Gender-specific regulation of pancreatic islet blood flow, insulin levels, and glycemia in spontaneously diabetic Goto-Kakizaki rats

Zhen Huang, Leif Jansson, Åke Sjöholm, Åke Sjöholm

Gender-specific regulation of pancreatic islet blood flow, insulin levels, and glycemia in spontaneously diabetic Goto-Kakizaki rats

Zhen Huang[1], Leif Jansson[2], and Åke Sjöholm[1*]

1) Department of Internal Medicine, Karolinska Institutet, Stockholm South Hospital, SE 118 83 Stockholm, Sweden
Phone # +4686163219 Fax # +4686163146 E-mail ake.sjoholm@sodersjukhuset.se
2) Department of Medical Cell Biology, University of Uppsala, SE-751 23 Uppsala, Sweden

*) To whom all correspondence and reprint requests should be addressed.

Key words: Pancreatic islet, diabetes, blood flow, gender, pravastatin, captopril, candesartan, irbesartan and palmitate

Running title: Diabetic islet blood flow

Abbreviations used in this paper: ABF, adrenal blood flow; ACE, angiotensin-converting enzyme; Ang II, angiotensin II; ELISA, enzyme-linked immunosorbent assay; IBF, islet blood flow; KBF, kidney blood flow; NO, nitric oxide; PBF, pancreatic blood flow; RAS, renin-angiotensin system.

Clinical Science Immediate Publication. Published on 21 Jan 2008 as manuscript CS20070386

Abstract

Diabetic patients are often treated with a statin against hyperlipidemia and an ACE inhibitor or angiotensin receptor antagonist against hypertension or albuminuria. These drugs may also exert beneficial metabolic effects, causing an improved glucose tolerance in patients. Gender-related differences have also been observed in the clinical responsiveness to these drugs, but the mechanism behind this is unclear. We now studied whether these drugs and the fatty acid palmitate influence pancreatic microcirculation, thereby impacting insulin secretion and glycemia *in vivo*, in spontaneously diabetic male and female Goto-Kakizaki rats. In male rats, islet blood flow (IBF) and total pancreatic blood flow (PBF) were increased significantly by pravastatin, captopril and irbesartan. Serum insulin levels were increased by pravastatin and captopril. Palmitate suppressed IBF and raised blood glucose. In female animals, IBF was stimulated by captopril, candesartan and irbesartan. PBF was increased by captopril, candesartan and irbesartan, and by pravastatin. Palmitate suppressed IBF and serum insulin secretion. In conclusion, the present study lends support to the view that a local pancreatic RAS and pravastatin may be selectively influencing pancreatic microcirculation and therefore affecting insulin secretion and glycemia. Free fatty acids impaired islet blood flow, suppressed insulin secretion, and raised blood glucose. Substantial gender-related differences in the vascular and metabolic responses to these drugs prevail in this diabetic animal model.

Clinical Science Immediate Publication. Published on 21 Jan 2008 as manuscript CS20070386

Introduction

The renin-angiotensin system (RAS) has been extensively studied since the first identification of renin by Tigerstedt and Bergmann in 1898 [1]. It plays an important role in regulating blood volume and systemic vascular resistance [2]. It has become increasingly clear that a local RAS exists in the pancreas and some other tissues [3-6]. Ang II, considered the main effective peptide of the RAS, has been shown to adversely influence pancreatic and islet blood flow through vasocontractive effects thereby suppressing insulin secretion [7, 8]. ACE, another component of RAS, has been detected in endothelial cells of the vascular wall [9-11] in human and rat [12-15]. This may be particular importance in diabetic patients since many of them are treated with ACE inhibitors or angiotensin receptor antagonists against hypertension or as part of a renal protection effort. Another salient feature of diabetic patients is hyperlipidemia and elevated serum levels of free fatty acids; therefore many diabetic patients use lipid lowering drugs, most notable statins. Interestingly, gender differences were observed in large clinical trials, with more pronounced negative effects of diabetes on the lipid profile and blood pressure in women compared with men [16, 17], and also the vascular responses to statins and RAS-interfering drugs seem to differ between men and women [18, 19]. Recently, we have shown the stimulatory effects of such drugs on pancreatic islet blood flow in normal male [20] and female [21] Wistar rats. However, it is not known how these drugs influence islet microcirculation, insulin secretion and glucose tolerance in diabetic animals. In the present study, we thus investigated gender-specific effects of ACE inhibition, Ang II receptor antagonism, statin treatment, and palmitate administration on islet blood flow, insulin levels and glycemia in Goto-Kakizaki rats, a widely used animal model of human type 2 diabetes [22].

Materials and Methods

Animals and drugs

Male (300-350 g) and female (200-250 g) Goto-Kakizaki rats were obtained from our

KISÖS breeding colony (Animal Department, Stockholm South Hospital, Stockholm, Sweden). The Stockholm GK rat colony was established at KISÖS in 2004 from breeding pairs graciously donated by Prof. Robert V. Farese (Tampa, FL). All animals had free access to pelleted food and tap water and were kept under standard conditions. The experiments were approved by the local ethical committee. Pravastatin and captopril were kindly given by Bristol Myers Squibb (New York, NY). Irbesartan was generously given by Sanofi-Aventis (Paris, France), whereas candesartan was graciously donated by Astra Zeneca (Södertälje, Sweden). Sodium palmitate was bought from Sigma-Aldrich (St Louis, MO) and dissolved in 10 % ethanol solution.

Surgical preparation

The rats were anesthetized with an intraperitoneal injection of sodium thiobutabarbital (120 mg/kg of body weight; Inactin™; Research Biochemicals International [Natick, MA]), then placed on a heated operating table in order to maintain body temperature. Catheters were inserted into the ascending aorta via the right carotid artery and into the left femoral artery. The catheter in the aorta was connected to a pressure transducer (model PDCR 75/1, Druck Ltd, Groby, Leicestershire, UK) in order to constantly monitor the mean arterial blood pressure. After the mean arterial blood pressure had remained stable for at least 20 min, the animals were injected intravenously with 1 ml of saline, 1 ml of pravastatin (0.5 mg/kg of body weight), 1 ml of captopril (3 mg/kg of body weight), 1 ml of candesartan (10 mg/kg of body weight), or 1 ml of irbesartan (3 mg/kg of body weight). The irbesartan was tested separately due to delayed delivery. All substances were dissolved in saline.

The palmitate experiments (60 mg/ml) were done in a separate series because the fatty acid had to be dissolved in 10 % ethanol, and thus controls were given equal amounts of this solvent only. To this end, 1 ml palmitate or solvent was injected i.v. exactly as described for the other agents above.

Clinical Science Immediate Publication. Published on 21 Jan 2008 as manuscript CS20070386

Blood flow measurements

The procedure was performed according to a protocol described in detail previously [23]. Briefly, 10 minutes after the injection of test substances, about 1.5-2.0 x 10^5 non-radioactive microspheres (IMT, Stason Labs, Irvine, CA), with a mean diameter of 10 μm, were injected into the ascending aorta during 10 s. Starting 5 s before microsphere injection and continuing for a total 60 s, an arterial blood reference sample was collected from the catheter in the femoral artery. The amount of blood was confirmed by weighting each reference sample. Additional arterial blood samples were collected at the end of the experiment (approximately 30 minutes after the surgical procedure has been done) for later measurement of blood glucose and serum insulin concentrations.

The animals were then killed by cervical dislocation, the whole pancreas and both adrenal glands, as well as a 100-mg slice of the left kidney (including both cortex and medulla), were removed, blotted, and weighted. The microsphere contents of the adrenals, part of kidney, pancreatic islets, and exocrine parenchyma were determined in frozen-thawed preparations separately [24]. The blood flow values were calculated according to the formula: $Q_{org} = Q_{ref} \times N_{org}/N_{ref}$, where Q_{org} is organ blood flow (ml/min), Q_{ref} is withdrawal rate of the reference sample (ml/min), N_{org} is the number of microspheres in the organ and N_{ref} is the number of microspheres in the reference sample. A less than 10 % difference in microsphere content between the two adrenal glands was taken to confirm an even distribution of the microspheres in the arterial blood stream.

Blood glucose and insulin concentration determinations

The blood glucose concentration was analyzed with test strips based on the glucose oxidase method (Medisense, Solna, Sweden), and serum insulin concentrations were measured with an ELISA kit (rat insulin ELISA; Mercodia, Uppsala, Sweden).

Statistical calculations

All values are given as the mean ± SEM. Probabilities of chance differences between experimental groups were analyzed with one-way ANOVA (SigmaStat) in combination with Student's two-tailed unpaired *t* test. $P<0.05$ was considered statistical significant.

Results

Gender-specific effects of captopril, candesartan, pravastatin, and irbesartan on blood flow

In *male* rats, captopril (3 mg/kg BW), pravastatin (0.5 mg/kg BW) and irbesartan (3 mg/kg BW) all enhanced PBF (Fig. 1a, 1b) and IBF (Fig. 2a, 2b) significantly, whereas candesartan had no discernable effects (Fig. 1a, 2a). KBF was enhanced markedly by pravastatin ($P<0.01$), candesartan ($P<0.05$) and irbesartan ($P<0.05$), but not significantly by captopril (Table 1). Only pravastatin increased ABF significantly ($P<0.05$), whereas captopril, candesartan and irbesartan failed to do so (Table 1).

In *female* rats, all test substances augmented PBF markedly (Fig 1a, 1b). Administration of captopril, candesartan and irbesartan induced an increase in IBF (Fig. 2a, 2b), whereas pravastatin did not. KBF was markedly increased by pravastatin ($P<0.001$), captopril ($P<0.001$), candesartan ($P<0.001$) and irbesartan ($P<0.01$) (Table 1). Captopril and candesartan increased ABF significantly (both $P<0.01$), whereas pravastatin and irbesartan had no such effects (Table 1).

Blood glucose concentrations, serum insulin levels, and mean arterial blood pressure

In *male* GK rats, pravastatin and captopril enhanced serum insulin levels (Table 2), whereas irbesartan and candesartan did not. The blood glucose concentration was unexpectedly increased by captopril (Table 2).

There were no significant differences between any groups in serum insulin levels in

the *female* animals (Table 3). Surprisingly, however, pravastatin and captopril slightly raised blood glucose, which might be due to surgical stress. Irbesartan had no effects on serum insulin or blood glucose levels (Table 3). No effects were found on mean arterial pressure by any of the treatments given (Table 2, 3).

Effects of palmitate on blood flow, insulin levels and glycemia

Palmitate significantly decreased islet blood flow in both *male* (Fig. 3) and *female* (Fig. 3) rats but had no significant changes on whole pancreatic blood flow in either gender (not shown). The reason for gender differences in basal islet blood flow (Figure 3) is unknown, but might be related to increased vascular sensitivity to ethanol in female rats (perhaps in part due to lower body weight than males). In *male* rats, palmitate markedly increased blood glucose (Table 2) along with a non-significant decrease in serum insulin (Table 2). In *female* rats, palmitate lowered serum insulin concentration significantly (Table 3) but failed to significantly impact glycemia in these animals (Table 3). In female rats only, palmitate evoked an increase in ABF but did not influence KBF in either gender (Table 1).

Discussion

Type 2 diabetes is increasing in prevalence globally and is seen in ever-younger age groups [25]. The disease is characterized not only by hyperglycemia, but also insulin resistance with attendant hypertension, dyslipidemia and increased levels of circulating free fatty acids. Most diabetic patients are being treated with one or more antidiabetic drugs, a lipid lowering statin, and an ACE inhibitor or angiotensin receptor antagonist against hypertension and/or albuminuria. In clinical trials, it has been repeatedly observed that these kinds of drugs also influence the rate of incident diabetes [26], causing an improved glucose tolerance in patients. Consistent with this, we have previously shown that these drugs exert substantial short-term impact on islet blood perfusion, insulin secretion and glucose tolerance *in vivo* in normal Wistar rats of both male and female gender [20, 21]. To evaluate whether these drugs also influence islet microcirculation, insulin levels and glycemia under diabetic conditions,

we have presently studied the effects of these drugs in spontaneously diabetic GK rats in both male and female groups.

Our findings reveal that irbesartan, an angiotensin II receptor antagonist, and captopril, an ACE inhibitor, induced a robust increase in islet and whole pancreatic blood flow both in male and female GK rats. This beneficial effect confirms our previous study with the administration of these drugs to normal Wistar rats of both genders [20, 21]. Captopril also significantly increased serum insulin concentrations in male GK rats. Previous publications show that locally produced Ang II suppressed islet blood flow [27, 4], lending support to our present findings. Additionally, recent evidence also suggests that irbesartan has the capacity to enhance vasodilatation [27, 4], which might be another mechanism contributing to our current findings.

Recently, another Ang II receptor antagonist, candesartan, proved efficacious in reducing fibrosis in and around the islets and prevented the loss of endothelial cells in diabetic mouse islets by long term treatment, suggesting that candesartan may partially prevent deterioration of glucose tolerance by affording protection against progressive β-cell damage [28, 29]. Our results reveal that candesartan increased islet blood flow and total pancreatic blood flow significantly only in female GK rats, but not in male animals. This gender difference was not noted for irbesartan or captopril. The underlying mechanism behind this notion remains elusive at this point, but, gender-related differences in the responsiveness to RAS-interfering drugs have been long noticed. A large body of evidence has been obtained through research in animals that components of the RAS are significantly influenced by gender at the tissue levels [30-37], while clinical studies in humans have predominantly evaluated gender effects on the circulating, *i.e.* systemic, RAS [38-45]. The characterization of an estrogen-responsive element in the 5'-flanking region of the angiotensinogen gene was an important early finding to prove an interaction with sex hormones and the RAS at the molecular level [46, 34]. More recent studies indicate that renin is suppressed by estrogen [44]. Collectively, these studies are in line with the observation that plasma renin levels are lower in women compared with men [47, 44]. In addition, AT1 receptors may also be down-regulated by estrogen while estrogen deficiency leads to up-regulation of this receptor [36]. In female transgenic rats with

activated RAS, it was shown that estrogen treatment may afford protection against hypertension, possibly by amplifying the vasodilator contributions of Ang-(1-7), while reducing the formation and vasoconstrictor actions of Ang II [48]. Conceivably, any of these mechanisms (or other) could contribute to our findings of gender-related differences in this system.

Pravastatin, a HMG-CoA reductase inhibitor, is clinically used in order to reduce the cholesterol level and thereby prevent dyslipidemia. Our study shows a preferential increase in pancreatic islet blood flow and pancreatic blood flow, as well as a significantly increased serum insulin level, in male GK rats by pravastatin administration. In contrast, in female animals total pancreatic blood flow was enhanced but not islet blood flow. In our previous studies in female non-diabetic Wistar rats, pravastatin actually increased both panceatic islet blood flow and total pancreatic blood flow [21]. The reason for this difference is currently unknown. However, with increasing age, persistent hyperglycemia may induce islet hypoperfusion in GK rats [49]. The islet blood hypoperfusion is accomanied by an islet capillary hypertension. Such shear stress changes are known to change the gene expression in surrounding cells [49], possibly impacting islet function. Dyslipidemia, a salient feature in type 2 diabetes, is known to impair endothelial-mediated vasodilatation [50, 51]. Pravastatin has beneficial anti-inflammatory effects on endothelial function [52-55], and therefore may significantly influence selective tissue perfusion.

When making comparisons with the diabetes preventive actions of pravastatin and RAS-interfering drugs noted in clinical trials [56], it should be kept in mind that our present results reflect only very acute effects of the substances tested. While reports on long-term effects of such drugs on islet blood flow are scarce [28, 6], this interesting issue is worthy of further investigation. However, it would be beyond the scope of the present study, whose aim was to monitor short-term actions.

Type 2 diabetes is commonly associated with elevated serum free fatty acids, due to insulin resistance and increased lipolysis [57, 27, 56, 25]. Free fatty acids are generally believed to be involved in endothelial dysfunction and vascular damage in type 2 diabetes [57, 27, 56, 25]. Our previous study showed that palmitate

preferentially suppressed pancreatic islet blood flow in non-diabetic male Wistar rats [20]. In the present study, palmitate decreased both whole pancreatic and islet blood flow in both male and female GK rats. This might be one mechanism by which free fatty acids may negatively impact β-cell function, *i.e.* through impeding nutritive islet blood flow and thereby further worsening the diabetic state by limiting the supply of insulin needed to curb hyperglycemia. Our results in this respect are at variance with many previous findings that acute effects of saturated fatty acids on glucose induced insulin secretion are normally positive (58), both *in vitro* and *in vivo*, and we have no obvious explanation for this apparent discrepancy that deserves further investigation in future work.

In summary, several vasoactive drugs that are frequently given to diabetic patients, and reportedly may improve their glucose tolerance, selectively enhanced pancreatic islet blood flow in this animal model of type 2 diabetes. Gender-related differences in the vascular responses to these drugs were also noted in this system. Free fatty acids may aggravate hyperglycemia in diabetes by limiting islet blood flow as described here.

Acknowledgments

The skilled technical assistance of Birgitta Bodin and Astrid Nordin is gratefully acknowledged. Financial support was received from Astra-Zeneca Sweden AB, the Swedish Research Council (72X-109, 72X-12550, 72X-14507, 72X-11564, 72P-12995, 72X-00034, 72X-09890, 72XS-12708, 72P-14787, and 12P-10151), EFSD/Novo Nordisk for Type 2 Diabetes Research Grant, the Swedish Diabetes Association, the Swedish-American Diabetes Research Program funded by the Juvenile Diabetes Foundation and the Wallenberg Foundation, the NOVO Nordic Research Fund, the Family Ernfors Fund, the Swedish Society of Medicine, the Sigurd and Elsa Golje Memorial Foundation, Svenska Försäkringsföreningen, Svenska Diabetesstiftelsen, Magn. Bergvall Foundation, Barndiabetesfonden, Åke Wiberg's Foundation, Torsten and Ragnar Söderberg's Foundations, Berth von Kantzow's Foundation, Harald Jeansson's and Harald and Greta Jeansson's

Foundations, Tore Nilson's Foundation for Medical Research, Fredrik and Inger Thuring's Foundation, and Syskonen Svensson's Fund.

References

1. Paul, M., Poyan Mehr, A. and Kreutz, R. (2006) Physiology of local renin-angiotensin systems. Physiol Rev. 86, 747-803.

2. Peach, M. J. (1977) Renin-angiotensin system: biochemistry and mechanisms of action. Physiol Rev. 57, 313-70.

3. Carlsson, P. O. (2001) The renin-angiotensin system in the endocrine pancreas. Jop. 2, 26-32.

4. Lau, T., Carlsson, P. O. and Leung, P. S. (2004) Evidence for a local angiotensin-generating system and dose-dependent inhibition of glucose-stimulated insulin release by angiotensin II in isolated pancreatic islets. Diabetologia. 47, 240-8.

5. Tahmasebi, M., Puddefoot, J. R., Inwang, E. R. and Vinson, G. P. (1999) The tissue renin-angiotensin system in human pancreas. J Endocrinol. 161, 317-22.

6. Tikellis, C., Wookey, P. J., Candido, R., Andrikopoulos, S., Thomas, M. C. and Cooper, M. E. (2004) Improved islet morphology after blockade of the renin-angiotensin system in the ZDF rat. Diabetes. 53, 989-97.

7. Carlsson, P. O., Berne, C. and Jansson, L. (1998) Angiotensin II and the endocrine pancreas: effects on islet blood flow and insulin secretion in rats. Diabetologia. 41, 127-33.

8. Olsson, R., Jansson, L., Andersson, A. and Carlsson, P. O. (2000) Local blood flow regulation in transplanted rat pancreatic islets: influence of adenosine, angiotensin II, and nitric oxide inhibition. Transplantation. 70, 280-7.

9. Corvol, P., Michaud, A., Soubrier, F. and Williams, T. A. (1995) Recent advances in knowledge of the structure and function of the angiotensin I converting enzyme. J Hypertens Suppl. 13, S3-10.

10. Costerousse, O., Allegrini, J., Huang, W. and Alhenc-Gelas, F. (1998) Angiotensin I-converting enzyme (kininase II) in cardiovascular and renal regulations and diseases. Biol Res. 31, 161-7.

11. Falkenhahn, M., Franke, F., Bohle, R. M., Zhu, Y. C., Stauss, H. M., Bachmann, S., Danilov, S. and Unger, T. (1995) Cellular distribution of angiotensin-converting enzyme after myocardial infarction. Hypertension. 25, 219-26.

12. Hirsch, A. T., Talsness, C. E., Schunkert, H., Paul, M. and Dzau, V. J. (1991) Tissue-specific activation of cardiac angiotensin converting enzyme in

experimental heart failure. Circ Res. 69, 475-82.

13. Kreutz, R., Fernandez-Alfonso, M. S., Liu, Y., Ganten, D. and Paul, M. (1995) Induction of cardiac angiotensin I-converting enzyme with dietary NaCl-loading in genetically hypertensive and normotensive rats. J Mol Med. 73, 243-8.

14. Paul, M., Wagner, J. and Dzau, V. J. (1993) Gene expression of the renin-angiotensin system in human tissues. Quantitative analysis by the polymerase chain reaction. J Clin Invest. 91, 2058-64.

15. Schunkert, H., Dzau, V. J., Tang, S. S., Hirsch, A. T., Apstein, C. S. and Lorell, B. H. (1990) Increased rat cardiac angiotensin converting enzyme activity and mRNA expression in pressure overload left ventricular hypertrophy. Effects on coronary resistance, contractility, and relaxation. J Clin Invest. 86, 1913-20.

16. Howard, B. V., Cowan, L. D., Go, O., Welty, T. K., Robbins, D. C. and Lee, E. T. (1998) Adverse effects of diabetes on multiple cardiovascular disease risk factors in women. The Strong Heart Study. Diabetes Care. 21, 1258-65.

17. Walden, C. E., Knopp, R. H., Wahl, P. W., Beach, K. W. and Strandness, E., Jr. (1984) Sex differences in the effect of diabetes mellitus on lipoprotein triglyceride and cholesterol concentrations. N Engl J Med. 311, 953-9.

18. Dale, K. M., Coleman, C. I., Shah, S. A., Patel, A. A., Kluger, J. and White, C. M. (2007) Impact of gender on statin efficacy. Curr Med Res Opin. 23, 565-74.

19. Juilliere, Y., Berder, V., Brembilla-Perrot, B. and Selton-Suty, C. (2004) [Response to drug therapy of cardiac failure according to gender]. Arch Mal Coeur Vaiss. 97, 1216-20.

20. Huang, Z., Jansson, L. and Sjoholm, A. (2006) Pancreatic islet blood flow is selectively enhanced by captopril, irbesartan and pravastatin, and suppressed by palmitate. Biochem Biophys Res Commun. 346, 26-32.

21. Huang, Z., Jansson, L. and Sjoholm, A. (2007) Vasoactive drugs enhance pancreatic islet blood flow, augment insulin secretion and improve glucose tolerance in female rats. Clin Sci (Lond). 112, 69-76.

22. Portha, B. (2005) Programmed disorders of beta-cell development and function as one cause for type 2 diabetes? The GK rat paradigm. Diabetes Metab Res Rev. 21, 495-504.

23. Jansson, L. and Hellerstrom, C. (1983) Stimulation by glucose of the blood flow to the pancreatic islets of the rat. Diabetologia. 25, 45-50.

24. Jansson L, H. C. (1981;113(3):371-4.) A rapid method of visualizing the

pancreatic islets for studies of islet capillary blood flow using non-radioactive microspheres. Acta Physiol Scand.

25. Zimmet, P., Alberti, K. G. and Shaw, J. (2001) Global and societal implications of the diabetes epidemic. Nature. 414, 782-7.

26. Abuissa, H., Jones, P. G., Marso, S. P. and O'Keefe, J. H., Jr. (2005) Angiotensin-converting enzyme inhibitors or angiotensin receptor blockers for prevention of type 2 diabetes: a meta-analysis of randomized clinical trials. J Am Coll Cardiol. 46, 821-6.

27. Gaede, P., Vedel, P., Larsen, N., Jensen, G. V., Parving, H. H. and Pedersen, O. (2003) Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. N Engl J Med. 348, 383-93.

28. Shao, J., Iwashita, N., Ikeda, F., Ogihara, T., Uchida, T., Shimizu, T., Uchino, H., Hirose, T., Kawamori, R. and Watada, H. (2006) Beneficial effects of candesartan, an angiotensin II type 1 receptor blocker, on beta-cell function and morphology in db/db mice. Biochem Biophys Res Commun. 344, 1224-33.

29. Shao, J. Q., Iwashita, N., Du, H., Wang, Y. T., Wang, Y. Y., Zhao, M., Wang, J., Watada, H. and Kawamori, R. (2007) Angiotensin II receptor blocker provides pancreatic beta-cell protection independent of blood pressure lowering in diabetic db/db mice. Acta Pharmacol Sin. 28, 246-57.

30. Armando, I., Jezova, M., Juorio, A. V., Terron, J. A., Falcon-Neri, A., Semino-Mora, C., Imboden, H. and Saavedra, J. M. (2002) Estrogen upregulates renal angiotensin II AT(2) receptors. Am J Physiol Renal Physiol. 283, F934-43.

31. Ellison, K. E., Ingelfinger, J. R., Pivor, M. and Dzau, V. J. (1989) Androgen regulation of rat renal angiotensinogen messenger RNA expression. J Clin Invest. 83, 1941-5.

32. Freshour, J. R., Chase, S. E. and Vikstrom, K. L. (2002) Gender differences in cardiac ACE expression are normalized in androgen-deprived male mice. Am J Physiol Heart Circ Physiol. 283, H1997-2003.

33. Gallagher, P. E., Li, P., Lenhart, J. R., Chappell, M. C. and Brosnihan, K. B. (1999) Estrogen regulation of angiotensin-converting enzyme mRNA. Hypertension. 33, 323-8.

34. Gordon, M. S., Chin, W. W. and Shupnik, M. A. (1992) Regulation of angiotensinogen gene expression by estrogen. J Hypertens. 10, 361-6.

35. Hinojosa-Laborde, C., Craig, T., Zheng, W., Ji, H., Haywood, J. R. and Sandberg, K. (2004) Ovariectomy augments hypertension in aging female Dahl

salt-sensitive rats. Hypertension. 44, 405-9.

36. Nickenig, G., Baumer, A. T., Grohe, C., Kahlert, S., Strehlow, K., Rosenkranz, S., Stablein, A., Beckers, F., Smits, J. F., Daemen, M. J., Vetter, H. and Bohm, M. (1998) Estrogen modulates AT1 receptor gene expression in vitro and in vivo. Circulation. 97, 2197-201.

37. Nickenig, G., Strehlow, K., Wassmann, S., Baumer, A. T., Albory, K., Sauer, H. and Bohm, M. (2000) Differential effects of estrogen and progesterone on AT(1) receptor gene expression in vascular smooth muscle cells. Circulation. 102, 1828-33.

38. Chidambaram, M., Duncan, J. A., Lai, V. S., Cattran, D. C., Floras, J. S., Scholey, J. W. and Miller, J. A. (2002) Variation in the renin angiotensin system throughout the normal menstrual cycle. J Am Soc Nephrol. 13, 446-52.

39. Danser, A. H., Derkx, F. H., Schalekamp, M. A., Hense, H. W., Riegger, G. A. and Schunkert, H. (1998) Determinants of interindividual variation of renin and prorenin concentrations: evidence for a sexual dimorphism of (pro)renin levels in humans. J Hypertens. 16, 853-62.

40. Goldhaber, S. Z., Hennekens, C. H., Spark, R. F., Evans, D. A., Rosner, B., Taylor, J. O. and Kass, E. H. (1984) Plasma renin substrate, renin activity, and aldosterone levels in a sample of oral contraceptive users from a community survey. Am Heart J. 107, 119-22.

41. Harvey, P. J., Wing, L. M., Savage, J. and Molloy, D. (1999) The effects of different types and doses of oestrogen replacement therapy on clinic and ambulatory blood pressure and the renin-angiotensin system in normotensive postmenopausal women. J Hypertens. 17, 405-11.

42. Hassager, C., Riis, B. J., Strom, V., Guyene, T. T. and Christiansen, C. (1987) The long-term effect of oral and percutaneous estradiol on plasma renin substrate and blood pressure. Circulation. 76, 753-8.

43. Laragh, J. H., Sealey, J. E., Ledingham, J. G. and Newton, M. A. (1967) Oral contraceptives. Renin, aldosterone, and high blood pressure. Jama. 201, 918-22.

44. Schunkert, H., Danser, A. H., Hense, H. W., Derkx, F. H., Kurzinger, S. and Riegger, G. A. (1997) Effects of estrogen replacement therapy on the renin-angiotensin system in postmenopausal women. Circulation. 95, 39-45.

45. Seely, E. W., Brosnihan, K. B., Jeunemaitre, X., Okamura, K., Williams, G. H., Hollenberg, N. K. and Herrington, D. M. (2004) Effects of conjugated oestrogen and droloxifene on the renin-angiotensin system, blood pressure and renal blood flow in postmenopausal women. Clin Endocrinol (Oxf). 60, 315-21.

46. Feldmer, M., Kaling, M., Takahashi, S., Mullins, J. J. and Ganten, D. (1991) Glucocorticoid- and estrogen-responsive elements in the 5'-flanking region of the rat angiotensinogen gene. J Hypertens. 9, 1005-12.

47. Fischer, M., Baessler, A. and Schunkert, H. (2002) Renin angiotensin system and gender differences in the cardiovascular system. Cardiovasc Res. 53, 672-7.

48. Brosnihan, K. B., Li, P., Ganten, D. and Ferrario, C. M. (1997) Estrogen protects transgenic hypertensive rats by shifting the vasoconstrictor-vasodilator balance of RAS. Am J Physiol. 273, R1908-15.

49. Olsson, R. and Carlsson, P. O. (2006) The pancreatic islet endothelial cell: emerging roles in islet function and disease. Int J Biochem Cell Biol. 38, 710-4.

50. Leung, W. H., Lau, C. P. and Wong, C. K. (1993) Beneficial effect of cholesterol-lowering therapy on coronary endothelium-dependent relaxation in hypercholesterolaemic patients. Lancet. 341, 1496-500.

51. Serne, E. H., Stehouwer, C. D., ter Maaten, J. C., ter Wee, P. M., Rauwerda, J. A., Donker, A. J. and Gans, R. O. (1999) Microvascular function relates to insulin sensitivity and blood pressure in normal subjects. Circulation. 99, 896-902.

52. Auer, J., Berent, R., Weber, T. and Eber, B. (2002) Clinical significance of pleiotropic effects of statins: lipid reduction and beyond. Curr Med Chem. 9, 1831-50.

53. Egashira, K., Hirooka, Y., Kai, H., Sugimachi, M., Suzuki, S., Inou, T. and Takeshita, A. (1994) Reduction in serum cholesterol with pravastatin improves endothelium-dependent coronary vasomotion in patients with hypercholesterolemia. Circulation. 89, 2519-24.

54. Muramatsu, J., Kobayashi, A., Hasegawa, N. and Yokouchi, S. (1997) Hemodynamic changes associated with reduction in total cholesterol by treatment with the HMG-CoA reductase inhibitor pravastatin. Atherosclerosis. 130, 179-82.

55. Williams, J. K., Sukhova, G. K., Herrington, D. M. and Libby, P. (1998) Pravastatin has cholesterol-lowering independent effects on the artery wall of atherosclerotic monkeys. J Am Coll Cardiol. 31, 684-91.

56. Sjoholm, A. and Nystrom, T. (2006) Inflammation and the etiology of type 2 diabetes. Diabetes Metab Res Rev. 22, 4-10.

57. Freeman, D. J., Norrie, J., Sattar, N., Neely, R. D., Cobbe, S. M., Ford, I., Isles, C., Lorimer, A. R., Macfarlane, P. W., McKillop, J. H., Packard, C. J., Shepherd, J. and Gaw, A. (2001) Pravastatin and the development of diabetes mellitus:

evidence for a protective treatment effect in the West of Scotland Coronary Prevention Study. Circulation. 103, 357-62.

58. Newsholme, P., Keane, D., Welters, H.J. and Morgan, N.G. Life and death decisions of the pancreatic beta-cell: the role of fatty acids. (2007) Clin Sci (Lond). 112, 27-42.

Legends to figures

Figure 1: Enhancement of pancreatic blood flow by pravastatin, captopril, candesartan and irbesartan in *male* and *female* GK rats.

Following i.v. injection of pravastatin (0.5 mg/kg BW), captopril (3 mg/kg BW) or candesartan (10 mg/kg BW) into *male* GK rats, rates of blood perfusion in the whole pancreas **(1a)** were measured using microsphere technique. Separate experiments with irbesartan (3 mg/kg BW) are shown in **(1b)**. Bars represent means ± SEM for 5 independent experiments. * and ** denote $P<0.05$ and $P<0.01$, respectively, for chance differences *vs* controls using ANOVA.

Figure 2: Enhancement of pancreatic islet blood flow by pravastatin, captopril, candesartan and irbesartan in *male* and *female* GK rats.

Following i.v. injection of pravastatin (0.5 mg/kg BW), captopril (3 mg/kg BW) or candesartan (10 mg/kg BW) into *male* GK rats, rates of blood perfusion in pancreatic islets (**2a**) were measured using a microsphere technique. Separate experiments with irbesartan (3 mg/kg BW) are shown in **(2b)**. Bars represent means ± SEM for 5 independent experiments. * and ** denote $P<0.05$ and $P<0.01$, respectively, for chance differences *vs* controls using ANOVA.

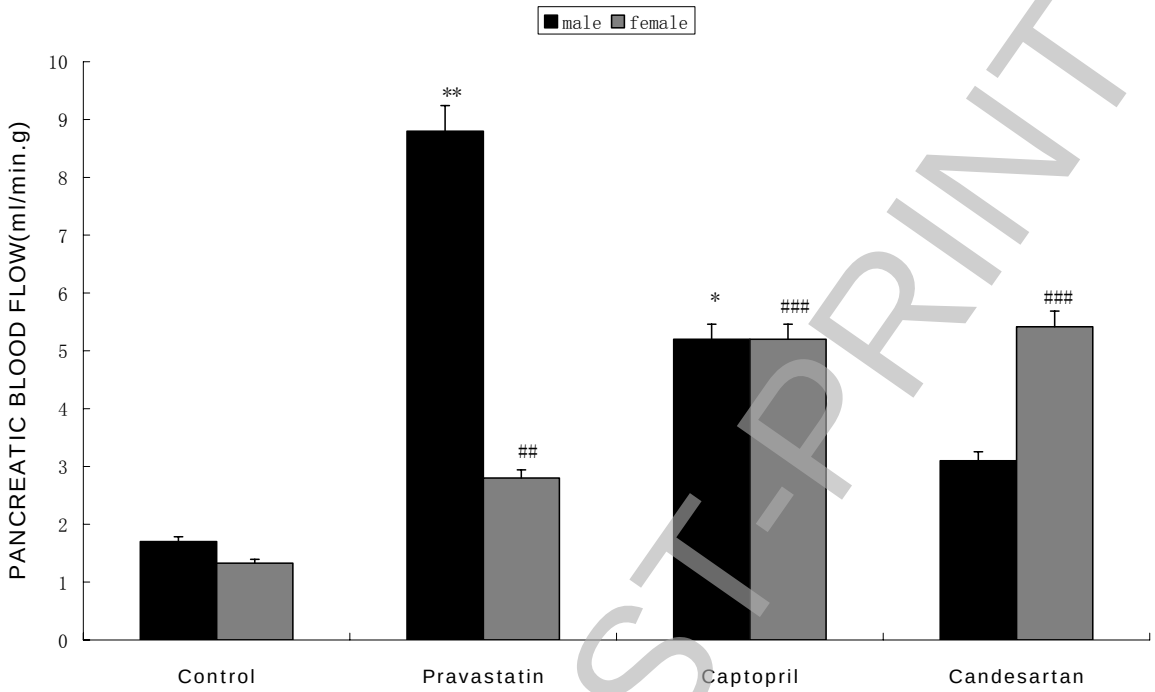
Figure 3: Suppression of pancreatic islet blood flow by palmitate in both *male* and *female* GK rats.

Following i.v. injection of palmitate (60 mg/kg BW) or ethanol (solvent) into *male* or *female* GK rats, pancreatic islet blood flow was measured using a microsphere technique. Bars represent means ± SEM for 5 independent experiments. * denotes $P<0.05$ for a chance difference *vs* controls using ANOVA.

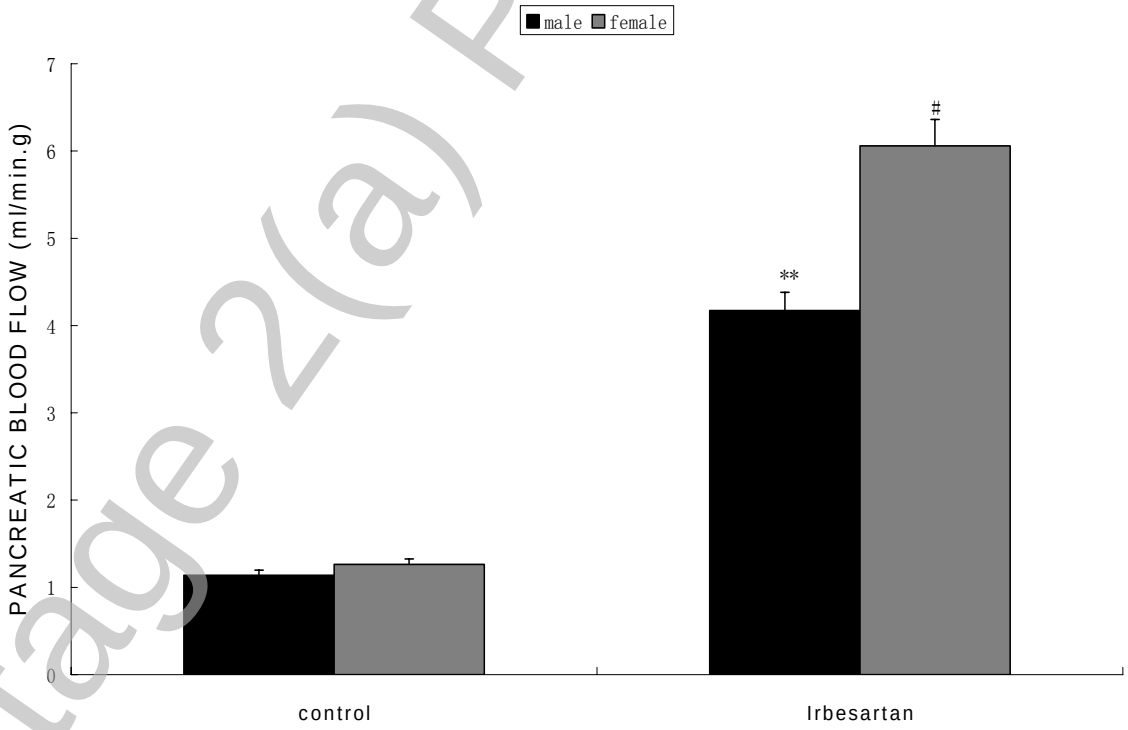
Clinical Science Immediate Publication. Published on 21 Jan 2008 as manuscript CS20070386

Figure 1

a.



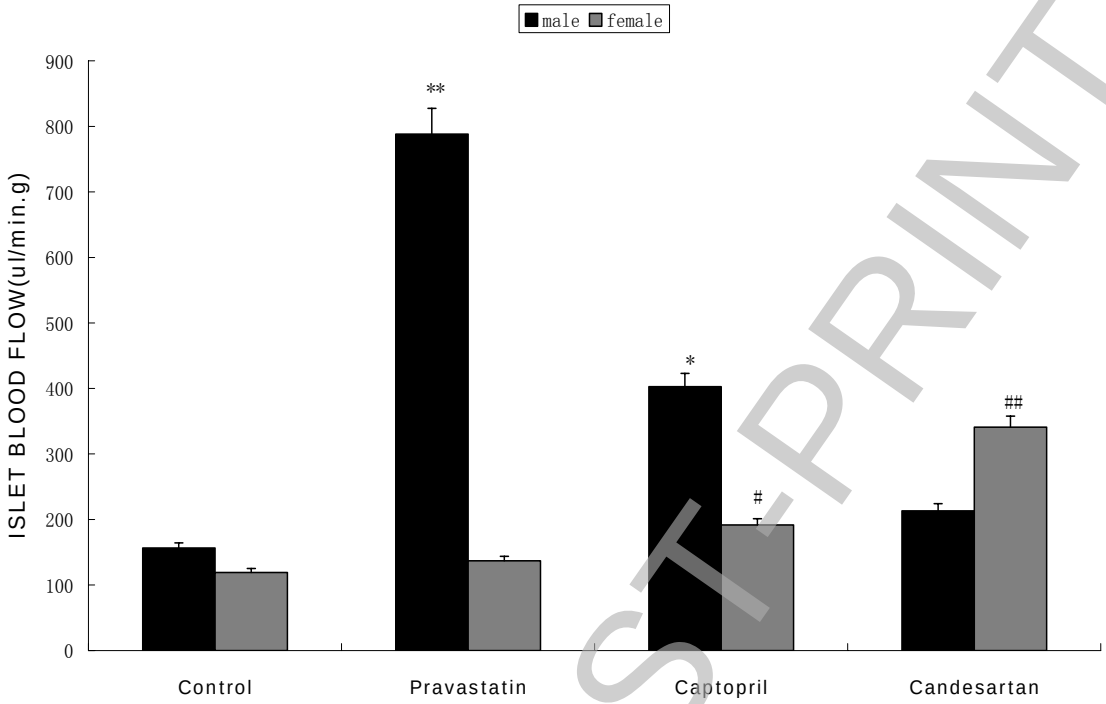
b.



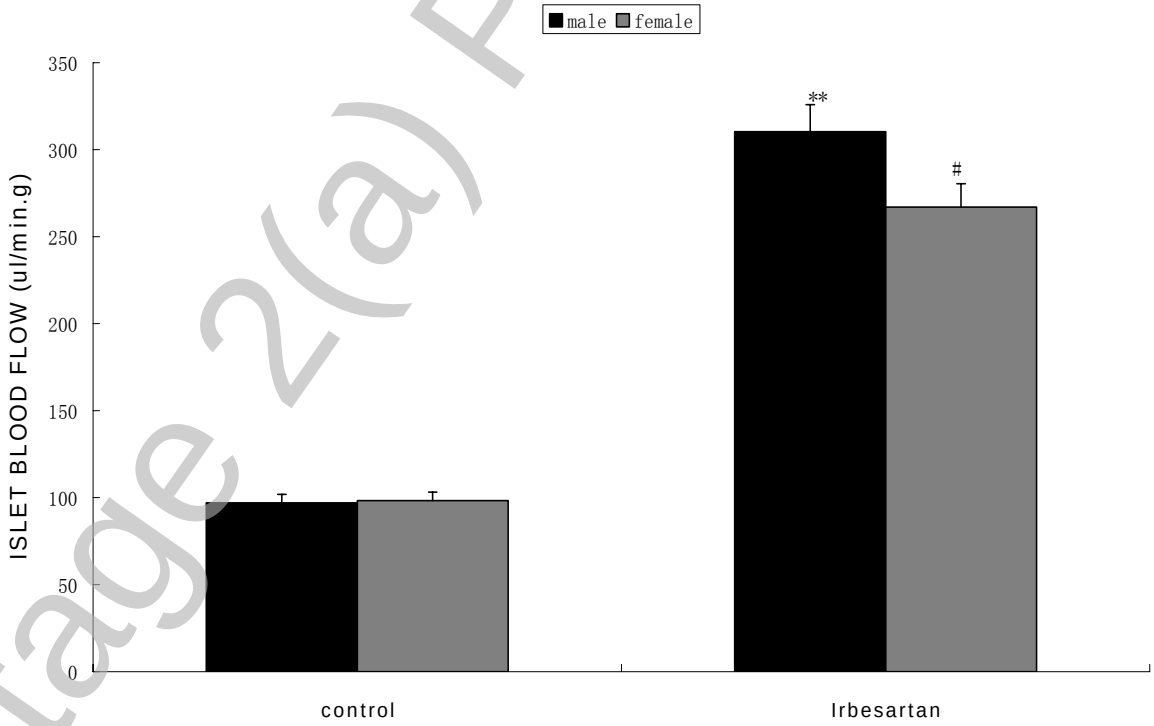
Clinical Science Immediate Publication. Published on 21 Jan 2008 as manuscript CS20070386

Figure 2

a.



b.



Clinical Science Immediate Publication. Published on 21 Jan 2008 as manuscript CS20070386

Figure 3

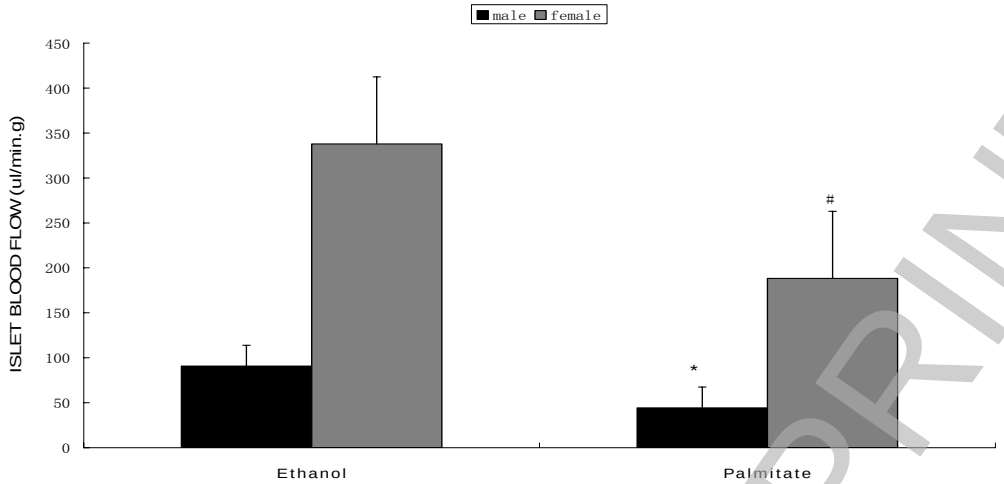


Table1

	Male		*Female*	
	KBF (ml/min/g)	**ABF** (ml/min/g)	**KBF** (ml/min/g)	**ABF** (ml/min/g)
Control	9.4±1.1	18.5±1.7	4.484±0.8	6.46±1.5
Pravastatin	30.5±4.6**	39.1±6.6**	17.245±0.97***	9.214±1.8
Captopril	16.6±4.8	19.9±5.9	30.935±3.4***	19.848±3**
Candesartan	22.7±6.7*	20.7±6.8	30.574±2.5***	17.895±2.5**
Control	7.75±2.5	7.8±1.9	23.791±5.7	18.23±3.9
Palmitate	6.7±1.4	4.2±1.2	29.509±5.5	28.281±1.8*
Control	9.1±0.8	20.8±0.9	5.1±0.6	11.7±2
Irbesartan	25.3±3.1*	36.9±1.3	25.6±3.1**	18.3±1.9

After i.v. injection of test substances into male or female GK rats, rates of blood perfusion in the kidney and adrenals were measured using a microsphere technique. Data are means ± SE. * $P<0.05$; ** $P<0.01$; *** $P<0.001$ when compared with the corresponding control strain (Student's unpaired *t* test and ANOVA).

Clinical Science Immediate Publication. Published on 21 Jan 2008 as manuscript CS20070386

Table 2.

In male GK group	Blood Glucose (mmol/l)	Serum Insulin (ng/ml)	Blood Pressure (mmHg)
Control	7.7 ± 0.4	4.46 ± 0.4	98.6 ± 2.7
Pravastatin	7.6 ± 0.6	7.19 ± 1.2 *	101.6 ± 3.4
Captopril	10.9 ± 1 **	8.89 ± 1.3 ***	108 ± 2.4
Candesartan	9.4 ± 1.4	5.35 ± 0.8	107 ± 2.7
Ethanol	8.2 ± 0.6	8.19 ± 1.9	110 ± 2.9
Palmitate	12.6 ± 1.9 *	5.49 ± 0.9	115 ± 4.7
Control	8.26 ± 0.3	4.08 ± 0.1	95.5 ±1.9
Irbesartan	7.58 ± 0.6	4.22 ± 0.4	103.7 ± 3.7

Blood glucose was measured with test strips based on the glucose oxidase method. Serum insulin concentrations were analyzed with an ELISA kit. Blood pressure was constantly monitored by a pressure transducer. Data are means ± SE. * $P<0.05$; ** $P<0.01$; *** $P<0.001$ when compared with the corresponding control strain (Student's unpaired *t* test and ANOVA).

Table 3.

In female GK group	Blood Glucose (mmol/l)	Serum Insulin (ng/ml)	Blood Pressure (mmHg)
Control	8.3 ± 0.6	3.05 ± 0.25	94.2 ± 3.5
Pravastatin	11.2 ± 0.4 ***	3.09 ± 0.26	98.1 ± 1.3
Captopril	11.3 ± 0.6 ***	3.01 ± 0.09	102 ± 3.6
Candesartan	9.7 ± 11	3.14 ± 0.12	105 ± 2.3
Ethanol	9.3 ± 0.8	4.15 ± 0.43	109 ± 5.9
Palmitate	9.95 ± 0.5	3.1 ± 0.17 *	110 ± 1.7
Control	9.16 ± 0.4	3.08 ± 0.1	95.1 ±1
Irbesartan	10.4 ± 0.6	3.1 ± 0.1	105.2 ± 6.1

Blood glucose was measured with test strips based on the glucose oxidase method. Serum insulin concentrations were analyzed with an ELISA kit. Blood pressure was constantly monitored by a pressure transducer. Data are means ± SE. * $P<0.05$; ** $P<0.01$; *** $P<0.001$ when compared with the corresponding control strain (Student's unpaired *t* test and ANOVA).