Effects of Stevioside on Glucose Transport Activity in Insulin-Sensitive and Insulin-Resistant Rat Skeletal Muscle

Narissara Lailerd, Vitoon Saengsirisuwan, Julie A. Sloniger, Chaivat Toskulkao, and Erik J. Henriksen

Stevioside (SVS), a natural sweetener extracted from *Stevia rebaudiana*, has been used as an antihyperglycemic agent. However, little is known regarding its potential action on skeletal muscle, the major site of glucose disposal. Therefore, the purpose of the present study was to determine the effect of SVS treatment on skeletal muscle glucose transport activity in both insulin-sensitive lean (*Fa/-*) and insulin-resistant obese (*fa/fa*) Zucker rats. SVS was administered (500 mg/kg body weight by gavage) 2 hours before an oral glucose tolerance test (OGTT). Whereas the glucose incremental area under the curve ($IAUC_{glucose}$) was not affected by SVS in lean Zucker rats, the insulin incremental area under the curve ($IAUC_{insulin}$) and the glucose-insulin index (product of glucose and insulin IAUCs and inversely related to whole-body insulin sensitivity) were decreased ($P < .05$) by 42% and 45%, respectively. Interestingly, in the obese Zucker rat, SVS also reduced the $IAUC_{insulin}$ by 44%, and significantly decreased the $IAUC_{glucose}$ (30%) and the glucose-insulin index (57%). Muscle glucose transport was assessed following in vitro SVS treatment. In lean Zucker rats, basal glucose transport in type I soleus and type IIb epitrochlearis muscles was not altered by 0.01 to 0.1 mmol/L SVS. In contrast, 0.1 mmol/L SVS enhanced insulin-stimulated (2 mU/mL) glucose transport in both epitrochlearis (15%) and soleus (48%). At 0.5 mmol/L or higher, the SVS effect was reversed. Similarly, basal glucose transport in soleus and epitrochlearis muscles in obese Zucker rats was not changed by lower doses of SVS (0.01 to 0.1 mmol/L). However, these lower doses of SVS significantly increased insulin-stimulated glucose transport in both obese epitrochlearis and soleus (15% to 20%). In conclusion, acute oral SVS increased whole-body insulin sensitivity, and low concentrations of SVS (0.01 to 0.1 mmol/L) modestly improved in vitro insulin action on skeletal muscle glucose transport in both lean and obese Zucker rats. These results indicate that one potential site of action of SVS is the skeletal muscle glucose transport system.

DIABETES MELLITUS is a group of metabolic diseases characterized by abnormally elevated levels of glucose in blood and urine. More than 90% of the cases of diabetes worldwide are classified as type 2 diabetes. The etiology of type 2 diabetes is complex and is associated with multiple defects, including impaired insulin secretion from pancreatic β cells and insulin resistance in peripheral tissues, primarily skeletal muscle.[1] Type 2 diabetes is a progressive disease, such that the initial development of hyperinsulinemia and skeletal muscle insulin resistance ultimately leads to a relative hypoinsulinemia and hyperglycemia. In order to regulate plasma glucose levels as close to normal as possible, dietary restrictions, exercise, and blood glucose–lowering agents are required. However, the present pharmacological approaches for the management of type 2 diabetes are far from ideal. Therefore, newer and safer agents for treatment of type 2 diabetes are needed. Currently, there is an enormous increase in the use of herbal and other alternative medicines for the treatment of diabetes.

Stevia rebaudiana is a shrub native to Brazil and Paraguay. The leaves from this plant contain a large amount of the glycoside stevioside (SVS), a noncaloric sweetener 300 times sweeter than sucrose. SVS is formed by 3 molecules of glucose and 1 molecule of steviol, a diterpenic carboxylic alcohol. Extracts from *Stevia rebaudiana* have been used for many years in South America in the treatment of diabetes. However, little is known regarding its action, especially in peripheral insulin-sensitive tissues, such as skeletal muscle. Several studies have reported that ingestion of extracts of *Stevia rebaudiana* causes a slight suppression of plasma glucose levels and a significantly increased glucose tolerance in normal adult humans.[2,3] Recently, Jeppesen et al[4] reported that SVS and steviol stimulate insulin secretion via a direct action on isolated β cells of rats. The insulinotropic action of SVS and steviol are independent of cyclic adenosine monophosphate and adenosine triphosphate-sensitive K^+ channel activity. This action is dependent on the prevailing glucose concentrations and diminishes when blood glucose levels decrease toward normal.[4] It is clear that additional potential mechanisms for the antihyperglycemic action of SVS and steviol need to be investigated.

The obese Zucker (*fa/fa*) rat is a well-defined animal model of glucose intolerance, dyslipidemia, and central obesity, and exhibits severe skeletal muscle insulin resistance attributable to defects in insulin signaling[5] and GLUT-4 glucose transporter protein translocation.[6,7] In order to evaluate the effect of SVS on whole-body and skeletal muscle insulin action in conditions of normal insulin sensitivity and insulin resistance, we examined the effect of in vivo and in vitro SVS treatment on glucose tolerance and skeletal muscle glucose transport activity in both insulin-sensitive lean Zucker rats and insulin-resistant obese Zucker rats.

From the Muscle Metabolism Laboratory, Department of Physiology, University of Arizona College of Medicine, Tucson, AZ; and the Department of Physiology, Faculty of Science, Mahidol University, Bangkok, Thailand.

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Address reprint requests to Erik J. Henriksen, PhD, Department of Physiology, Ina E. Gittings Building #93, University of Arizona, Tucson, AZ 85721-0093.

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MATERIALS AND METHODS

Animals

Female lean (*Fa/-*) and obese (*fa/fa*) Zucker rats (Harlan, Indianapolis, IN) were received at 11 to 12 weeks of age and weighed 150 to 170 g and 300 to 320 g, respectively, at the time of use. The animals were housed in a temperature-controlled room (20 to 22°C) at the Central Animal Facility of the University of Arizona. A 12:12-hour light-dark cycle (lights on 7 AM to 7 PM) was maintained. Animals had free access to water and chow (Purina, St Louis, MO). All procedures were approved by the University of Arizona Animal Use and Care Committee.

Oral Glucose Tolerance Test

Lean and obese Zucker rats were randomly divided into 2 groups: a vehicle-treated control group and an acute SVS-treated group. The rats were restricted to 4 g of chow after 6 PM of the evening before the oral glucose tolerance test (OGTT). At 8 AM on the day of the test, the rats were administered either 200 or 500 mg/kg body weight SVS by gavage. These doses conform to the estimated acceptable daily intake (ADI, which represents a level of daily intake that should result in no health hazard from a particular food additive) for rats, as calculated from the ADI of SVS in humans of 7.94 mg/kg/d,[8] and allowing for a 100-fold safety factor. These doses of SVS are only a small fraction of the LD_{50} for SVS (15 g/kg) reported for the rat.[9] Two hours after the oral adminstration of SVS, the rats were given a 1 g/kg body weight glucose load by gavage. Blood was drawn from a cut at the tip of the tail at 0, 15, 30, 60, and 120 minutes after the glucose feeding, thoroughly mixed with EDTA (18 mmol/L final concentration), and centrifuged at 13,000 × *g* to separate the plasma. The plasma was stored at −80°C and subsequently assayed for glucose (Sigma Chemical, St Louis, MO), insulin (Linco Research, St Charles, MO), and free fatty acids (FFA; Wako, Richmond, VA). Immediately after completion of the OGTT, each animal was given 2 mL of sterile 0.9% saline subcutaneously to compensate for plasma loss.

The incremental area under the curve for glucose ($IAUC_{glucose}$) or insulin ($IAUC_{insulin}$) was calculated as the integrated area under the respective curve above the basal (time 0) value over the 120-minute sampling period. The total area under the curve for glucose ($TAUC_{glucose}$) or insulin ($TAUC_{insulin}$) was calculated as the integrated area under the respective curve above zero over this period. The glucose-insulin index was calculated as the product of the respective glucose and insulin AUCs and is inversely related to whole-body insulin sensitivity.[10]

Measurement of In Vitro Glucose Transport Activity in Skeletal Muscle

At least 4 days separated the OGTT and the assessment of in vitro glucose transport activity. The determination of muscle glucose transport activity was initiated at 8 AM after an overnight food restriction as described above. On the day of the experiment, the animals were weighed and deeply anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg body wt). One soleus and both epitrochlearis muscles were dissected and prepared for in vitro incubation as described previously.[11] Whereas epitrochlearis muscles were incubated intact, soleus muscles were prepared into 2 strips (~25 to 30 mg) and incubated in the unmounted state. Each muscle was initially incubated for 1 hour at 37°C in 3 mL of oxygenated (95% O_2 to 5% CO_2) Krebs-Henseleit buffer (KHB) supplemented with 8 mmol/L glucose, 32 mmol/L mannitol, and 0.1 % bovine serum albumin (BSA; radioimmunoassay grade, Sigma Chemical) in the presence or absence of various concentrations of SVS. A stock of SVS was prepared in dimethyl sulfoxide (DMSO; Sigma Chemical), and the control buffer contained an equal final concentration of DMSO (<1%). For insulin-stimulated glucose transport experiments, muscles were incubated in the presence of a maximally effective concentration of insulin (2 mU/mL; Humulin R, Eli Lilly, Indianapolis, IN) with or without SVS.

After this initial incubation period, the muscles were rinsed for 10 minutes at 37°C in 3 mL of oxygenated KHB containing 40 mmol/L mannitol, 0.1 % BSA, and insulin or SVS, if previously present. Thereafter, the muscles were transferred to 2 mL of KHB containing 1 mmol/L 2-[1,2-^{3}H] deoxyglucose (2-DG, 300 μCi/mmol; Sigma Chemical), 39 mmol/L [U-^{14}C] mannitol (0.8 μCi/mmol; ICN Radiochemicals, Irvine, CA), 0.1% BSA, and insulin or SVS, if previously present. After this final 20-minute incubation, the muscles were removed, trimmed of excess fat and connective tissue, quickly frozen between aluminum blocks cooled to the temperature of liquid N_2, weighed, and dissolved in 0.5 mL of 0.5 mmol/L NaOH. After the muscles were completely solubilized, 5 mL of scintillation cocktail were added, and samples were analyzed for radioactivity in the ^{3}H and ^{14}C channels. The radioactivity in the ^{14}C channel and the specific activity of the incubation medium were used to determine the extracellular space, whereas the specific uptake of 2-DG was calculated by subtracting the ^{3}H activity in the extracellular space from the total ^{3}H activity in each sample. All values for in vitro 2-DG uptake are expressed as picomoles of 2-DG per milligram muscle wet weight per 20 minutes.

Table 1. Effects of Acute Oral SVS Treatment (500 mg/kg body weight) on Fasting Plasma Glucose, Plasma Insulin, and Plasma FFA Levels in Lean and Obese Zucker Rats

Group	Plasma Glucose (mg/dL)	Plasma Insulin (μU/mL)	Plasma FFA (mmol/L)
Lean + vehicle	92.6 ± 10.1	11.6 ± 0.9	0.84 ± 0.25
Lean + stevioside	93.1 ± 2.6	10.9 ± 0.5	0.82 ± 0.16
Obese + vehicle	113.0 ± 1.8	79.2 ± 10.1	1.97 ± 0.07
Obese + stevioside	117.0 ± 5.0	121.0 ± 20.4	1.85 ± 0.14

NOTE. Values are means ± SE for 5 animals per group.

Statistic Analysis

All values are expressed as means ± SE. The significance differences among experimental groups were tested by analysis of variance (ANOVA), or paired and unpaired Student's *t* test, as appropriate. A *P* value of .05 or less was considered statistically significant.

RESULTS

Plasma Glucose, Insulin, and FFAs

The fasting plasma levels of glucose, insulin, and FFA in the experimental groups are shown in Table 1. After the 2-hour feeding with SVS at 500 mg /kg body weight, plasma glucose, insulin, and FFA levels were not significantly different from vehicle-treated controls in the lean and obese groups, although the plasma insulin level in the SVS-treated obese animals tended to increase compared to the obese vehicle-treated control group. Treatment with 200 mg/kg body weight SVS likewise did not affect these fasting plasma variables (data not shown).

OGTT Responses

Treatment with 200 mg/kg body weight SVS did not alter the plasma glucose and insulin responses during OGTT in either the lean or obese Zucker rats (data not shown). The glucose and insulin responses during the OGTT after treatment with 500 mg/kg body weight SVS in lean and obese Zucker rats are

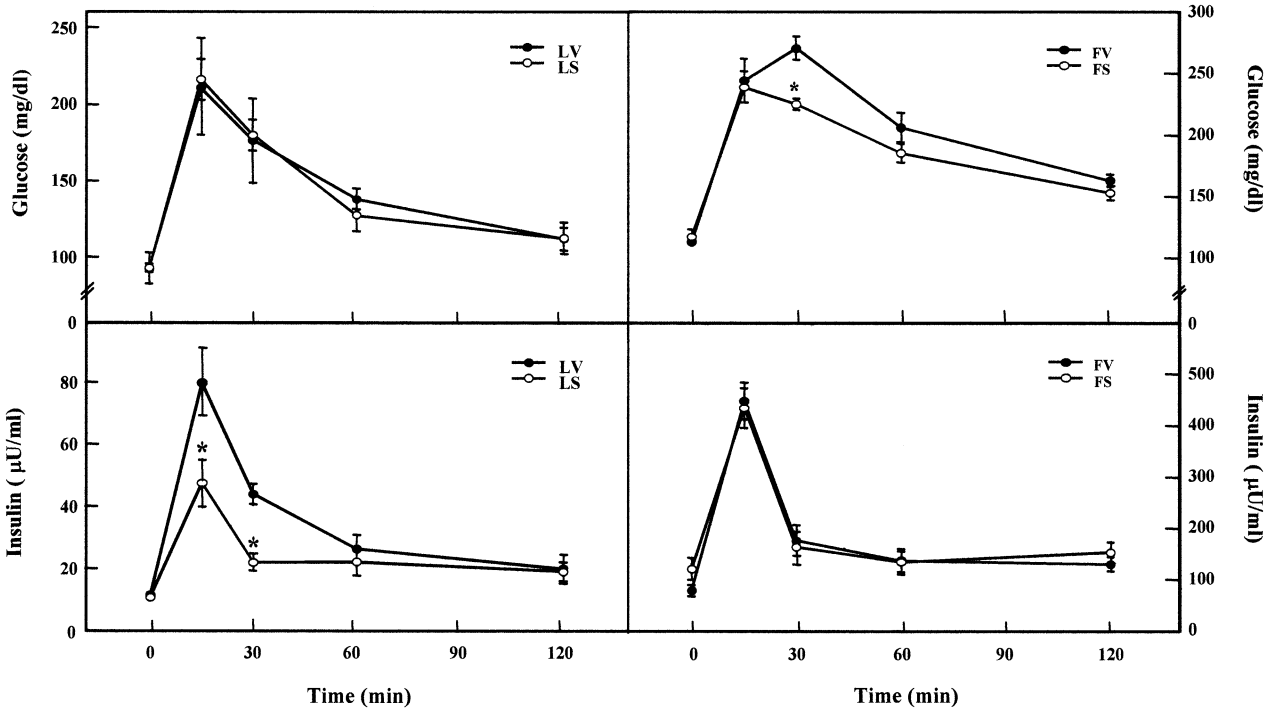

Fig 1. Glucose (top) and insulin (bottom) responses during an OGTT in lean Zucker rats (left panels) and in obese Zucker rats (right panels) after 2 hours treatment with SVS (500 mg/kg body weight). LV, lean vehicle-treated group; LS, lean SVS-treated group; FV, obese vehicle-treated group; FS, obese SVS-treated group. Values are means ± SE for 5 animals per group. *$P < .05$ *v* respective vehicle-treated control.

shown in Fig 1. SVS treatment had no effect on the plasma glucose response in lean Zucker rats, but it significantly reduced the plasma insulin values at 15 and 30 minutes compared to vehicle-treated controls. The $TAUC_{glucose}$ (Fig 2A) was therefore not affected by SVS treatment, whereas the $TAUC_{insulin}$ (Fig 2B) was decreased by 29% and the glucose-insulin index (Fig 2C) was reduced by 34% (both $P < .05$). The $IAUC_{glucose}$ (Fig 2D) in the lean group was likewise not altered by SVS, but the $IAUC_{insulin}$ (Fig 2E) and the glucose-insulin index using IAUCs were decreased by 42% and 45%, respectively, in these animals (Fig 2, lower panels).

As shown in Fig 1, the plasma insulin response in the obese Zucker rat was not altered by SVS treatment, whereas the plasma glucose response in obese SVS-treated animals was significantly reduced at 30 minutes after glucose load and tended to return to control levels after 120 minutes. The result-

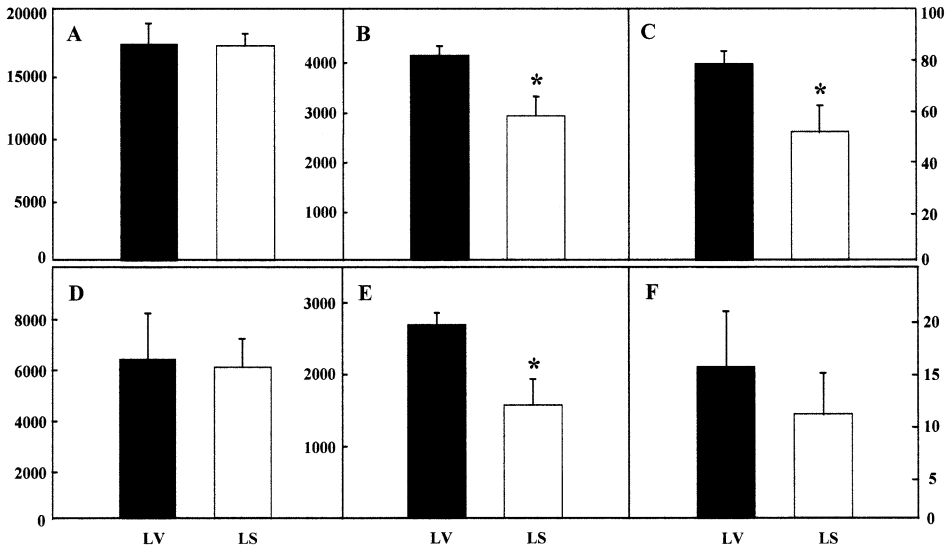

Fig 2. TAUCs (upper panels) and IAUCs (lower panels) for glucose (panel A for TAUC, panel D for IAUC; mg · dL^{-1} · min^{-1}) and insulin (panel B for TAUC, panel E for IAUC; μU · mL^{-1} · min$_{-1}$) during the OGTT and the glucose-insulin index (panel C from TAUC, panel F from IAUC; mg · dL^{-1} · min^{-1} × μU · mL^{-1} · min^{-1} × 10^{6}) in lean Zucker rats after 2 hours treatment with SVS (500 mg/kg body weight). Data for TAUC and IAUC were taken from Fig 1. Values are means ± SE for 5 animals per group. *$P < .05$ *v* LV.

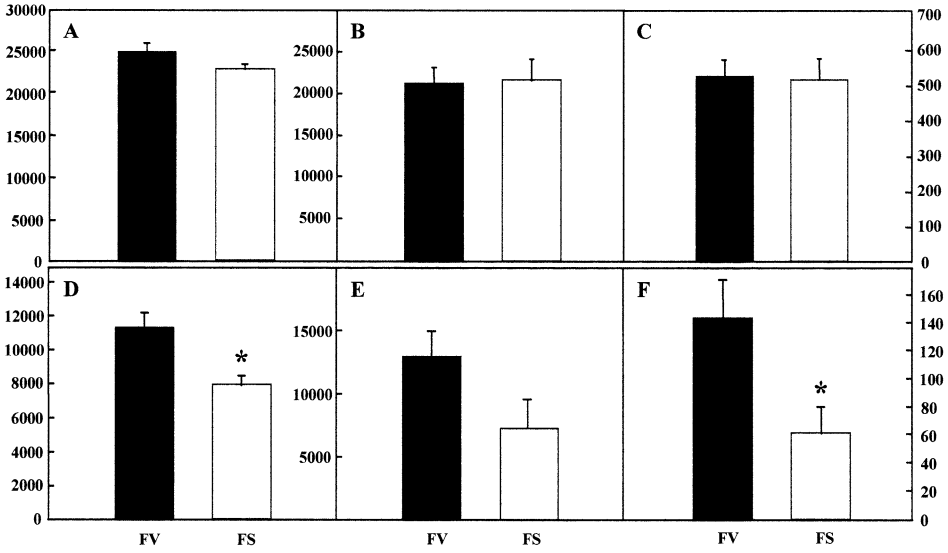

Fig 3. TAUCs (upper panels) and IAUCs (lower panels) for glucose (panel A for TAUC, panel D for IAUC; mg · dL^{-1} · min^{-1}) and insulin (panel B for TAUC, panel E for IAUC; μU · mL^{-1} · min^{-1}) during the OGTT and the glucose-insulin index (panel C from TAUC, panel F from IAUC; mg · dL^{-1} · min^{-1} × μU · mL^{-1} · min^{-1} × 10^{6}) in obese Zucker rats after 2 hours treatment with SVS (500 mg/kg body weight). Data for TAUC and IAUC were taken from Fig 1. Values are means ± SE for 5 animals per group. *$P < .05$ *v* FV.

ing $TAUC_{glucose}$ (Fig 3A) was slightly decreased (9%) in obese Zucker rats that received SVS. The $TAUC_{insulin}$ (Fig 3B) and the glucose-insulin index derived from the $TAUC_{glucose}$ and the $TAUC_{insulin}$ (Fig 3C) in the obese animals were not altered by SVS treatment when compared to vehicle-treated obese controls. However, reductions in the $IAUC_{glucose}$ (Fig 3D; 30%, $P < .05$), the $IAUC_{insulin}$ (Fig 3E; 44%, $P < .1$), and glucose-insulin index using IAUCs (Fig 3F; 57%, $P < .05$) were observed in the obese SVS treatment group.

Muscle Glucose Transport

To examine whether SVS had any effects on the skeletal muscle glucose transport system, basal and insulin-stimulated

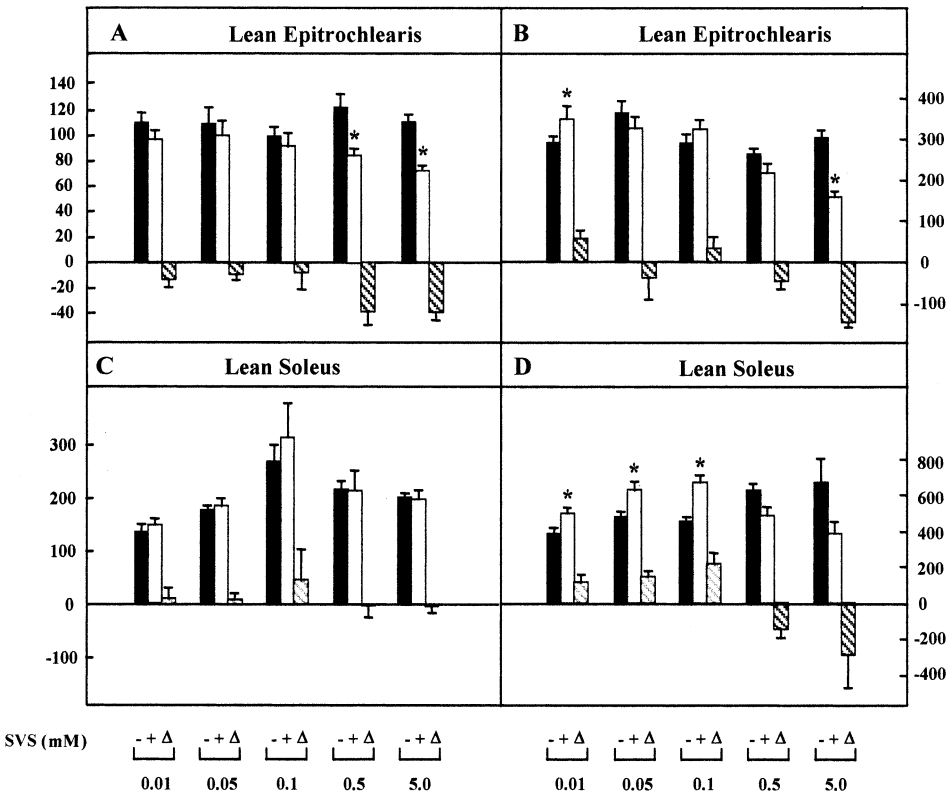

Fig 4. In vitro basal rates of 2-DG uptake in epitrochlearis (A) and insulin-stimulated rates of 2-DG uptake in epitrochlearis (B) and basal rates of 2-DG uptake in soleus (C) and insulin-stimulated rates of 2-DG uptake in soleus (D) muscles from lean Zucker rats in the presence or absence of various concentrations of SVS. Units are pmol/mg muscle/20 min. Values are means ± SE for 5 animals per group. *$P < .05$ *v* vehicle-treated control.

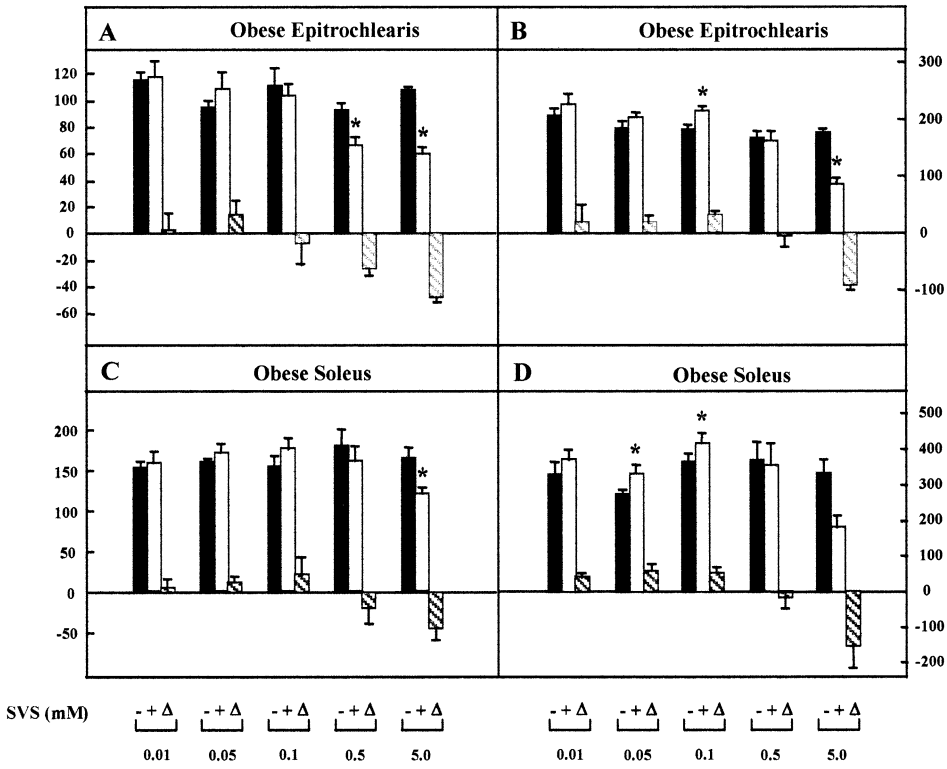

Fig 5. In vitro basal rates of 2-DG uptake in epitrochlearis (A) and insulin-stimulated rates of 2-DG uptake in epitrochlearis (B) and basal rates of 2-DG uptake in soleus (C) and insulin-stimulated rates of 2-DG uptake in soleus (D) muscles from obese Zucker rats in the presence or absence of various concentrations of SVS. Units are pmol/mg muscle/20 min. Values are means ± SE for 5 animals per group. *$P < .05$ *v* vehicle-treated control.

(2 mU/mL) 2-DG uptake in isolated type IIb epitrochlearis and type I soleus muscles were determined. As seen in Fig 4A, in epitrochlearis muscles from lean Zucker rats, low doses (0.01 to 0.1 mmol/L) of SVS showed little or no effect on basal 2-DG uptake, whereas higher doses of SVS (0.5 and 5.0 mmol/L) significantly decreased (31% to 34%; $P < .05$) basal 2-DG uptake. In contrast, 0.01 mmol/L SVS significantly enhanced the rate of insulin-stimulated 2-DG uptake in the epitrochlearis (20%, $P < .05$) (Fig 4B), whereas at 5.0 mmol/L, the rate of insulin-stimulated 2-DG uptake was significantly reduced (48%, $P < .05$) in this muscle (Fig 4B). In the soleus, the basal 2-DG uptake was not significantly affected by any dose of SVS (Fig 4C). Interestingly, the rate of insulin-stimulated 2-DG uptake was substantially increased by low doses (0.01 to 0.1 mmol/L) of SVS (29% to 48%, $P < .05$), but at 0.5 mmol/L or higher; this positive effect of SVS was no longer detected (Fig 4D).

Similar to the responses in lean Zucker rats, the basal 2-DG uptake in the epitrochlearis from obese Zucker rats was not altered by low doses of SVS (0.01 to 0.1 mmol/L). However, basal 2-DG uptake was again significantly decreased (29% to 45%, $P < .05$) at higher (>0.5 mmol/L) SVS concentrations (Fig 5A). Moreover, low doses of SVS (0.01 to 0.1 mmol/L) enhanced the rate of insulin-stimulated 2-DG uptake in the obese epitrochlearis, with a significant increase of 18% ($P < .05$) realized at 0.1 mmol/L (Fig 5B). SVS at 5.0 mmol/L significantly reduced (52%, $P < .05$) the rate of insulin-stimulated 2-DG uptake in this muscle (Fig 5B). In the obese soleus, SVS treatment had no effect on basal 2-DG uptake except 5.0 mmol/L SVS, where a significant reduction in basal 2-DG uptake was observed (Fig 5C). Importantly, the rate of insulin-stimulated 2-DG uptake was significantly enhanced in the obese soleus by low doses of SVS (0.05 to 0.1 mmol/L, $P < .05$) (Fig 5D).

DISCUSSION

The antihyperglycemic action of SVS has been the focus of several studies in recent years because of its potential of being a new intervention in the management of type 2 diabetes. It is generally accepted that a major defect in type 2 diabetes is tissue insulin resistance (especially muscle and liver), which develops initially and is followed by progressive deterioration of pancreatic β-cell function, leading to the hyperglycemic state.[12] Therefore, in the present study, we evaluated the effect of SVS on whole-body insulin sensitivity and skeletal glucose transport activity in the insulin-resistant obese Zucker rat, and compared these responses with those observed in the insulin-sensitive lean Zucker rat.

The results from this study have confirmed that insulin-resistant obese Zucker rat displays marked hyperinsulinemia, dyslipidemia, and glucose intolerance, compared to insulin-sensitive lean Zucker rat (Table 1 and Fig 1). Moreover, we found that the acute administration of SVS (500 mg/kg body weight) did not significantly affect fasting plasma glucose, insulin, and FFA levels in either lean or obese Zucker rats (Table 1). Our results are consistent with those of Suanarunsawat and Chaiyabutr,[13] who demonstrated that the plasma glucose level was not affected by SVS feeding in normal rats. The lack of an immediate effect of SVS in lowering the plasma

glucose and insulin levels is supported by the results of an in vitro study reporting that SVS stimulated insulin release from isolated mouse islets at glucose concentrations of 8.3, 11.1, and 16.7 mmol/L, but not at glucose concentrations of 3.3 mmol/L or less.[4] However, a 3-day period of oral SVS treatment in normal adult human caused a significant decrease in the fasting plasma glucose concentration.[2,3]

Our results clearly demonstrate that the acute oral administration of SVS reduced the glucose-insulin index, the product of glucose and insulin IAUCs and inversely related to whole-body insulin sensitivity,[10] in both lean and obese Zucker rats (Figs 2 and 3). In insulin-sensitive lean Zucker rats, the improvement of glucose tolerance following SVS treatment was associated with a significantly smaller increase in the insulin response during the OGTT compared to the vehicle treatment (Figs 1 and 2). It appears from this finding that the improvement of glucose tolerance was due to increased whole-body insulin sensitivity. However, Jeppesen et al[14] found that the bolus intravenous injection of SVS induced a transient insulin secretion during an intravenous glucose tolerance test (IVGTT) in anesthetized, normal Wistar rats without any change of the plasma glucose level. In contrast, Suanarunsawat and Chaiyabutr[13] reported a slight increase in plasma glucose level in response to a SVS infusion (150 mg/mL) in normal Wistar rats. They also showed that the elevation of the plasma glucose level during and after a SVS infusion was not due to the reduction of the plasma insulin level. The reason for these different results in various normal animal models is not apparent.

We have made the novel and important observation that the acute oral administration of SVS also improved glucose tolerance and decreased the glucose-insulin index in the insulin-resistant obese Zucker rat. We found that acute SVS treatment caused a significantly reduction of the plasma glucose response and a decreased $IAUC_{glucose}$, as well as a decreased $IAUC_{insulin}$ (Figs 1 and 3). These findings indicate that acute SVS treatment in this insulin-resistant animal model causes an enhancement of whole-body insulin sensitivity. Consistent with our results, Jeppesen et al[14,15] reported that SVS treatment, either via a bolus intravenous injection or following a 6-week period of feeding, significantly suppressed the glucose response during a IVGTT in diabetic Goto-Kakizaki rat, and also increased the insulin response and suppressed the glucagon level in these animals.

There are, however, several important differences between the present study and previous investigations of the actions of SVS. Previous studies using SVS have used different animal models. The diabetic Goto-Kakizaki rat used by Jeppesen et al[14,15] represents a mild form of non-obese type 2 diabetes characterized by moderate hyperglycemia and hypoinsulinemia.[16] We have used the obese Zucker rat, which is a model of obesity-associated insulin resistance and marked hyperinsulinemia, but not hyperglycemia, and therefore would correspond to a prediabetic state exhibiting marked glucose intolerance. The second difference is that in previous reports, the glucose tolerance test was performed in anaesthetized animals, whereas we have performed the OGTT in conscious animals. These different conditions may have had an effect on plasma glucose, insulin, and other variables related to these hormones. All of these issues should be considered, as insulin secretion by the β cells is a complex event modulated by a number of variables, including the nature and quantity of the secretagogue, the route of its administration, the glucose concentration at the time of administration of the stimulus, and the prevailing degree of insulin sensitivity.[17] In addition, other possible factors that could be involved in the decreased plasma glucose response to the OGTT due to SVS treatment include increased tissue glycolysis, increased muscle glycogen storage, less hepatic glycogenolysis, or increased urinary glucose loss. It has been reported that the total aqueous extract from the leaves of *Stevia rebaudiana*, including SVS, steviol, isosteviol, and steviolbioside, exert an inhibitory action on adenosine triphosphate (ATP) phosphorylation and on nicotinamide adenine dinucleotide (NADH)-oxidase activity in rat liver mitochondria, contributing to an inhibition of adenosine diphosphate (ADP)/ATP exchange, and resulting in increased glycolysis and decreased gluconeogenesis.[18] Furthermore, it has been shown that an intravenous infusion of SVS at doses higher than 8 mg/kg/h causes an increased urinary glucose clearance in rats.[19]

It is well established that the fasting plasma FFA levels have been found to correlate inversely with whole-body insulin sensitivity,[20-23] possibly via impaired action on peripheral glucose uptake and suppression of hepatic glucose output[24] or inhibition of insulin receptor substrate-1 (IRS-1) phosphorylation and IRS-1–associated phosphatidylinositol-3-kinase activity.[25] However, in the present study, the acute oral administration of SVS did not affect fasting FFA levels in either lean and obese Zucker rats (Table 1), and this factor appears unrelated to the SVS-induced enhancement of whole-body insulin sensitivity in lean and obese Zucker rats.

We also examined the direct effect of in vitro SVS treatment on glucose transport activity in skeletal muscle, the primary tissue of glucose disposal. Glucose transport activity in type I soleus and type IIb epitrochlearis muscles was investigated in both basal and insulin-stimulated (2 mU/mL) conditions. Our results clearly indicate that the basal rates of 2-DG uptake in soleus and epitrochlearis muscles from both lean and obese Zucker rats were not significantly affected by lower concentrations of SVS (0.01 to 0.1 mmol/L) (Figs 4 and 5). In addition, higher concentrations of SVS significantly reduced the basal rate of 2-DG uptake in these muscles. However, we report here the novel finding that lower concentrations of SVS (0.01 to 0.1 mmol/L) significantly enhanced the rate of insulin-stimulated 2-DG uptake in soleus and epitrochlearis muscles from both lean and obese Zucker rats (Figs 4 and 5). The absolute effect of SVS on insulin-stimulated 2-DG uptake was greater in the type I soleus compared to the type IIb epitrochlearis in both lean and obese animals, indicating that there may be fiber type-specific effects of the compound. Again, this effect of SVS was reversed at the highest concentrations of the compound. These results indicate that SVS improves the insulin action on the skeletal muscle glucose transport system in both insulin-sensitive lean and insulin-resistant obese Zucker rats in a dose-dependent fashion. Clearly, further investigations to elucidate the possible cellular mechanism(s) whereby SVS interacts with the insulin-dependent glucose transport system in skeletal muscle are warranted.

It is not possible from the available information to determine

whether the concentrations of SVS that were effective in positively modulating in vitro glucose transport in insolated skeletal muscle can be achieved in vivo following oral administration of the compound. A limited amount of information is in the literature regarding the bioavailability and metabolism of SVS in rats. Nakayama et al[26] administered [^{3}H] SVS orally to Wistar rats at a dose of 125 mg/kg. The level of radioactivity in the blood increased slowly to a maximum of 4.83 μg/mL at 8 hours, and exhibited a biological half-life of 24 hours. However, it should be noted that all of the SVS was metabolized to other products. In this context, Toskulkao et al[9] administered SVS orally to rats at 125 mg/kg and showed that 94% of the ingested SVS was absorbed and rapidly metabolized by various tissues. SVS itself in the plasma was undetectable, even at high doses (1 g/kg body weight). However, the principle metabolite of SVS, steviol-16, 17 α-epixide, was detectable in plasma at 20 to 27 nmol/L during the first 5 hours after the administration, and another metabolite, 15α-hydroxysteviol, was found in plasma at 150 to 366 nmol/L after 24 hours.[9] Because of this rapid metabolism of SVS by tissues, measurement of SVS in the plasma is likely not a reliable tool for assessing whether an oral dose of SVS will be effective in eliciting metabolic alteration in target tissues, such as muscle.

In summary, the present study provides the first information regarding the beneficial effect of SVS in improving whole-body insulin sensitivity and insulin-stimulated glucose transport activity in skeletal muscle from both insulin-sensitive lean and insulin-resistant obese Zucker rats. These results also provide evidence that the skeletal muscle glucose transport system may be at least one important site of action of SVS. However, further information is needed on the mechanism of SVS action in this tissue.

REFERENCES

1. American Diabetes Association: Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 26:S5-S20, 2003 (suppl 1)
2. Curi R, Alvarez M, Bazotte RB, et al: Effects of Stevia rebaudiana on glucose tolerance in normal adult humans. Braz J Med Biol Res 19:771-774, 1986
3. Oviedo CA, Franciani G, Marenu R, et al: Action hipoglycemiante de la Stevia rebaudiana Bertoni (Kaa-he-e). Seventh Congress of the International Diabetes Federation, Buenos Aires, Argentina, 1970 (abstr 208)
4. Jeppesen PB, Gregersen S, Poulsen CR, et al: Stevioside acts directly on pancreatic beta-cells to secrete insulin: Action independent of cyclic adenosine monophosphate and adenosine. Metabolism 49: 208-214, 2000
5. Anai M, Funaki M, Ogihara T, et al: Altered expression levels and impaired steps in pathways to phosphatidylinositol-3-kinase activation via insulin receptor substrates 1 and 2 in Zucker fatty rats. Diabetes 47:13-23, 1998
6. Brozinick JT, Etgen GJ, Yaspelkis BB, et al: Glucose uptake and GLUT-4 protein distribution in skeletal muscle of the obese Zucker rat. Am J Physiol Regul Integr Comp Physiol 267:R236-R243, 1994
7. Etgen GT, Jensen J, Wilson CM, et al: Exercise training reverses insulin resistance in muscle enhanced recruitment of GLUT-4 to the cell surface. Am J Physiol Endocrinol Metab 272:E864-E869, 1997
8. Xili L, Chengjiany B, Eryi X, et al: Chronic oral toxicity and carcinogenicity study of stevioside in rats. Food Chem Toxicol 30:957-965, 1992
9. Toskulkao C, Chaturat L, Temcharoen P, et al: Acute toxicity of stevioside, a natural sweetener, and its metabolites, steviol, in several species. Drug Chem Toxicol 20:31-44, 1997
10. Cortez MY, Torgan CE, Brozinick JT, et al: Insulin resistance of obese Zucker rats exercise trained at two difference intensities. Am J Physiol Endocrinol Metab 261:E613-E619, 1991
11. Saengsirisuwan V, Kinnick TR, Schmit MB, et al: Interactions of exercise training and lipoic acid on skeletal muscle glucose transport in obese Zucker rats. J Appl Physiol 91:145-53, 2001
12. Petersen KF, Shulman GI: Pathogenesis of skeletal muscle insulin resistance in type 2 diabetes mellitus. Am J Cardiol 90:11G-18G, 2002 (suppl)
13. Suanarunsawat T, Chaiyabutr N: The effect of stevioside on glucose metabolism in rat. Can J Physiol Pharmacol 75:976-982, 1997
14. Jeppesen PB, Gregersen S, Alstrup KK, et al: Stevioside induces antihyperglycemic, insulinotropic and glucagonostatic effects in vivo: Studies in the diabetic Goto-Kakizaki (GK) rats. Phytomedicine 9:9-14, 2002
15. Jeppesen PB, Gregersen S, Rolfen SE, et al: Antihyperglycemic and blood pressure-reducing effects of stevioside in the diabetic Goto-Kakizaki rat. Metabolism 52:372-378, 2003
16. Movassat J, Saulnier C, Serradas P, et al: Impaired development of pancreatic beta-cell mass is a primary event during the progression to diabetes in the GK rat. Diabetologia 40:916-925, 1997
17. Ward WK, Beard JC, Halter JB, et al: Pathophysiology of insulin secretion in non-insulin-dependent diabetes mellitus. Diabetes Care 7:491-502, 1984
18. Bracht AK, Alvarez M, Bracht A: Effects of Stevia rebaudiana natural products on rat liver mitochondria. Pharmacol 34:873-882, 1985
19. Melis MS: Renal excretion of stevioside in rats. J Nat Prod 55:688-690, 1992
20. Baldeweg SE, Golay A, Natali A, et al: Insulin resistance, lipid and fatty acid concentrations in 867 healthy Europeans. Eur J Clin Invest 30:45-52, 2000
21. Goodpaster BH, Theriault R, Watkins SC, et al: Intramuscular lipid content is increased in obesity and decreased by weight loss. Metabolism 49:467-472, 2000
22. Krssak M, Falk Petersen K, Dresner A, et al: Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: A ^{1}H NMR spectroscopy study. Diabetologia 42:113-116, 1999
23. Perseghin G, Ghosh S, Gerow K, et al: Metabolic defects in lean nondiabetic offspring of NIDDM parents. A cross sectional study. Diabetes 46:1001-1009, 1997
24. Yolanta TK, Dorothy SW, Jachelle O, et al: Fatty acid-induced insulin resistance: Decreased muscle PI3K activation but unchanged Akt phosphorylation. J Clin Endocrinol Metab 87:226-234, 2002
25. Griffin ME, Marcucci MJ, Cline GW, et al: Free fatty acid-induced insulin resistance is associated with activation of protein kinase C theta and alterations in the insulin signaling cascade. Diabetes 48:1270-1274, 1999
26. Nakayama K, Kasahara D, Yamamoto F: Absorption, distribution, metabolism and excretion of stevioside in rats. J Food Hyg Soc Jpn 27:1-8, 1986