The prostacyclin analog beraprost sodium (BPS) ameliorates characteristics of metabolic syndrome in obese Zucker (fatty) rats.

**Running title:** BPS ameliorates diabetes mellitus

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Objective-The prostacyclin analog, beraprost sodium (BPS), was examined for its potential to improve the symptoms of obesity-type diabetes, i.e., hyperglycemia, hyperinsulinemia, dyslipidemia, histopathological change and diabetic complications.

Research Design And Methods-Obese Zucker rats, an experimental model of genetic obese type 2 diabetes mellitus, were repeatedly administered BPS at oral doses of 0.2 or 0.6 mg/kg/day b.i.d. for 12 weeks, and serum chemistry, urinalysis and histopathological examination were performed.

Results- BPS dose-dependently suppressed serum glucose, insulin, triglyceride, and cholesterol levels in obese animals. In oral glucose tolerance test, BPS suppressed the post-glucose-loading elevation of serum glucose in a dose-dependent manner. Urinary N-acetyl-β-D-glucosaminidase (NAG) was significantly lower in BPS-treated obese animals compared with control animals, although no significant differences were observed in urinary protein levels between the BPS-treated groups and the control group. In addition, histopathological examination revealed significant protective effects of BPS against renal disorder in obese animals. Histopathologically, BPS also inhibited the progression of hepatic steatosis, hypertrophy of adipose tissue, and pancreatic fibrosis. Furthermore, thermographic analysis of the hind limb sole skin surface indicated a significant increase in temperature in BPS-treated animals, compared with control animals, which was likely due to improved blood circulation by administration of BPS.

Conclusions- BPS suppressed the pathogenesis and development of diabetic mellitus and its complication, nephropathy, which was presumably accompanied by improving glucose intolerance and insulin resistance in obese Zucker rats.
Diabetes mellitus has become a major health concern worldwide. In fact, the number of patients with diabetes mellitus is still increasing and presently accounts for at least 5% of the adults in the world. Among them, about 30% of diabetes patients develop nephropathy. The mechanisms underlying the pathogenesis of diabetes mellitus and subsequent nephropathy are extremely complex and have not yet been fully elucidated. Glucose intolerance and insulin resistance, however, commonly occur and are often accompanied by hyperglycemia and hyperinsulinemia in diabetic patients, especially in those with obese type 2 diabetes mellitus. These abnormalities are also regarded as risk factors leading to macrovascular and microvascular complications, such as myocardial infarction, nephropathy, retinopathy, and neuropathy (1).

Skeletal muscle, liver, and adipose tissues are insulin-responsive organs that are considered to be closely associated with glucose intolerance and insulin resistance. Among them, skeletal muscle glucose disposal is a primary defect that leads to the development of glucose intolerance and type 2 diabetes mellitus (2-3). In addition, glucose imbalance in diabetic patients is presumed to be closely associated with poor secretion of the endogenous vasodilator, nitric oxide (NO), from endothelial cells and the resultant reduction of blood flow (4). This reduction in blood flow presumably triggers further resistance of the skeletal muscle to glucose disposal. It is well known that variety of activity and distribution were observed in NO synthase (NOS) according to its subtypes (5-8). Since there were contradictory reports for involvement of subtypes such as iNOS to diabetic condition in obese Zucker rats (9-10), the role of NOS subtypes was still unclear. However, in case of eNOS, decrease in its activity would be closely related to the exacerbation of diabetic conditions (6).

BPS, the stable prostacyclin analog, has potent vasodilating activity and activation effect of NO synthase (eNOS) expression in the endothelium (11-12). Based on the vasodilating activity together with protection of the endothelium, anti-inflammatory, anti-platelet activities and increasing of eNOS production, BPS improves regional blood flow (13-14). Accordingly, BPS is expected to improve insulin resistance, at least in part, by improving blood flow in muscle and consequently to ameliorate other pathogenesis seen in type 2 diabetes mellitus. However, the potential of BPS against these pathological conditions remains to be elucidated.

Obese Zucker rats are widely used as a useful experimental model for genetically obese type 2 diabetes mellitus because development of the disease shares many features with that in humans. Namely, these rats are obese and develop progressive insulin resistance, glucose intolerance, hyperinsulinemia and hyperlipidemia (15-17). Therefore, to verify the hypothesis that BPS can improve insulin resistance and ameliorate the symptoms and complications of type 2 diabetes mellitus, we have utilized Zucker rats as an animal model.

The present report describes the changes in some serum chemistry parameters related to glucose and lipid metabolism and some urinary biomarkers, indicative of renal function, in male obese Zucker rats treated with repeated oral BPS. Histopathological examination, focusing on changes in the pancreas, liver, adipose tissue, and kidney, and blood pressure and thermographic analysis of changes in blood flow are also described.

**RESEARCH DESIGN AND METHODS**

**Animals:** Male obese and lean Zucker rats (ZUC-Leprfa/Leprfa rats) were purchased from Charles River Japan (Tokyo, Japan). Animals were housed in an animal room where the light cycle and temperature were
maintained from 7:00 to 19:00, and 23±2°C, respectively. Animals were allowed free access to drinking water and standard rat chow (MF, Oriental Yeast, Tokyo, Japan). All experiments were performed in compliance with the ethical standards for animal studies in Toray Ind., Inc.

**Drug treatment:** BPS was administrated orally to obese Zucker rats at 0.2 or 0.6 mg/kg/day b.i.d. from 7 to 19 weeks of age. Animals in the control group were administered distilled water as a vehicle control, in place of BPS. For each animal, body weight was measured at the beginning of each week throughout the administration period. The body weight data were recorded as a parameter for monitoring the general condition of the animal and were used to determine the dosing volume administered to the animal each week.

**Food consumption:** Food consumption was measured at the time of urinalysis.

**Blood:** Blood was collected from animals once a week during the administration period via the tail artery under ether anesthesia. Serum fractions were obtained by centrifugation and used for the analysis of the following parameters with the respective enzyme assay kits: glucose (N-assay GLU-UL Nittobo, Nittobo Medical, Tokyo, Japan), triglyceride (TG) (N-assay L TG-H Nittobo, Nittobo Medical), and total cholesterol (Tcho) (N-assay L T-CHO-H Nittobo, Nittobo Medical). Insulin was analyzed using a commercially available ELISA kit (Rat Insulin ELISA, Mercodia, Uppsala, Sweden).

HbA1c was analyzed at 19 weeks of age using a commercially available kit (NycoCard HbA1c, AXIS-SHIELD PoC AS, Kimbolton, UK). The value under the lower limit of quantification (below 3.0%) was treated as zero.

**Urine:** At 7, 13 and 17 weeks of age, animals were housed individually in metabolic cages, and urine was collected in metabolic sampling bottles over twenty-four hours. Urine was analyzed for the following parameters using commercially available enzyme assay kits: total protein (Micro TP Test Kit Wako, Osaka, Japan) and NAG (N-assay L NAG Nittobo, Nittobo Medical).

**Oral glucose tolerance test (OGTT):** At 18 weeks of age, after receiving the twice-daily administration of BPS for 11 weeks, animals were subjected to an oral glucose tolerance test. Animals were administered the second daily dose and then fasted overnight (16 hours). Animals were administered 2g/kg of glucose, and blood samples were collected via the tail vein at 0, 0.5, 1, 2, 4 hrs after the oral glucose load. The blood samples were analyzed for serum glucose and insulin, as described above.

**Blood pressure:** Systolic blood pressure (SBP) and heart rate were measured in conscious rats at 12 weeks of age using an automated non-invasive sphygmomanometer (BP-98A, Softron, Tokyo, Japan) equipped with a tail-cuff sensor.

**Skin temperature:** At 19 weeks of age, after receiving the twice-a-day administration of BPS for 12 weeks, the skin temperature of the animals was assessed using a thermograph (Neo Thermo TVS-700, NIPPON AVIONICS, Tokyo, Japan). For each animal, the thermal image of the left hind leg was recorded at 0 and 2 hrs after the first administration of the day. The image was analyzed for temperature at about 4000 points in the area corresponding to each hind leg, and the mean temperature was calculated using the software PE professional (NIPPON AVIONICS).

**Processing tissues for histopathological evaluation:** At the end of the administration period, animals were exsanguinated via the abdominal aorta under ether anesthesia. The kidney, pancreas, liver, and adipose tissue were removed from each animal, fixed in 10% neutral buffered formalin, and embedded in paraffin. Thin sections were prepared from the paraffin blocks and stained with hematoxylin...
and eosin (HE) and periodic acid-Schiff (PAS).

For the quantification of areas of lipid accumulation in the liver, HE-stained images of the lean, control, and BPS groups were imported into a computer for analysis. In each liver, five high-power fields were randomly selected to extract porosity areas and the rate of lipid accumulation area was calculated in each section by an image analysis system (MacSCOPE, Mitani Corporation, Tokyo, Japan).

Glomerular lesions and tubulointerstitial fibrosis were evaluated semi-quantitatively. In brief, glomerular lesions were graded according to the severity of lesions for each glomerulus, from 0 to 4+ as follows; 0: no lesion, 1+: expansion of the mesangial area was observed, but there was no glomerulosclerosis, 2+: sclerosis of less than 50%, 3+: 50 to 75% sclerosis, 4+: more than 75% of glomerular tuft. Tubulointerstitial fibrosis was graded according to the area of injury in 20 randomly-selected high-power fields (×40) and were graded according to the area of alteration from 0 to 5+ as follows: 0: no lesion, 1+: < 10%, 2+: 10-25%, 3+: 25-50%, 4+: 50-75%, 5+: > 75% in each left kidney.

Statistical analysis: Body weight, food consumption, serum and urine chemistry, blood pressure, heart rate, skin temperature, and histopathological data were expressed as the mean ± SE. Statistical significance between BPS treated group and control group was assessed using a two-way analysis of variance, followed by parametric-Williams’ test (body weight, food consumption, systolic blood pressure, hart rate, skin temperature and hepatic steatosis) and non-parametric Williams’ test (serum and urinary parameter, and renal injury), and lean group and control obese group by t test (body weight, food consumption, systolic blood pressure, hart rate, sole temperature and hepatic steatosis) and Welch's test (serum and urinary parameter, and renal injury). The probability value of $P$ less than 0.05 was considered statistically significant.

RESULTS

Body weight and food consumption:
The body weight was significantly greater in obese Zucker rats in the control group than in that for lean rats throughout the administration period. In comparison, among the groups of obese rats, the body weight was significantly greater in the 0.6 mg/kg BPS group than in the control group from 14 weeks of age to the end of the administration period (Fig. 1A). In the 0.2 mg/kg BPS group, the body weight was only higher than the control group from 16 to 17 weeks of age. Food consumption was significantly high in obese Zucker rats in the control group than in that for lean rats, but there was no difference between control rats and BPS treated rats (Fig. 1B).

Hematological examination: Serum glucose was significantly higher in obese Zucker rats than in lean rats from 11 weeks of age until the end of the administration period. During this period, obese Zucker rats treated with 0.6 mg/kg BPS exhibited significantly lower serum glucose levels than the control Zucker rats (Fig. 1C). Serum insulin was markedly elevated in control untreated obese Zucker rats from 8 weeks of age, compared with lean rats. No such elevation was observed in obese Zucker rats in the 0.6 mg/kg BPS group throughout the administration period. Indeed, insulin levels were significantly lower in the 0.6 mg/kg BPS group compared with the control group (Fig. 1D). HbAlc tended to be dose dependently lowered by BPS treatment (Fig. 1E).

Regarding the parameters related to lipid metabolism, serum TG was significantly higher in control untreated obese Zucker rats compared with lean animals throughout the administration period. Serum TG levels, however, were significantly lower in the 0.6 mg/kg BPS group compared with the control group (Fig. 1F). Similarly, serum Tcho was
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significantly higher in control Zucker rats than in lean animals throughout the administration period. Significantly lower Tcho levels were observed in the 0.6 mg/kg BPS group, compared with those in the control group, from 10 to 15 weeks of age, but not for other ages (Fig. 1G).

**Oral glucose tolerance test:** After the glucose loading, control untreated obese Zucker rats showed rapid and remarkable elevation of serum glucose, whereas serum glucose elevation was relatively slow and brief in lean rats (Fig. 2A). After reaching a plateau 0.5-1 hr post-loading, serum glucose was maintained at an essentially constant level in either control untreated obese or lean animals, respectively, until 4 hrs post-loading. Accordingly, serum glucose levels were significantly higher in control untreated obese animals than in lean rats. The 0.6 mg/kg BPS group, however, showed a less rapid and less remarkable serum glucose elevation, when compared with the control group.

Serum insulin was remarkably higher in control untreated obese rats than in lean rats, even at the baseline, and throughout the 4-hr post-loading observation period (Fig. 2B). Compared with the control group, the 0.6 mg/kg BPS group showed significantly lower insulin levels at 0, 0.5 and 4 hr post-loading.

**Urinalysis:** As for urinary protein, a remarkable elevation was observed in control untreated obese Zucker rats, while lean animals exhibited only a slight elevation (Fig. 3A). Thus, urinary protein was significantly higher than in lean rats from 7 weeks of age until the end of the administration period. BPS apparently suppressed the elevation of urinary protein level observed in obese Zucker rats during the administration period; although these values were not significantly different from the control group.

As for urinary NAG, gradual elevations were observed in control untreated obese Zucker rats as well as in lean rats, although the levels were significantly higher in the former than in the latter group (Fig. 3B). BPS suppressed the elevation observed in obese Zucker rats, and a significant difference from the control group was detected in the 0.6 mg/kg BPS group at 17 weeks of age.

Serum creatinine level was not elevated in obese Zucker rats and there were no differences between each group (Data not shown).

**Blood pressure:** The systolic blood pressure of obese Zucker rats was higher than that of lean rat (P=0.054) at 12 weeks of age. While BPS inhibited the elevation of blood pressure (Fig. 3C), the heart rate was not different in each group (Fig. 3D).

**Skin temperature:** Immediately before the daily administration, thermographic image analysis revealed that there was no apparent change in the temperature in the area corresponding to the left hind leg between BPS-treated obese Zucker rats, control untreated obese Zucker rats, and lean rats (data not shown). Two hours after administration, the skin temperature in the hind leg area was significantly higher in the 0.6 mg/kg BPS treated group than in the control group (Fig. 4A, 4B).

**Morphology:** Compared with the pancreatic islets in lean animals, control untreated obese Zucker rats exhibited obviously severe hypertrophic, lobed, and fibrotic changes. In obese rats treated repeatedly with BPS for 12 weeks, these changes were less extensive (Fig. 5).

Severe hepatic steatosis, characterized by the accumulation of higher levels of lipid in hepatic intracellular vesicles and ballooning degeneration of hepatocytes, was evident in obese Zucker rats (Fig. 6A). By contrast, BPS treatment markedly reduced lipid accumulation and ballooning degeneration of hepatocytes (Fig. 6A, 6B).

Adipocytes were obviously larger in obese rats compared with lean animals. Adipose tissues from BPS-treated rats,
however, exhibited greater populations of much smaller adipocytes (Fig. 7).

Compared with kidney tissues from lean rats, control untreated obese Zucker rats exhibited focal and segmental glomerulosclerosis and expansion of glomerular matrix (Fig. 8A, arrowhead). Furthermore, tubular damage such as tubular atrophy, interstitial fibrosis, thickening of the tubular basement membranes, and infiltration of inflammatory cells were also observed in obese Zucker rats (Fig. 8A, arrow). These changes were comparatively mild in kidney tissues from obese Zucker rats treated repeatedly with BPS. In fact, semiquantitative histopathological analysis indicated that both glomerular and tubular abnormality scores were significantly lower in obese Zucker rats treated with BPS at either 0.2 or 0.6 mg/kg compared with in control untreated obese rats (Fig 8B).

DISCUSSION

In the present study, we examined the effects of the prostacyclin analog beraprost sodium (BPS) to improve various diabetic markers in obese Zucker rats; an animal model whose pathological condition resembles that seen in human genetic obese type 2 diabetes mellitus. Specifically, BPS inhibited the increases in blood glucose, insulin, triglyceride, total cholesterol and blood pressure, and ameliorated the development of glucose intolerance and insulin resistance. Histopathologically, BPS inhibited the progression of hepatic steatosis, hypertrophy of the adipose cells, pancreatic fibrosis, and renal damage. Thus, we revealed that BPS suppressed the pathogenesis and development of diabetes mellitus, which was presumably accompanied by improving glucose intolerance and insulin resistance.

Insulin resistance plays a central role in the development of diabetes mellitus. The muscles, liver, and adipocyte are deeply involved in the utilization of glucose as insulin-responsive organs, and are, thus, closely related to insulin resistance. The transport of glucose into muscles and the liver is decreased with increasing levels of glucose and lipids in blood (2, 18-21). In adipocyte, the secretion of cytokines, such as TNF-α and MCP-1, is increased consistent with enlargement of adipocyte caused by excessive accumulation of adipose and with infiltration of inflammatory cells, such as macrophages. It is known that these changes may decrease insulin signaling (22-23).

When the action of insulin decreased through insulin resistance, abnormal metabolism of glucose and lipid are result in further development of diabetes mellitus. Therefore, it is especially important to improve insulin resistance to prevent development of diabetes mellitus.

In this study, we demonstrated that BPS inhibited the development of diabetes mellitus in obese Zucker rats, probably by improvement of insulin resistance. While the mechanisms underlying the findings of this study remain uncertain, we discussed the following hypothesis.

In patients with diabetes mellitus, the vasodilating response was decreased by reduced production of NO from the endothelium, and structural and functional disorder of capillary blood vessels and decreasing muscle blood flow was caused (24-25). In Zucker rats, in addition to insulin resistance, microvascular density, blood flow, and responsiveness of insulin-stimulated glucose metabolism in muscles (26-27), together with activity of PGI$_2$ synthase and eNOS in blood vessels (5), were decreased. In addition, endothelium-dependent relaxation was ameliorated together with enhanced eNOS activity and reduced superoxide anion release in the aorta of obese Zucker rats in case by polyphenols treatment (6). Based on these observations, it was considered that reduced production of NO by decreased eNOS activity together with microvascular disturbances...
would be closely related to exacerbated diabetic conditions, especially insulin resistance. Reduced PGI$_2$ also might be involved in induction of insulin resistance, either directly, or through lowered NO production.

BPS, a PGI$_2$ derivative, exhibits direct actions on smooth muscle cells to elicit vasodilatation, and increase NO production by facilitating expression of eNOS mRNA in endothelial cells as well (11-12). It is also known that BPS can protect the endothelium from high levels of glucose and other cytotoxic agent (28). In addition, BPS ameliorated the decrease in NO production in the endothelial cells of STZ-induced diabetes mellitus model rats (29). Therefore, BPS is supposed to improve insulin resistance through its vasodilating activity, protective effects on vascular endothelial cells, and the facilitation of NO production, and, as a result, amelioration of blood flow in muscles. In this study, increased muscle blood flow was confirmed, based on the data regarding skin temperature obtained after BPS administration.

It is also known that diabetes mellitus may be improved by increasing fatty acid oxidation and energy consumption in muscles (30), and it was confirmed that insulin resistance was improved by increased thermogenesis and energy consumption in Zucker rats (31). Therefore, it is suggested that BPS improved insulin resistance by increased thermogenesis and energy metabolism, as a consequence of increasing blood flow in muscles.

Adipocytes also play a central role in energy metabolism, and can store energy, especially through the intake of FFA, in an insulin-dependent manner. When the amount of accumulated fat is increased by hyperlipidemia, enlarged adipocyte and infiltrating macrophages produce cytokines, such as MCP-1 and TNF-$\alpha$, which are considered to be closely related to the deterioration of insulin resistance. In this study, it was clarified that increases in blood TG and enlargement of adipocyte were inhibited by BPS treatment. It was reported that serum level of TNF-$\alpha$ and MCP-1 were increased in Zucker rats (32-33), and BPS could exhibit an inhibitory action on the production of MCP-1 and TNF-$\alpha$ in another models (34-35). Moreover, lipolytic activity was increased when production of PGI$_2$ was increased in adipose tissues (36). Based on these facts, it was supposed that BPS improved insulin resistance by reducing the enlargement of adipocyte and production of development factors, such as MCP-1 and TNF-$\alpha$.

In recent years, it has also been reported that BPS can enhance PPAR$\delta$ (37). GW501516, PPAR$\delta$ agonist, is considered to facilitate fatty acid oxidation in muscles, and improve insulin resistance and obesity (30). Thus, this action by BPS may be involved in the improvement of insulin resistance in Zucker rats.

In histopathological examination, we clarified that BPS would have effects, not only to improve the functional disturbance of insulin resistance, but also to improve tissue disturbance in the pancreas, liver, and kidney, which are characteristic of patients with diabetes mellitus. In this model, fibrosis, caused due to deterioration of the pancreas when insulin secretion associated with insulin resistance was increased continuously, was improved by the administration of BPS. The possibility exists that both direct protective action of BPS on the pancreas, including the inhibition of vascular endothelial disorders and indirect inhibitory action on increased insulin by improving insulin resistance, might be involved in the improvement of fibrosis.

Regarding the liver, it is known that hepatic steatosis often occurs in patients with obese type 2 diabetes mellitus, and as mentioned above, insulin resistance is deeply involved. In this study, vacuoles, caused by the accumulation of adipose in hepatic cells, were observed in Zucker rats and were
improved by BPS. Since BPS inhibits increases of blood glucose and TG, and improves insulin resistance, these actions are considered to be comprehensively involved in the inhibition of the development of hepatic steatosis. In addition, the direct protective action of BPS on hepatic cells (38) may contribute to improvements in the hepatic steatosis.

Diabetic nephropathy is one of the major complications among patients with diabetes mellitus. It is important to inhibit the development of renal disorder for improving the prognosis of diabetes patients. In this study, characteristic morphological changes such as segmental glomerulosclerosis, expansion of glomerular matrix, tubular damage such as tubular atrophy, interstitial fibrosis, thickening of the tubular basement membranes, and infiltration of inflammatory cells, which were also seen in human diabetic nephropathy, were observed, although nodular glomerulosclerosis did not observed up to 19 weeks of age in this study, and these changes were improved by the administration of BPS. In Zucker rats, decreased NO synthase (eNOS) expression in the kidney was observed concomitant with renal injury (39), and renal damage was caused when NO production was inhibited by administration of L-NAME (40). Thus, it was suggested that decreased production of NO would be involved in the development of renal disorder and there is the possibility that increased NO production upon administration of BPS may inhibit the development of nephropathy. In addition, since it is known that insulin resistance is involved in renal injury in this model (41), the improvement in insulin resistance by BPS also might contribute to the improvement of renal damage. In OLETF rats, other obesity-type diabetes model, renal disorder was also inhibited by BPS. By contrast, since blood glucose did not change prominently at the stage of OLETF rats, effect of BPS against blood glucose was unclear (42). Furthermore, it has been reported that in the renal failure model with no evidence of diabetes, BPS could inhibit the development of renal disorder (35, 43). Therefore, there is the another possibility that in addition to the inhibitory effect on development observed in improvement of diabetes mellitus, the direct inhibitory action against renal disorder may be somehow involved in the inhibition of renal damage by BPS observed in Zucker rats. BPS maintained blood pressure over time in Zucker rats to the same level as that in lean rats. Since increased blood pressure and renal damage are observed in patients with diabetes mellitus (44) and these are correlated with each other (45-46), inhibition of blood pressure by BPS may be related to inhibition on development of renal disorder.

Like other anti-diabetes agent (47-48), diabetic condition such as hyperglycemia and hyperlipidemia was ameliorated despite constant increasing of body weight by BPS treatment. Hence food consumption was not increased and edema was not observed in BPS treated rats, we supposed that mild increase in body weight of BPS treated rats might be the ameliorating effect of glucose utilization by BPS treatment.

As for the involvement of increased NO production and eNOS activity for the beneficial effect of BPS, further investigation would be needed focusing on the local expression of eNOS and other NOS subtypes in the artery, kidney, and other related organs. Taken together, by use of Zucker rats, an experimental model of genetic obese type 2 diabetes mellitus, we succeed to reveal for the first time that BPS, a prostacyclin analog, would improve a variety of markers of obesity-type diabetes and nephropathy, one of diabetic complications, in addition to tissue disturbance in the liver, adipocyte, and pancreas. And it is considered that improvement of insulin resistance by BPS treatment may contribute to ameliorate these pathological conditions.
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REFERENCES


47. de Souza C J, Eckhardt M, Gagen K, Dong M, Chen W, Laurent D, Burkey BF: Effects of pioglitazone on adipose tissue remodeling within the setting of obesity and insulin resistance. *Diabetes* 2001; 50(8):1863-1871

FIGURE LEGENDS

FIG. 1. Body weight (A), food consumption (B), serum glucose (C), insulin (D), HbA1c (E), triglyceride (F), and total cholesterol (G) in control obese Zucker rats (●), obese Zucker rats treated with BPS (△; 0.2mg/kg/day, ▲; 0.6mg/kg/day), and Zucker lean rats (○) as line graph, and control obese Zucker rats (□), Zucker rats treated with BPS ( △; 0.2mg/kg/day, ■; 0.6mg/kg/day), and Zucker lean rats ( □ ) as bar graph. Data are mean± SE of 7-8 rats. *P<0.05, **P<0.01 vs. control rats by parametric Williams' test (body weight, food consumption) and non-parametric Williams' test (glucose, insulin, triglyceride and total cholesterol). #P<0.05, ##P<0.01 vs. control by t test (body weight, food consumption) and Welch's test (glucose, insulin, triglyceride and total cholesterol).

FIG. 2. Time-course changes in the level of serum glucose and insulin during oral glucose tolerance test (OGTT) in control obese Zucker rats (●), obese Zucker rats treated with BPS (△; 0.2mg/kg/day, ▲; 0.6mg/kg/day), and Zucker lean rats (○). Data are mean± SE of 7-8 rats. *P<0.05, **P<0.01 vs. control rats by non-parametric Williams' test. ##P<0.01 vs. control by Welch's test.

FIG. 3. Urinary protein (A), NAG (B), systolic blood pressure (C), and heart rate (D) in control obese Zucker rats (●), obese Zucker rats treated with BPS (△; 0.2mg/kg/day, ▲; 0.6mg/kg/day), and Zucker lean rats (○) as line graph, and control obese Zucker rats (□), Zucker rats treated with BPS (△; 0.2mg/kg/day, ■; 0.6mg/kg/day), and Zucker lean rats ( □ ) as bar graph. Data are mean± SE of 7-8 rats. *P<0.05 vs. control rats by non-parametric (NAG) and parametric (SBP) Williams' test. #P<0.05, ##P<0.01 vs. control by Welch's test.

FIG. 4. The level of sole temperature after administration. (A) Representative thermal images of each groups at 2 hour after administration. (B) Sole temperature at 2 hours after administration in control obese Zucker rats ( □ ) and obese Zucker rats treated with BPS ( △; 0.2mg/kg/day, ■; 0.6mg/kg/day). Data are mean± SE of 4 rats. *P<0.05 vs. control rats by parametric Williams' test.

FIG. 5. Photomicrographs of HE staining of the pancreas in control obese Zucker rats, obese Zucker rats treated with high-dose of BPS, and Zucker lean rat.

FIG. 6. Photomicrographs of HE staining of the liver in control obese Zucker rats, obese Zucker rats treated with BPS, and Zucker lean rat (A) and quantitative analysis of vesicles in control obese Zucker rats ( □ ) and obese Zucker rats treated with BPS (△; 0.2mg/kg/day, ■; 0.6mg/kg/day) (B). Data are mean± SE of 7-8 rats. **P<0.01 vs. control by parametric Williams' test.

FIG. 7. Photomicrographs of HE staining of the adipose tissues in control obese Zucker rats, obese Zucker rats treated with high-dose of BPS, and Zucker lean rat.

FIG. 8. Photomicrographs of PAS staining of the kidney in control obese Zucker rats, obese Zucker rats treated with BPS, and Zucker lean rat (A) and semiquantitative analysis of glomerular (B: left) and tubular (B: right) injuries in control obese Zucker rats ( □ ), obese Zucker rats treated with BPS (△; 0.2mg/kg/day, ■; 0.6mg/kg/day), and Zucker lean rats ( □ ). Data are mean± SE of 7-8 rats. *P<0.05, **P<0.01 vs. control by non-parametric Williams' test. #P<0.01 vs. control by Wilcoxon test.
Figure 1

A

B

C

D

**BPS ameliorates diabetes mellitus**
BPS ameliorates diabetes mellitus

E

![Graph showing HbA1c (%)]

F

![Graph showing TG (mg/dL)]

G

![Graph showing Tcho (mg/dL)]
Figure 2

A

B

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**Figure 3**

**A**
- Graph showing urinary protein (mg/day) over weeks of age for Lean, Control, BPS 0.2mg/kg, and BPS 0.6mg/kg groups.
- Significant differences indicated by symbols.

**B**
- Graph showing urinary NAG (IU/day) over weeks of age for Lean, Control, BPS 0.2mg/kg, and BPS 0.6mg/kg groups.
- Significant differences indicated by symbols.

**C**
- Graph showing SBP (mmHg) for Lean, Control, BPS 0.2, and BPS 0.6 groups.
- P-value of 0.054 indicated.

**D**
- Graph showing heart rate for Lean, Control, BPS 0.2, and BPS 0.6 groups.

Figure 4

A

Control

BPS 0.2 mg/kg

BPS 0.6 mg/kg

B

Sole temperature (°C)

Control 0.2 0.6

BPS (mg/kg/day)
Figure 5

Lean

Control

BPS 0.6 mg/kg
Figure 6

A

Lean    Control

BPS 0.2 mg/kg          BPS 0.6 mg/kg

B

Area of vacuoration (%)

Control    0.2    0.6

BPS (mg/kg/day)
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Figure 7

Lean | Control

BPS 0.6 mg/kg
Figure 8

A

Lean    Control

BPS 0.2 mg/kg    BPS 0.6 mg/kg

B

Glomerular score

Tubular score

Lean  Control  0.2  0.6  BPS (mg/kg/day)

Lean  Control  0.2  0.6  BPS (mg/kg/day)