conditions, species, or even splice variants of SUR2 (6). One postulated role for the KATP channel is to control electrical activity of the cell membrane in response to alterations in the metabolic state of the muscle fibers. The channels become activated during metabolic inhibition, and opening of the channels inhibits massive depolarization of the cell membrane and shortens the duration of action potential (13). The channels are also activated during fatigue development (20). Activation
of the K\textsubscript{ATP} channels with pinacidil induces a faster decrease in tetanic force and reduces the development of resting tension during fatigue, whereas it improves force recovery after fatigue (23). These actions probably function to conserve energy (i.e., ATP) during fatigue or metabolic inhibition (13, 23). We generated Kir6.2-deficient [Kir6.2(−/−)] mice (25) and confirmed that the K\textsubscript{ATP} channels in skeletal muscle are involved in determining resting tension during fatigue development (11).

In addition to the role in contractile activity of skeletal muscle, K\textsubscript{ATP} channels also may be involved in glucose uptake in skeletal muscles. Sulfonilureas have been shown to improve glycemic control in patients with type 2 diabetes mellitus by acting on extrapancreatic tissues to reduce insulin resistance (22). However, no direct evidence has been reported of the involvement of skeletal muscle K\textsubscript{ATP} channels in the extrapancreatic effects of sulfonilureas. Using Kir6.2(−/−) mice, we found that the glucose-lowering effect of insulin is enhanced in Kir6.2(−/−) mice (25). The involvement of K\textsubscript{ATP} channels in glucose uptake was also indicated by SUR2-deficient [SUR2(−/−)] mice (7).

In the present study, we confirmed that there is no K\textsubscript{ATP} channel activity on the plasma membrane of skeletal muscles of Kir6.2(−/−) mice. We found both in vivo and in vitro insulin-stimulated glucose uptake to be significantly increased in specific skeletal muscles of Kir6.2(−/−) mice compared with WT mice. We also found that insulin-stimulated glucose uptake of adipose tissues in Kir6.2(−/−) mice is enhanced significantly in vivo but not in vitro. These data indicate that Kir6.2-containing K\textsubscript{ATP} channels are involved in glucose uptake in both skeletal muscles and adipocytes and that they are involved in the regulation of glucose uptake in skeletal muscles directly and in adipose tissues indirectly.

**MATERIALS AND METHODS**

**Animals.** Kir6.2(−/−) mice were generated as described previously (25). Animals were cared for according to guidelines established by the Animal Care Committees of Chiba and Ottawa Universities.

**Insulin Tolerance Test**

Various doses (0.05, 0.1, 0.2, 0.5, and 0.75 U/kg) of human insulin (Humulin R, Eli Lilly, Indianapolis, IN) were injected intraperitoneally in awake male mice (18–22 wk) after a 16-h fast. Blood samples were taken from the orbital sinus to determine blood glucose levels. Blood glucose levels were measured in whole blood with Antosense Glucose II (Sankyo, Tokyo, Japan) as previously described (25).

**Northern Blotting of Phosphoenolpyruvate Carboxykinase mRNA**

After food deprivation for 0, 6, 12, 18, or 24 h, 16-wk-old male mice were killed, and total RNA was extracted from the liver. Sixteen-week-old male mice were injected orally with 10 cal/kg of mixed meal (Twinline, Otsuka Pharmaceuticals, Tokushima, Japan), and total RNA of the liver was extracted 1 h after the meal. Ten micrograms of total RNA samples were fractionated by electrophoresis and subjected to Northern blot analysis. The [\textsuperscript{32}P]dCTP-labeled partial fragment (nt +121−+947) of mouse phosphoenolpyruvate carboxykinase (PEPCK) cDNA was used as the probe. The consistency of the relative amounts of RNA loaded into each lane was evaluated by estimating the 28S and 18S ribosomal RNA (not shown) and also by probing the blots with [\textsuperscript{32}P]dCTP-labeled human glyceraldehyde-3-phosphate dehydrogenase (GAPDH).

**Measurement of the Glycogen Content**

The glycogen content in liver was measured as follows. The liver was lysed in 0.6 ml of 1 N NaOH and heated for 45 min at 65°C. Twenty microliters of the solution was incubated for 30 min at 25°C in a buffer containing 1.14 mg/ml of amyloglucosidase. The glucose produced was measured by F-kit glucose (Roche Diagnostics, Basel, Switzerland). The protein was measured by Bradford’s method.

**Glucose Uptake in Vivo**

The experiment was started at 10:00 AM. To establish the rate constant of basal glucose uptake, male mice aged 20–24 wk were fasted for 16 h and injected via the caudal vein with 12.5 μCi of 2-deoxy-D-[\textsuperscript{3H}]glucose (2-[\textsuperscript{3H}]DG; Amersham Pharmacia Biotech, Buckinghamshire, UK) and 2.5 μCi of [\textsuperscript{14}C]glucose (Amersham Pharmacia Biotech) dissolved in 0.05 ml of saline. Blood was taken before and 0, 2, 5, and 10 min after injection of radioactive tracers. The mice were killed by cervical dislocation 20 min after injection, and skeletal muscles [extensor digitorum longus (EDL), soleus, and red and white part of gastrocnemius] and epidyndidal white adipose tissues (WAT) were rapidly dissected and weighed. The rate constant of the 2-[\textsuperscript{3H}]DG uptake was calculated as previously described (38). For measurement of insulin-stimulated glucose uptake, the fed mice were injected with 0.5 U/kg of human insulin intraperitoneally. Fifteen minutes after insulin injection, the injection of the radioactive tracers, dissection of the tissues, and calculation of the rate constant of 2-[\textsuperscript{3H}]DG uptake were done as in the basal glucose uptake measurement.

**Electrophysiological Recordings of Skeletal Muscles**

Muscle fibers were isolated from flexor digitorum brevis of 2-wk-old wild-type (WT) and Kir6.2(−/−) mice as described previously (12). The fibers were cultured for 1–3 days before recordings. K\textsuperscript{+} channel currents were recorded from the sarcolemma by patch-clamp technique in the excised inside-out membrane configuration (16). The pipette and bath solutions were the same as those described previously (16). K\textsubscript{ATP} channel recordings were made at a membrane potential of −60 mV.

**Glucose Uptake by Skeletal Muscles in Vitro**

Glucose uptake capacity by skeletal muscles in vitro was measured using 2-[\textsuperscript{3H}]DG, a nonmetabolized glucose marker as described by Hansen et al. (14) with a few modifications. Briefly, mice were fasted for 16 h before the uptake measurements. After excision, EDL and soleus muscles were incubated for 1 h in 1.8 ml of physiological saline solutions containing (in mM) 115.5 NaCl, 4.7 KCl, 2.4 CaCl\textsubscript{2}, 3.1 MgCl\textsubscript{2}, 25 NaHCO\textsubscript{3}, and 2 NaH\textsubscript{2}PO\textsubscript{4}, supplemented with 8 mM glucose or 2-DG, 32 mM mannitol, and 0.1% albumin (Sigma-Aldrich, St. Louis, MO). Solutions were continuously exposed to 95% O\textsubscript{2}-5% CO\textsubscript{2} to maintain pH at 7.4. 2-[\textsuperscript{3H}]DG uptakes were then measured by exposing muscles for 10 or 20 min to 2.0 μCi/ml of 2-[\textsuperscript{3H}]DG (Amersham Pharmacia Biotech, Buckinghamshire, UK) and 2.5 μCi/ml of [\textsuperscript{3H}]glucose (Amersham Pharmacia Biotech).
Insulin-Induced Glucose Uptake in Skeletal Muscles

1 ml of 6% PCA. Briefly, the fat was minced and promptly removed. Adipocytes were isolated by a protocol described above, except that muscles were exposed to 1, 100, or 300 U/ml of Humulin R for 15 min before addition of D-[U-14C]glucose. Glucose uptake was stopped by adding 13.3 μM cytochalasin B and 0.2 mM phloretin. After cell separation by spinning the suspension with oil, glucose uptake into the cells was measured by counting cell pellets solubilized in ACS II liquid scintillation analysis cocktail (Amersham Pharmacia Biotech).

RESULTS

Response of Kir6.2(−/−) Mice to Blood Glucose-Lowering Stress

Dose-response relationship of insulin-induced hypoglycemia. As we reported previously (25), the glucose-lowering effect of intraperitoneal insulin injection is significantly enhanced in Kir6.2(−/−) mice (Fig. 1A). The decrease in blood glucose levels was significantly larger in Kir6.2(−/−) than in WT mice, when 0.1, 0.2, or 0.5 U/kg of insulin was injected. Thus the differences between Kir6.2(−/−) and WT mice were apparent when the mice were injected with relatively low doses (0.1 or 0.2 U/kg) of insulin.

Effects of fasting on blood glucose and serum insulin levels. We then examined changes in blood glucose levels during food deprivation to determine whether a massive fall in blood glucose occurs during prolonged fasting in Kir6.2(−/−) mice. The blood glucose levels of Kir6.2(−/−) mice were significantly lower than those of WT mice at 6, 12, 18, and 24 h. In particular, Kir6.2(−/−) mice exhibited a rapid and severe fall in blood glucose levels between 6 and 12 h after food deprivation (Fig. 1B): blood glucose levels fell by 3.93 ± 0.52 mM in Kir6.2(−/−) mice (n = 8), whereas only a slight decrease was observed in WT mice (0.97 ± 0.51 mM, n = 8). After 9 h of fasting, the percent decrease in serum insulin levels was similar in Kir6.2(−/−) and WT mice, as the insulin levels decreased to 46.4 and 56.3% of those in the fed state, respectively (Kir6.2(−/−), fed state, 1,052 ± 104 ng/ml; 9-h fast, 487 ± 71 ng/ml, n = 8; WT, fed state, 1,997 ± 181 ng/ml; 9-h fast, 1,118 ± 190 ng/ml, n = 8).

Gluconeogenesis and Glycogenolysis in the Liver During Fasting

The liver is the major source of glucose during fasting, the glucose being produced from gluconeogenesis or glycogenolysis. We examined PEPCK (a key gluconeogenic enzyme) mRNA expression and glycogen...
content during fasting as markers of gluconeogenesis and glycogenolysis, respectively.

**PEPCK mRNA expression.** In WT mice, PEPCK mRNA expression in liver was markedly suppressed 1 h after the mice had ingested 10 cal/kg of mixed meal, whereas its expression gradually increased during food deprivation (Fig. 2). The increase in PEPCK mRNA expression levels during fasting was less in the fed state and after 6 h of fasting in Kir6.2(−/−) mice than in WT mice, whereas after extended fasting (longer than 12 h), the increase was comparable to that in WT mice. These results suggest that delayed increased PEPCK mRNA might have contributed to the early, abrupt drop in blood glucose levels after food deprivation in Kir6.2(−/−) mice.

**Liver glycogen content.** Because glycogenolysis is promoted during fasting, the glycogen content of the liver was measured before and after 24 h of food deprivation. The liver glycogen content of Kir6.2(−/−) mice fed ad libitum was similar to that of WT mice [Kir6.2(−/−), 246 ± 49 μg/mg protein; WT, 299 ± 77 μg/mg protein, n = 4] and decreased to almost the same extent as in controls during fasting [Kir6.2(−/−), 69 ± 14 μg/mg protein; WT, 56 ± 12 μg/mg protein, n = 4].

**Absence of the Sarcolemmal KATP Channel in Skeletal Muscles of Kir6.2(−/−) Mice**

To ascertain that Kir6.2 is the pore-forming subunit of the KATP channels in skeletal muscles, we carried out electrophysiological recordings by use of flexor digitorum brevis muscles of 2-wk-old mice. Figure 3A shows a representative example of single KATP channel current recorded from a muscle fiber isolated from WT mice. The channel activity was reversibly inhibited by bath application of 0.01 mM or higher concentrations of ATP. Similar KATP channel activity was observed in 35 of 38 patches excised from Kir6.2(+/+) flexor digitorum brevis muscle fibers, with channel density of 1–5 channels/patch (−1 μm of an opening diameter of the pipette tip). In contrast, no KATP current was detected in 20 patches excised from Kir6.2(−/−) muscle fibers (Fig. 3B). At positive holding membrane potentials, activity of Ca2+-activated K+ channels was frequently observed in both WT and Kir6.2(−/−) muscles in the presence of elevated cytoplasmic Ca2+, confirming that the membrane patches were excised in the inside-out configuration (data not shown).

**Glucose Uptake in Skeletal Muscle and Adipose Tissues**

**Glucose uptake by skeletal muscles and adipose tissues in vivo.** Glucose uptake in skeletal muscles and WAT was examined in vivo. Uptake was measured both in the basal state (after a 16-h fast and without exogenous insulin) and after intraperitoneal insulin injection (Fig. 4A). The basal uptake by EDL muscle and white and red gastrocnemius muscles in Kir6.2(−/−) was significantly greater than that in WT mice. The uptake by soleus muscle was, on the other hand, similar in WT and Kir6.2(−/−) mice. Insulin, at 0.5 U/kg body weight, significantly increased glucose uptake in all muscles. The insulin effect was significantly greater in Kir6.2(−/−) muscles than in WT muscles, especially in soleus and red gastrocnemius. Similarly, both the basal and insulin-stimulated glucose uptake in WAT of Kir6.2(−/−) mice was significantly greater than that in the WT tissues (Fig. 4B).

**Glucose uptake by skeletal muscles in vitro.** In vivo glucose uptake in skeletal muscle is affected not only by insulin but also by exogenous factors and muscle activity (15). To better understand the difference in basal and insulin-stimulated glucose uptake measured in vivo, we also measured glucose uptake in vitro. Basal glucose uptake was also determined with 8, 15, and 32 mM unlabeled 2-DG over a 20-min period. Basal glucose uptake of 2-DG was significantly greater

---

**Fig. 3.** Electrophysiological recordings of isolated skeletal muscles. Representative traces of inside-out patch clamp experiments of WT (A) and Kir6.2(−/−) (B) mice are shown.

**Fig. 4.** 2-Deoxy-[3H]glucose (2-[3H]DG) uptake in skeletal muscles (A) and adipose tissues (B) in vivo. Gastro, gastrocnemius; EDL, extensor digitorum longus; WAT, white adipose tissue. Basal (no insulin stimulation) and insulin-stimulated glucose uptake values in WT (open bars) and Kir6.2(−/−) mice (filled bars) are shown. Values are means ± SE of 4–7 independent experiments for each group. *P < 0.02, **P < 0.05.
2-DG concentration (32 mM) (Fig. 5A) uptake in soleus muscle of Kir6.2(−/−) and 1, 100 or 10,000 U/ml insulin. Values are means ± SE of 5 muscles isolated from WT (open bars) and Kir6.2(−/−) (filled bars) mice. *P < 0.05.

in Kir6.2(−/−) than in WT EDL muscle only at high 2-DG concentration (32 mM) (Fig. 5A). Basal glucose uptake in soleus muscle of Kir6.2(−/−) mice tended to be greater than that of WT mice (Fig. 5B), but the difference was not significant.

Insulin-induced glucose uptake of skeletal muscles in vitro. We determined whether the increased insulin-induced glucose uptake by skeletal muscle also occurs in vitro. Insulin at 100 and 10,000 U/ml increased 2-DG uptake in EDL and soleus muscle (Fig. 6, A and B). In soleus muscle, 100 U/ml of insulin increased 2-DG uptake in Kir6.2(−/−) more than in WT mice, whereas there was no difference in the insulin effect between Kir6.2(−/−) and WT mice at 10,000 U/ml (Fig. 6B). Contrary to the in vivo observations (Fig. 4A), the insulin effect in vitro was not different in Kir6.2(−/−) EDL than in WT muscle (Fig. 6A).

Glucose uptake of adipose tissues in vitro. Glucose uptake in adipocytes isolated from epididymal fat also was compared. Glucose uptake in the adipocytes of Kir6.2(−/−) and WT mice was similar in the presence of insulin at all concentrations from 1 to 300 μU/ml (Fig. 7).

DISCUSSION

K<sub>ATP</sub> channels are expressed in many cells and tissues, and they regulate various cellular functions by linking the metabolic state of the cell to membrane potential (1, 3, 35). We previously showed that the channel is critical in glucose-induced insulin secretion in pancreatic β-cells by use of transgenic mice expressing dominant-negative Kir6.2 specifically in pancreatic β-cells (27). The finding was further confirmed in Kir6.2-deficient [Kir6.2(−/−)] mice, in which the Kir6.2 gene, which encodes for the pore subunit of the β-cell K<sub>ATP</sub> channel, is disrupted, resulting in complete lack of K<sub>ATP</sub> channel activity (25).

Studies using Kir6.2(−/−) mice demonstrate not only the importance of the K<sub>ATP</sub> channel in glucose-induced insulin secretion but also that the glucose-lowering effect of insulin is significantly enhanced in mice lacking these channels (25). In the present study, we found that a relatively low dose (0.1 and 0.2 U/kg) of insulin lowered blood glucose levels in Kir6.2(−/−) mice significantly more than in WT mice (Fig. 1A). This study also shows that fasting leads to enhanced hypoglycemia in Kir6.2(−/−) mice compared with WT mice (Fig. 1B). Furthermore, analysis of glucose-responsive neurons in the VMH shows that Kir6.2(−/−) mice have a defect in glucose sensing during hypoglycemia, which leads to impaired glucagon secretion from the pancreatic α-cells under those conditions (24). Therefore, the enhanced effects of insulin and fasting on blood glucose levels may be due to 1) low capacity of the liver to produce glucose because of the lack of glucagon stimulation or 2) enhanced glucose uptake in peripheral tissues. Both possibilities were investigated.

Glucose Production During Hypoglycemia

Glucagon is known to increase glucose release from the liver by activating gluconeogenesis. The rate-lim-
Glucose Uptake in Skeletal Muscle and Adipose Tissues

Blood glucose levels during fasting are determined not only by glucose supply from the liver but also by glucose utilization in the peripheral tissues, including skeletal muscles and adipose tissues. Although K\textsubscript{ATP} channels are known to be present in skeletal muscles (37), there is no report of functional expression in adipose tissues (29). Electrophysiological recording clearly shows that Kir6.2-containing K\textsubscript{ATP} channels are present in skeletal muscles. The channel in skeletal muscles opens when the intracellular ATP concentration is decreased by metabolic inhibition (13) or muscle fatigue (20). Sulfonylureas such as glibenclamide and glimepiride (4, 9) have been used to investigate the role of the K\textsubscript{ATP} channels in glucose uptake in skeletal muscles, but whether or not the extrapancreatic effects of sulfonylureas also are mediated by closure of K\textsubscript{ATP} channels is controversial (8, 28).

The gene-targeting technique is the most straightforward strategy to clarify the involvement of molecules in cellular functions. Using Kir6.2-deficient mice, we found that basal glucose uptake in the skeletal muscles in vivo after a 16-h fast was significantly enhanced in Kir6.2\textsubscript{(-/-)} gastrocnemius and EDL muscles compared with those of WT mice but that it was not in soleus muscle (Fig. 4A). Similarly, in vitro basal glucose uptake was greater in Kir6.2\textsubscript{(-/-)} EDL muscle than in WT EDL muscle, but no difference was observed in the soleus muscles (Fig. 5). The effect of insulin on glucose uptake also was greater in Kir6.2\textsubscript{(-/-)} muscles than in WT muscles, except for the EDL muscle in vitro (Figs. 4 and 5). These results thus provide direct evidence for the involvement of the K\textsubscript{ATP} channel in glucose uptake in skeletal muscle, in agreement with previous findings in SUR2-deficient mice (7). This study, however, clearly shows that the effects of disruption of the K\textsubscript{ATP} channels on glucose uptake differ in muscles composed of different types of fibers. Although not observed in vivo, the EDL but not the soleus muscles of Kir6.2\textsubscript{(-/-)} mice showed enhanced capacity for basal glucose uptake compared with WT muscles. The enhanced insulin effect on glucose uptake in Kir6.2\textsubscript{(-/-)} mice was much greater in soleus than in EDL muscle both in vivo and in vitro, whereas in vivo, it was greater in red gastrocnemius than in white gastrocnemius.

Both soleus and red gastrocnemius muscles of both Kir6.2\textsubscript{(-/-)} and WT mice are composed primarily of type I and IIA fibers, which are slow-twitch oxidative fibers (36, 39, and M. Thabet and J.-M. Renaud, unpublished results), whereas white gastrocnemius and EDL muscles are composed primarily of type IIX and IIB fibers, which are fast-twitch glycolytic fibers (32, 36, and M. Thabet and J.-M. Renaud, unpublished results). Thus it appears that lack of K\textsubscript{ATP} channel activity affects insulin-sensitive glucose uptake to a greater extent in type I and IIA fibers than in type IIX and IIB fibers, although the basal glucose uptake capacity is increased more in the latter. It is interesting to note that the stimulatory effect of insulin on glucose transport is also greater in soleus than in EDL muscle (19).

We found that glucose uptake in Kir6.2\textsubscript{(-/-)} adipose tissues also is enhanced in vivo but not in vitro. Because K\textsubscript{ATP} channels have not been detected in adipocytes (29), the effect of increased glucose uptake in Kir6.2\textsubscript{(-/-)} adipocytes is most likely indirect.

Four mouse models lacking K\textsubscript{ATP} channels have been generated: Kir6.2\textsubscript{(-/-)} (25), SUR1\textsubscript{(-/-)} (35), SUR2\textsubscript{(-/-)} (7), and Kir6.1\textsubscript{(-/-)} (26). Although the glucose-lowering effect of a low dose of insulin is not increased in SUR1\textsubscript{(-/-)} (34), insulin sensitivity is enhanced in both SUR2\textsubscript{(-/-)} (7) and Kir6.2\textsubscript{(-/-)} (25) mice. When we consider these observations together with the findings of the present study, we can conclude that the Kir6.2/SUR2 channel apparently participates in glucose uptake in skeletal muscles directly and that the Kir6.2-containing channel is involved in glucose uptake in adipose tissues indirectly.

This work was supported by a Grant-in-Aid for Creative Scientific Research (10NP0201) and for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology, Japan; by a Scientific Research Grant from the Ministry of Health, Labour, and Welfare, Japan; and by grants from Novo Nordisk Pharma, Takeda Chemical Industries, the Yamanouchi Foundation for Research on Metabolic Disorders, and the National Science and Engineering Research Council of Canada.

Present address of Y. Minokoshi: Division of Endocrinology, Diabetes and Metabolism, Beth Israel Deaconess Medical Center and Department of Medicine, Harvard Medical School, Boston, MA 02215.

REFERENCES


