Akita mice were elevated compared with WT mice; however, they remained >4% lower than those of Akita mice, even at 40 weeks of age (Fig. 1*D*). Average HbA_{1c} levels were 11.4 ± 0.9% in Akita, 7.5 ± 1.1% in LepTg:Akita, 3.9 ± 0.3% in LepTg, and 4.1 ± 0.9% in WT mice from 5 to 40 weeks of age.

Time course of body weight and food intake. The body weight of all groups of mice increased gradually from birth (Fig. 1*E*). LepTg:Akita mice weighed significantly less than did Akita mice from 5 to 20 weeks of age. Akita mice lost body weight after 20 weeks of age. Percent body fat at 18 weeks of age was 27.6 ± 1.9, 29.9 ± 0.5, 2.1 ± 0.5, and 1.0 ± 0.4% for WT, LepTg, Akita, and LepTg:Akita mice, respectively.

The food intake of Akita mice was nearly double that of the other three groups of mice during the observation period (Fig. 1*F*). Food intake did not differ significantly between the WT, LepTg, and LepTg:Akita mice at any time during the study.

Pair-feeding experiments. To investigate whether leptin's ability to decrease food intake is the main reason for its efficacy in improving diabetes, Akita mice were pair fed to achieve the ad libitum food intake of LepTg:Akita mice for from 3 weeks to 8 weeks of age. Although pair feeding reduced the body weight of Akita mice to that of LepTg:Akita mice (Fig. 1*G*), it did not significantly improve the blood glucose levels in the Akita mice (Fig. 1*H*).

Glucose tolerance and insulin sensitivity. GTTs were performed to evaluate glucose metabolism further (Fig. 2*A*). At 8 weeks of age, Akita mice had reduced glucose tolerance; however, LepTg:Akita mice exhibited normal glucose tolerance similar to that of the LepTg and WT mice of the same age. At 16 weeks of age, Akita mice developed more severe glucose intolerance than that at 8 weeks of age. Although glucose tolerance was impaired in 16-week-old LepTg:Akita mice compared with that of LepTg and WT mice, it was better than that of 8- and 16-week-old Akita mice. These results indicate that hyperleptinemia significantly improved glucose tolerance during the progressive course of diabetes in Akita mice.

ITTs were performed to determine whether the improved glucose tolerance observed in LepTg:Akita mice was associated with insulin sensitivity (Fig. 2*B*). At 8 weeks of age, both LepTg and LepTg:Akita mice showed similar, exaggerated hypoglycemic responses to insulin relative to WT and Akita mice (Fig. 2*C*). At 18 weeks of age, the effect of insulin was blunted in LepTg mice compared with that in LepTg:Akita mice and was comparable with those in WT and Akita mice. At 28 weeks of age, glucose responses to insulin in LepTg and WT mice were severely impaired compared with those in Akita and LepTg:Akita mice. Insulin sensitivities in LepTg and WT mice deteriorated in parallel with advancing age and increasing body weight. In contrast, insulin sensitivities in Akita mice did not deteriorate with age, and the enhanced sensitivity in LepTg:Akita mice did not change at all during the course of our study.

Secretion and production of insulin and glucagon. Insulin secretion in response to a maximal glucose challenge was assessed. Plasma insulin levels were measured after an injection of glucose (3 g/kg body wt i.p.) (Fig. 3*A*). The fasting insulin levels were similar in LepTg, Akita, and LepTg:Akita mice at 8 weeks of age, and all were significantly lower than in WT mice at the same age.

However, the fasting plasma insulin-to-glucose ratio was about three times higher in LepTg:Akita mice than in Akita mice. Both WT and LepTg mice showed an acute insulin response to glucose, Akita mice had virtually no response, and LepTg:Akita mice maintained a slow and slight response.

Plasma glucagon concentration was measured in ad libitum–fed mice at 22 weeks of age (Fig. 3*B*). Despite marked hyperglycemia, the glucagon level in Akita mice was nearly twice that in WT and LepTg mice. By contrast, LepTg:Akita mice had a normal plasma glucagon level, equivalent to that in the WT and LepTg mice.

Total pancreatic insulin contents of the Akita and LepTg:Akita mice decreased similarly to about one-tenth of those in the WT and LepTg mice (Fig. 3*C*). The pancreatic glucagon content of the Akita mice was twice that of the WT and LepTg mice; the glucagon content of the LepTg:Akita was half that in Akita mice (Fig. 3*D*).

Immunohistochemical examination of the pancreas revealed that Akita mice had profoundly abnormal islet histology with few active β-cells and a higher proportion of α-cells compared with WT and LepTg mice (Fig. 3*E* and *F*). LepTg:Akita mice had fewer β-cells, but α-cell hyperplasia was suppressed relative to Akita mice (Fig. 3*E* and *F*). These characteristics agree with the plasma hormone levels (Fig. 3*A* and *B*) and pancreatic hormone contents (Fig. 3*C* and *D*).

Lipids and ketones. Transgenic leptinemia did not significantly affect plasma levels of TG, NEFA, and β-hydroxybutyrate in WT mice (Fig. 4*A–C*). Akita mice had lower levels of both NEFA and β-hydroxybutyrate, possibly reflecting their lower adipose mass (Fig. 4*B* and *C*). None of the Akita mice developed ketonuria as determined by a urine ketone dipstick test (data not shown). In the Akita mice, leptin significantly decreased plasma TG levels by half (LepTg:Akita 37.4 ± 11.6 vs. Akita 89.4 ± 13.6 mg/dL; $P < 0.05$) (Fig. 4*A*) but did not change plasma NEFA or β-hydroxybutyrate levels (Fig. 4*B* and *C*).

Systemic oxidative stress. The plasma level of TBARS was examined as a marker of systemic oxidative stress (Fig. 4*D*). Akita mice exhibited the highest TBARS levels (35.1 ± 4.9 μmol/L) of the four genotypes at 18 weeks of age; the plasma TBARS levels were similar in WT, LepTg, and LepTg:Akita mice (WT 12.7 ± 1.3, LepTg 16.3 ± 1.8, and LepTg:Akita 17.8 ± 2.3 μmol/L).

Diabetic nephropathy. The renoprotective effects of leptin were investigated in Akita mice (Fig. 5*A*). Akita mice developed overt albuminuria at 12 weeks of age, and urinary albumin excretion was >200 μg/day during the follow-up period. By contrast, the increase in albuminuria was largely attenuated in LepTg:Akita mice throughout the follow-up period.

The increase in mesangial matrix (defined as mesangial area) observed in Akita mice was prevented completely in LepTg:Akita mice at 22 weeks of age (Fig. 5*B* and Table 1). Systolic and diastolic blood pressure and heart rates did not differ significantly between the four groups of mice (Table 1).

in WT, LepTg, Akita, and LepTg:Akita mice at 18 weeks of age ($n \geq 4$in each group). Data are expressed as means ± SE. ††$P < 0.01$ for WT vs. LepTg, §§$P < 0.01$ for WT vs. Akita, ‡$P < 0.05$, ‡‡$P < 0.01$ for WT vs. LepTg:Akita, ¶$P < 0.05$, ¶¶$P < 0.01$ for LepTg vs. Akita, ★$P < 0.05$, ★★$P < 0.01$ for LepTg vs. LepTg:Akita, *$P < 0.05$, and **$P < 0.01$ for Akita vs. LepTg:Akita. (A high-quality digital representation of this figure is available in the online issue.)

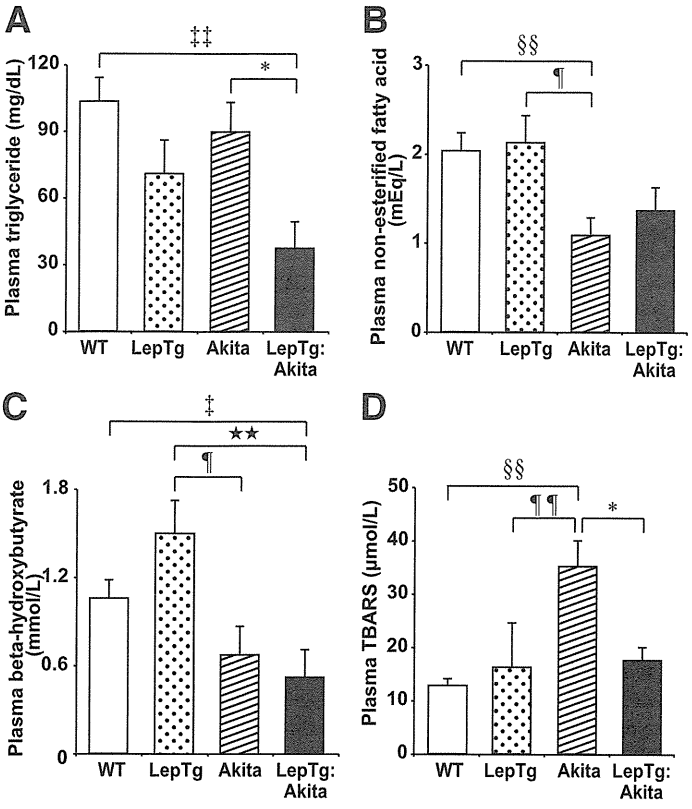

FIG. 4. Plasma levels of TG, NEFA, β-hydroxybutyrate, and TBARS. *A* and *B*: Fasting plasma levels of TG (*A*) and NEFA (*B*) concentrations in WT, LepTg, Akita, and LepTg:Akita mice at 18 weeks of age ($n \geq 4$ in each group). *C*: Fasting plasma levels of β-hydroxybutyrate concentrations in WT, LepTg, Akita, and LepTg:Akita mice at 11 weeks of age ($n \geq 4$ in each group). *D*: Fasting plasma levels of TBARS concentrations in WT, LepTg, Akita, and LepTg:Akita mice at 18 weeks of age ($n \geq 4$ in each group). Data are expressed as means ± SE. §§$P < 0.01$ for WT vs. Akita, ‡$P < 0.05$, ‡‡$P < 0.01$ for WT vs. LepTg:Akita, ¶$P < 0.05$, ¶¶$P < 0.01$ for LepTg vs. Akita, ★★$P < 0.01$ for LepTg vs. LepTg:Akita, and *$P < 0.05$ for Akita vs. LepTg:Akita.

Survival rate. As shown in Fig. 6, the first Akita mouse died at 27 weeks of age, and half of the mice died within 40 weeks. Before death, Akita mice exhibited rough coats and decreased activity. Blood analysis showed liver and kidney dysfunction and extreme hyperglycemia ~1,000 mg/dL without overt ketone body production.

By contrast, the life span was significantly longer in LepTg:Akita mice than in Akita mice ($n \geq 14$, $P < 0.01$), and none of the LepTg:Akita mice died during the observation period of 1 year (Fig. 6). Considering that the median survival time of male C57BL/6 mice is ~120 weeks (The Jackson Laboratory, Bar Harbor, ME), the survival rate did not appear to be affected significantly in LepTg:Akita mice.

DISCUSSION

The current study demonstrates that transgenic expression of leptin raised its plasma concentration to the level observed in morbidly obese individuals, markedly reduced mortality, and prolonged the survival time in Akita mice. The extension of life span in LepTg:Akita mice was accompanied by various beneficial effects on the course of diabetes.

We have pursued the therapeutic potential of leptin as an antidiabetic agent using transgenic skinny mice and propose that leptin could be used therapeutically in the treatment of diabetes of different etiology and pathophysiology (2,5–8,11–13). Leptin effectively improves glucose and lipid metabolism in streptozotocin (STZ)-induced insulinopenic diabetic mice (7) and in mildly obese mice fed a high-fat diet and administered low-dose STZ (6). Transgenic overexpression of leptin can delay the onset of insulin resistance and diabetes in KKAy mice at younger ages, when they are of normal weight (8). These findings suggest that leptin alone is effective in treating diabetes without obesity-induced leptin resistance. We also found that transgenic overexpression of leptin can rescue insulin resistance and diabetes in a mouse model of lipoatrophic diabetes, showing that leptin should be effective in the treatment of lipoatrophic diabetes (5). Therapeutic leptinemia can be achieved clinically by subcutaneous injection of recombinant human leptin (11,12). Leptin has been used in the treatment of human diabetes in patients with leptin deficiency and lipodystrophy (11–14).

Leptin delayed the onset and progression of diabetes in Akita mice. Hyperglycemia after a 16-h fast was prevented completely for >10 months in LepTg:Akita mice. The onset of the increase in the 6-h fasting blood glucose and HbA_{1c} levels was also delayed for at least several weeks. Good metabolic control was achieved after the onset of diabetes in LepTg:Akita mice. There are several possible explanations for this glucose-lowering effect of leptin. This study demonstrated that the constitutive hyperleptinemia (approximately 10 times higher than control) strongly and stably increased insulin sensitivity in Akita mice. Leptin increases the effects of insulin in suppressing hepatic glucose production and stimulating muscular glucose uptake (2). The mechanisms through which leptin regulates insulin signaling are not understood completely, although reduction in ectopic fat deposition, especially in the liver and muscle, and activation of AMP-activated protein kinase in the peripheral tissues via stimulation of the hypothalamic-autonomic nervous system are important components (6,15,16). Increased mitochondrial biogenesis and oxygen consumption in skeletal muscle and adipose tissue may also contribute to the increase in glucose disposal independent of insulin action (15,17,18). Previous studies also demonstrated the antidiabetic effects of leptin in insulin-deficient diabetic rodents (19,20).

This study showed no attenuation of the biological effects of leptin (increase in insulin sensitivity and decrease in food intake) in Akita mice throughout the long follow-up. Most of the obese patients have elevated plasma leptin levels (21,22), implying leptin resistance for weight control (23). The basis for such leptin resistance is not understood enough, although such resistance coexists with hyperinsulinemia and insulin resistance (24). This context suggests that leptin has potent therapeutic effects on insulin-deficient diabetes with minimum insulin intervention (7). We and others reported that exogenously administered leptin can normalize hyperglycemia in STZ-induced diabetes, when fed plasma insulin levels were >0.10 ng/mL (7,25). The glycemic control of LepTg:Akita mice worsened gradually when the plasma insulin levels became extremely low (<0.10 ng/mL). These results show that the threshold of plasma insulin level is ~0.10 ng/mL, above which leptin can prevent hyperglycemia.

Akita mice, which have low plasma insulin and leptin levels, have increased food intake. Transgenic hyperleptinemia prevented hyperphagia in Akita mice. Although short-term food restriction did not affect hyperglycemia in Akita mice, continuous reduction in food intake might

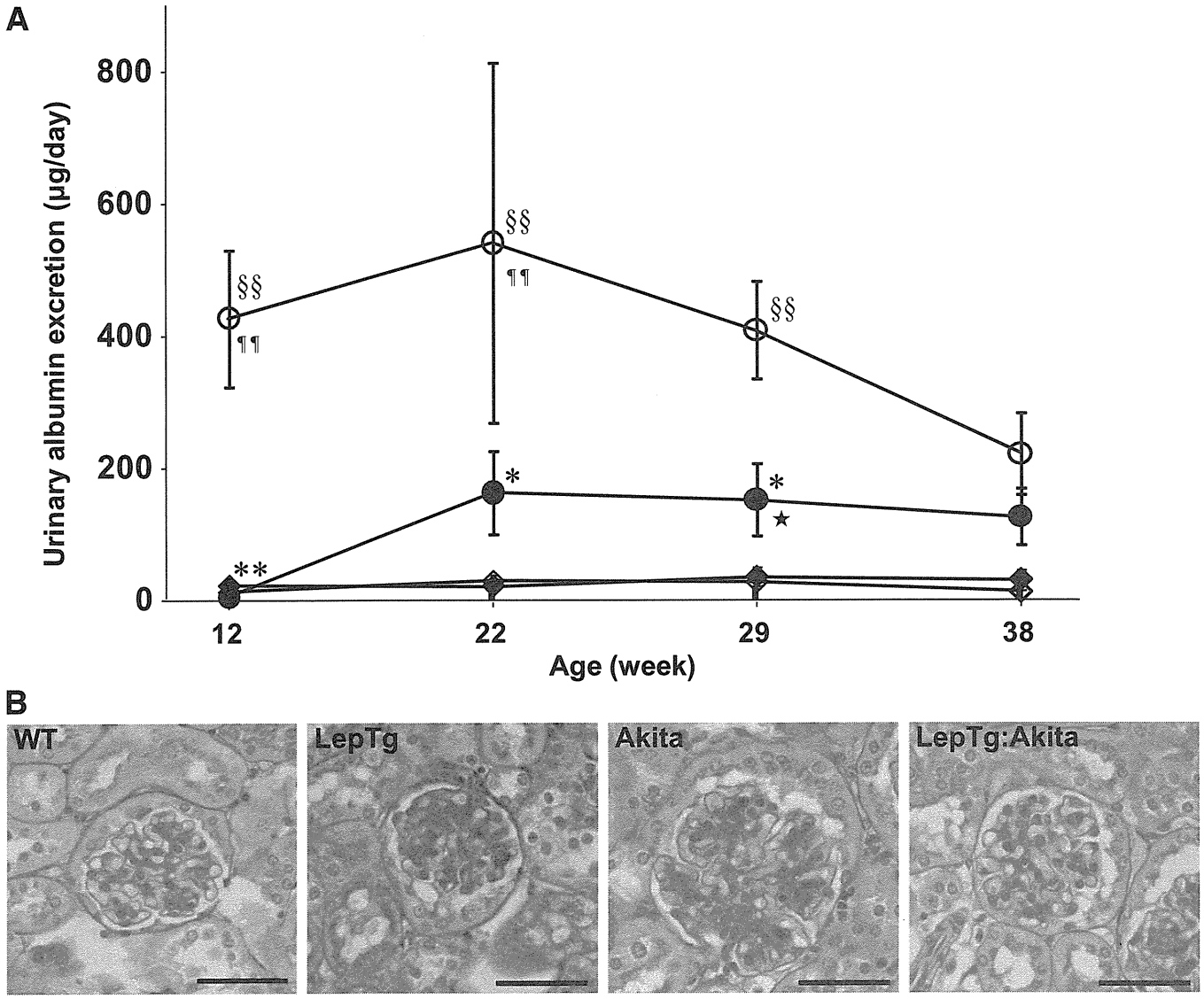

FIG. 5. Urinary albumin excretion and histology of glomeruli. *A*: Time course of urinary albumin excretion of WT (◇), LepTg (◆), Akita (○), and LepTg:Akita (●). Data are expressed as means ± SE ($n \geq 4$ in each group). §§$P < 0.01$ for WT vs. Akita, ¶¶$P < 0.01$ for LepTg vs. Akita, ★$P < 0.01$ for LepTg vs. LepTg:Akita, *$P < 0.05$, and **$P < 0.01$ for Akita vs. LepTg:Akita. *B*: PAS-staining of representative glomeruli from WT, LepTg, Akita, and LepTg:Akita mice at 22 weeks of age. Scale bar indicates 50 μm. (A high-quality color representation of this figure is available in the online issue.)

play a role in the antidiabetic effect of leptin. In insulin-deficient diabetic animals, adiposity and plasma leptin levels decrease and food intake increases concomitantly (26). In our previous report, leptin administration reversed hyperphagia by correcting the imbalance in the hypothalamic neuropeptide expression in STZ-administered mice (7). Upregulation of orexigenic neuropeptides (neuropeptide Y and agouti-related peptide) and downregulation of anorexigenic neuropeptides (proopiomelanocortin) were observed in the hypothalamus of insulin-deficient diabetic mice (7,27). Leptin and insulin are crucial signals that convey "adiposity negative feedback" information to the hypothalamus. Our previous and present results indicate that leptin is useful for preventing diabetic hyperphagia.

Glucose-stimulated insulin secretion and pancreatic insulin content were markedly lower in both LepTg:Akita and Akita mice compared with WT and LepTg mice; however, both the plasma concentration and pancreatic content of glucagon decreased to the normal level in LepTg:Akita mice. Leptin is reported to suppress the synthesis and secretion of insulin by pancreatic β-cells (28,29). Our results did not reveal any negative effect of leptin on β-cell function in Akita mouse but showed that systemic hyperleptinemia plays a role in restoring the insulin-glucagon balance to proper equilibrium, which is lost in Akita mice. Wang et al. (30) reported recently that like insulin, leptin suppresses glucagon secretion in NOD mice. Our current data suggest that the antiglucagon and insulin-sensitizing effects of leptin on glucoregulatory hormones are therapeutically useful actions.

We found that chronic overexpression of leptin effectively prevented the development of diabetic nephropathy in Akita mice, as we have also demonstrated in lipoatrophic diabetic A-ZIP/F1 mice (31). Leptin suppressed the induction of massive albuminuria and the expansion of mesangial matrix in the glomeruli of Akita mice. Increased urinary albumin excretion and mesangial matrix accumulation are well-established features of diabetic nephropathy. Akita mice manifest the typical renal injury observed in human diabetic nephropathy that is associated

TABLE 1
Renal characteristics of 22-week-old F1 mice

	WT	LepTg	Akita	LepTg:Akita
Albuminuria (μg/day)	32.0 ± 9.4	23.5 ± 13.1	544.6 ± 272.7	165.9 ± 62.1*
Urine volume (mL/day)	1.7 ± 0.4	0.9 ± 0.1	27.4 ± 4.6	7.3 ± 2.2**
Mesangial area (μm^2)	1,210 ± 50	1,278 ± 50	2,025 ± 76	1,222 ± 67**
Body weight (g)	37.1 ± 1.7	38.4 ± 1.3	26.2 ± 0.9	25.0 ± 0.5
Kidney weight (g)	0.21 ± 0.03	0.18 ± 0.02	0.26 ± 0.04	0.21 ± 0.01
sBP (mmHg)	96.8 ± 0.3	93.5 ± 1.9	98.5 ± 4.1	107.7 ± 1.3
dBP (mmHg)	44.0 ± 0.5	42.3 ± 3.8	47.6 ± 3.9	52.2 ± 4.7
Heart rate (bpm)	714 ± 18	726 ± 7	611 ± 32	730 ± 30

Values are expressed as the mean ± SE ($n = 4$). sBP, systolic blood pressure; dBP, diastolic blood pressure. *$P < 0.05$. **$P < 0.01$, LepTg:Akita vs. Akita.

with renal hypertrophy, glomerular hypertrophy, mesangial expansion, and overt proteinuria (32). The nephropathy in Akita mice is more similar to that seen in human patients with diabetes than is the nephropathy in chemically induced diabetic mice (32). Although several reports suggest that leptin exerts profibrotic action in the kidney, which has caused concern about possible pathogenic roles of leptin in obesity-related glomerulopathy (31,33), the present results clearly show that leptin prevented renal injury in Akita mice. It is likely that leptin is beneficial for nephropathy in patients with insulin-dependent diabetes.

Reduced insulin action in diabetes elevates plasma TG levels by decreasing lipoprotein lipase activity and increasing hormone-sensitive lipase activity. We demonstrated previously a significant reduction in plasma VLDL-TG level in LepTg mice (34). Leptin suppresses the activities of liver lipogenic enzymes (35,36). In LepTg:Akita mice, decreased levels of plasma lipids may also result from dwindling body fat stores (orthotopic and ectopic) because of augmented effects of leptin. Since hypertriglyceridemia is reported to be an independent cardiovascular risk factor in patients with glucose intolerance (37), our observation of the TG-lowering effects of leptin may be useful in preventing and treating diabetic cardiovascular complications.

Lipid peroxidation is a well-established mechanism of cellular injury as a diabetes complication and is used as an indicator of oxidative stress. Increased oxidative stress also participates in the development and progression of diabetes and its complications (38). LepTg:Akita mice maintained normal levels of plasma TBARS, in contrast to Akita mice. Increased levels of serum TBARS were reported in patients with peripheral arterial disease, ischemic heart disease, hypertension, and diabetes (39). Our finding that leptin relieved systemic oxidative stress in Akita mice is of interest because TBARS level does not depend only on the blood glucose or lipid level but reflects the complex net redox balance (40,41).

Various interventions have been reported to improve the metabolic profiles in Akita mice (42,43). Targeted disruption of the transcription factor C/EBP homologous protein gene or C/EBP-β gene alleviates endoplasmic reticulum stress in pancreatic β-cells and improves hyperglycemia in Akita mice (42). Intracerebroventricular administration of adeno-associated viral vector expressing leptin also attenuates hyperglycemia in Akita mice (43). However, it is unclear whether those interventions are directly applicable to the human therapeutic settings. Therapeutic leptinemia in LepTg mice is induced by transgenic overexpression. Leptinemia can be achieved clinically by subcutaneous injection of leptin, as shown in leptin-replacement therapy (11,12,44). Whether leptin affects the immunological processes of type 1 diabetes remains to be established. Leptin, a cytokine-like hormone, is suggested to be involved in linking nutritional status and immune response (45). Leptin administration accelerates autoimmune diabetes in NOD mice, and the incidence of diabetes is significantly reduced in NOD mice and BB/Wor rats with Ob-R mutation (46–48). However, another study that assessed NOD mice with defective leptin signaling (*Ay*, *db/db*, and *ob/ob*) has shown that leptin is not essential for the development of autoimmune diabetes (49). Whether the beneficial effects of leptin in Akita mice can be translated to type 1 diabetes in humans will be important to determine.

In conclusion, the current study demonstrates that leptin has a therapeutic impact on the onset and progression of glucose intolerance, diabetes complications, and longevity in a mouse model of insulin-deficient nonobese diabetes. These data offer proof of concept that leptin may be useful as a long-term therapeutic agent for treating human diabetes.

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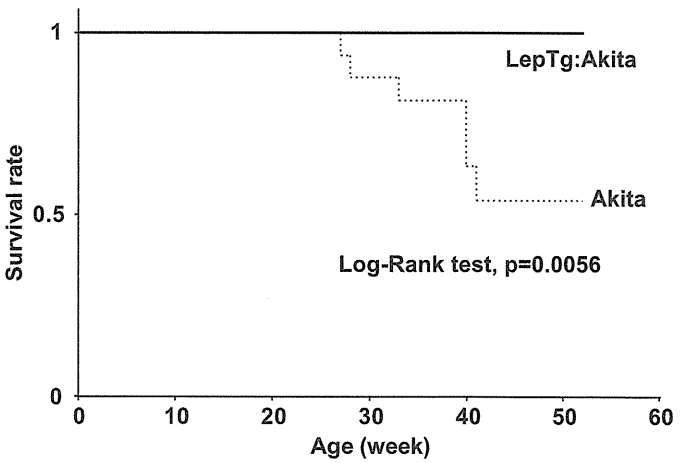

FIG. 6. Survival rates. Survival curves of Akita (dotted line) and LepTg:Akita (solid line) mice ($n \geq 11$ in each group). Survival rate of Akita mice markedly decreased relative to LepTg:Akita mice ($P < 0.01$) and was ~50% at 52 weeks of age.

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M.N. researched data. J.F. researched data and wrote the manuscript. K.E. contributed to discussion. F.M. researched data. H.Y. researched data and contributed to discussion. T.K., Y.Y., C.S., M.M., K.H., and K.N. contributed to discussion.

REFERENCES

1. Friedman JM, Halaas JL. Leptin and the regulation of body weight in mammals. Nature 1998;395:763–770
2. Ogawa Y, Masuzaki H, Hosoda K, et al. Increased glucose metabolism and insulin sensitivity in transgenic skinny mice overexpressing leptin. Diabetes 1999;48:1822–1829
3. Frederich RC, Hamann A, Anderson S, Löllmann B, Lowell BB, Flier JS. Leptin levels reflect body lipid content in mice: evidence for diet-induced resistance to leptin action. Nat Med 1995;1:1311–1314
4. Considine RV, Sinha MK, Heiman ML, et al. Serum immunoreactive-leptin concentrations in normal-weight and obese humans. N Engl J Med 1996; 334:292–295
5. Ebihara K, Ogawa Y, Masuzaki H, et al. Transgenic overexpression of leptin rescues insulin resistance and diabetes in a mouse model of lipoatrophic diabetes. Diabetes 2001;50:1440–1448
6. Kusakabe T, Tanioka H, Ebihara K, et al. Beneficial effects of leptin on glycaemic and lipid control in a mouse model of type 2 diabetes with increased adiposity induced by streptozotocin and a high-fat diet. Diabetologia 2009;52:675–683
7. Miyanaga F, Ogawa Y, Ebihara K, et al. Leptin as an adjunct of insulin therapy in insulin-deficient diabetes. Diabetologia 2003;46:1329–1337
8. Masuzaki H, Ogawa Y, Aizawa-Abe M, et al. Glucose metabolism and insulin sensitivity in transgenic mice overexpressing leptin with lethal yellow agouti mutation: usefulness of leptin for the treatment of obesity-associated diabetes. Diabetes 1999;48:1615–1622
9. Yoshioka M, Kayo T, Ikeda T, Koizumi A. A novel locus, Mody4, distal to D7Mit189 on chromosome 7 determines early-onset NIDDM in nonobese C57BL/6 (Akita) mutant mice. Diabetes 1997;46:887–894
10. Han BG, Hao CM, Tchekneva EE, et al. Markers of glycemic control in the mouse: comparisons of 6-h- and overnight-fasted blood glucoses to Hb A1c. Am J Physiol Endocrinol Metab 2008;295:E981–E986
11. Ebihara K, Masuzaki H, Nakao K. Long-term leptin-replacement therapy for lipoatrophic diabetes. N Engl J Med 2004;351:615–616
12. Ebihara K, Kusakabe T, Hirata M, et al. Efficacy and safety of leptin-replacement therapy and possible mechanisms of leptin actions in patients with generalized lipodystrophy. J Clin Endocrinol Metab 2007;92:532–541
13. Nakao K, Yasoda A, Ebihara K, Hosoda K, Mukoyama M. Translational research of novel hormones: lessons from animal models and rare human diseases for common human diseases. J Mol Med 2009;87:1029–1039
14. Dardeno TA, Chou SH, Moon HS, Chamberland JP, Fiorenza CG, Mantzoros CS. Leptin in human physiology and therapeutics. Front Neuroendocrinol 2010;31:377–393
15. Tanaka T, Hidaka S, Masuzaki H, et al. Skeletal muscle AMP-activated protein kinase phosphorylation parallels metabolic phenotype in leptin transgenic mice under dietary modification. Diabetes 2005;54:2365–2374
16. Long YC, Zierath JR. AMP-activated protein kinase signaling in metabolic regulation. J Clin Invest 2006;116:1776–1783
17. Kakuma T, Wang ZW, Pan W, Unger RH, Zhou YT. Role of leptin in peroxisome proliferator-activated receptor gamma coactivator-1 expression. Endocrinology 2000;141:4576–4582
18. Li L, Pan R, Li R, et al. Mitochondrial biogenesis and peroxisome proliferator-activated receptor-γ coactivator-1α (PGC-1α) deacetylation by physical activity: intact adipocytokine signaling is required. Diabetes 2011; 60:157–167
19. Yu X, Park BH, Wang MY, Wang ZV, Unger RH. Making insulin-deficient type 1 diabetic rodents thrive without insulin. Proc Natl Acad Sci USA 2008;105:14070–14075
20. Fujikawa T, Chuang JC, Sakata I, Ramadori G, Coppari R. Leptin therapy improves insulin-deficient type 1 diabetes by CNS-dependent mechanisms in mice. Proc Natl Acad Sci USA 2010;107:17391–17396
21. Hosoda K, Masuzaki H, Ogawa Y, et al. Development of radioimmunoassay for human leptin. Biochem Biophys Res Commun 1996;221:234–239
22. Maffei M, Halaas J, Ravussin E, et al. Leptin levels in human and rodent: measurement of plasma leptin and ob RNA in obese and weight-reduced subjects. Nat Med 1995;1:1155–1161
23. Enriori PJ, Evans AE, Sinnayah P, et al. Diet-induced obesity causes severe but reversible leptin resistance in arcuate melanocortin neurons. Cell Metab 2007;5:181–194
24. Knight ZA, Hannan KS, Greenberg ML, Friedman JM. Hyperleptinemia is required for the development of leptin resistance. PLoS ONE 2010;5:e11376
25. Chinookoswong N, Wang JL, Shi ZQ. Leptin restores euglycemia and normalizes glucose turnover in insulin-deficient diabetes in the rat. Diabetes 1999;48:1487–1492
26. Sivitz WI, Walsh S, Morgan D, Donohoue P, Haynes W, Leibel RL. Plasma leptin in diabetic and insulin-treated diabetic and normal rats. Metabolism 1998;47:584–591
27. Asakawa A, Toyoshima M, Inoue K, Koizumi A. Ins2Akita mice exhibit hyperphagia and anxiety behavior via the melanocortin system. Int J Mol Med 2007;19:649–652
28. Seufert J. Leptin effects on pancreatic beta-cell gene expression and function. Diabetes 2004;53(Suppl. 1):S152–S158
29. Kulkarni RN, Wang ZL, Wang RM, et al. Leptin rapidly suppresses insulin release from insulinoma cells, rat and human islets and, in vivo, in mice. J Clin Invest 1997;100:2729–2736
30. Wang MY, Chen L, Clark GO, et al. Leptin therapy in insulin-deficient type I diabetes. Proc Natl Acad Sci USA 2010;107:4813–4819
31. Suganami T, Mukoyama M, Mori K, et al. Prevention and reversal of renal injury by leptin in a new mouse model of diabetic nephropathy. FASEB J 2005;19:127–129
32. Gurley SB, Clare SE, Snow KP, Hu A, Meyer TW, Coffman TM. Impact of genetic background on nephropathy in diabetic mice. Am J Physiol Renal Physiol 2006;290:F214–F222
33. Wolf G, Hamann A, Han DC, et al. Leptin stimulates proliferation and TGF-beta expression in renal glomerular endothelial cells: potential role in glomerulosclerosis [see comments]. Kidney Int 1999;56:860–872
34. Matsuoka N, Ogawa Y, Masuzaki H, et al. Decreased triglyceride-rich lipoproteins in transgenic skinny mice overexpressing leptin. Am J Physiol Endocrinol Metab 2001;280:E334–E339
35. Gallardo N, Bonzón-Kulichenko E, Fernández-Agulló T, et al. Tissue-specific effects of central leptin on the expression of genes involved in lipid metabolism in liver and white adipose tissue. Endocrinology 2007; 148:5604–5610
36. Cohen P, Miyazaki M, Socci ND, et al. Role for stearoyl-CoA desaturase-1 in leptin-mediated weight loss. Science 2002;297:240–243
37. Keech A, Simes RJ, Barter P, et al.; FIELD study investigators. Effects of long-term fenofibrate therapy on cardiovascular events in 9795 people with type 2 diabetes mellitus (the FIELD study): randomised controlled trial. Lancet 2005;366:1849–1861
38. Maritim AC, Sanders RA, Watkins JB 3rd. Diabetes, oxidative stress, and antioxidants: a review. J Biochem Mol Toxicol 2003;17:24–38
39. Oberley LW. Free radicals and diabetes. Free Radic Biol Med 1988;5: 113–124
40. Altomare E, Vendemiale G, Chicco D, Procacci V, Cirelli F. Increased lipid peroxidation in type 2 poorly controlled diabetic patients. Diabete Metab 1992;18:264–271
41. Fujiwara Y, Kondo T, Murakami K, Kawakami Y. Decrease of the inhibition of lipid peroxidation by glutathione-dependent system in erythrocytes of non-insulin dependent diabetics. Klin Wochenschr 1989;67:336–341
42. Oyadomari S, Koizumi A, Takeda K, et al. Targeted disruption of the Chop gene delays endoplasmic reticulum stress-mediated diabetes. J Clin Invest 2002;109:525–532
43. Ueno N, Inui A, Kalra PS, Kalra SP. Leptin transgene expression in the hypothalamus enforces euglycemia in diabetic, insulin-deficient nonobese Akita mice and leptin-deficient obese ob/ob mice. Peptides 2006;27:2332–2342
44. Ozata M, Ozdemir IC, Licinio J. Human leptin deficiency caused by a missense mutation: multiple endocrine defects, decreased sympathetic tone, and immune system dysfunction indicate new targets for leptin action, greater central than peripheral resistance to the effects of leptin, and spontaneous correction of leptin-mediated defects. J Clin Endocrinol Metab 1999;84:3686–3695
45. Matarese G, Leiter EH, La Cava A. Leptin in autoimmunity: many questions, some answers. Tissue Antigens 2007;70:87–95
46. Matarese G, Sanna V, Lechler RI, et al. Leptin accelerates autoimmune diabetes in female NOD mice. Diabetes 2002;51:1356–1361
47. Lee CH, Reifsnyder PC, Naggert JK, et al. Novel leptin receptor mutation in NOD/LtJ mice suppresses type 1 diabetes progression: I. Pathophysiological analysis. Diabetes 2005;54:2525–2532
48. Guberski DL, Butler L, Manzi SM, Stubbs M, Like AA. The BBZ/Wor rat: clinical characteristics of the diabetic syndrome. Diabetologia 1993;36:912–919
49. Nishimura M, Miyamoto H. Immunopathological influence of the Ay, db, ob and nu genes placed on the inbred NOD background as murine models for human type I diabetes. J Immunogenet 1987;14:127–130

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Additional Information: Information on the NCRR is available at http://www.ncrr.nih.gov/. Information on the Reengineering the Clinical Research Enterprise can be obtained from http://nihroadmap.nih.gov.

1. Markel H. "I swear by Apollo"—on taking the Hippocratic oath. *N Engl J Med.* 2004;350(20):2026-2029.
2. Orr RD, Pang N, Pellegrino ED, Siegler M. Use of the Hippocratic Oath: a review of twentieth century practice and a content analysis of oaths administered in medical schools in the US and Canada in 1993. *J Clin Ethics.* 1997;8(4):377-388.
3. Kao AC, Parsi KP. Content analyses of oaths administered at US medical schools in 2000. *Acad Med.* 2004;79(9):882-887.
4. Graham D. Revisiting Hippocrates: does an oath really matter? *JAMA.* 2000; 284(22):2841-2842.
5. McHugh PR. Hippocrates à la mode. *Nat Med.* 1996;2(5):507-509.
6. Antiel RM, Curlin FA, James KM, Tilburt JC. Physicians' beliefs and U.S. health care reform—a national survey. *N Engl J Med.* 2009;361(14):e23.
7. Temkin O. *Hippocrates in a World of Pagans and Christians.* Baltimore, MD: Johns Hopkins University Press; 1991.
8. Pellegrino ED. Bioethics at century's turn: can normative ethics be retrieved? *J Med Philos.* 2000;25(6):655-675.

COMMENTS AND OPINIONS

Considering Selection Bias When Developing a Search Strategy

We read with great interest the article by Sciarretta et al[1] on antihypertensive treatment and development of heart failure in hypertension. They performed the largest network meta-analysis in essential hypertension, to our knowledge, and showed that the use of diuretics and renin-angiotensin system inhibitors are the most effective first-line antihypertensive drug for preventing heart failure. In this meta-analysis, however, the search strategy and study selection are somewhat unclear.

The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial, published in 2008,[2] assessed cardiovascular outcomes in high-risk hypertensive patients receiving either candesartan or amlodipine. The primary end point of the CASE-J trial was a composite of cardiovascular morbidity and mortality, including heart failure. We think that this trial meets the inclusion criteria in the

See also page 384

meta-analysis by Sciarretta et al,[1] but it was not included despite a careful search using 2 databases by 2 investigators. Also, neither the KYOTO HEART study[3] nor the Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Artery Disease (HIJ-CREATE)[4] was included. The authors also checked in the references of a previous meta-analysis by Verdecchia et al.[5] This could not be enough to identify all randomized controlled trials to evaluate a wide range of antihypertensive drugs because this previous meta-analysis by Verdecchia et al[5] aimed to compare old antihypertensive drugs (diuretics and β-blockers) or placebo with new drugs (renin-angiotensin system inhibitors or calcium channel blockers). We are afraid that other important clinical trials are not included in the meta-analysis by Sciarretta et al.[1]

Although a meta-analysis can provide more precise estimates of interventions, it always has a potential for selection bias. Therefore, we would like to know in more detail the search strategy used in this meta-analysis based on the Preferred Reporting Items for Systematic Reviews and Meta-analysis statement.

Yoko M. Nakao, MD
Kenji Ueshima, MD, PhD
Satoshi Teramukai, PhD
Sachiko Tanaka, PhD
Shinji Yasuno, MD, PhD
Akira Fujimoto, MS
Koji Kawakami, MD, PhD
Kazuwa Nakao, MD, PhD

Author Affiliations: Department of Pharmacoepidemiology, Kyoto University Graduate School of Medicine and Public Health (Drs Y. M. Nakao and Kawakami), EBM Research Center (Drs Y. M. Nakao, Ueshima, Tanaka, Yasuno, Fujimoto, and K. Nakao) and Department of Medicine and Clinical Science (Dr K. Nakao), Kyoto University Graduate School of Medicine, and Division of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital (Dr Teramukai), Kyoto, Japan.
Correspondence: Dr Ueshima, EBM Research Center, Kyoto University Graduate School of Medicine, Yoshida-konoe-cho Sakyo-ku, Kyoto 606-8501, Japan (kenji.ueshima@at3.ecs.kyoto-u.ac.jp).
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1. Sciarretta S, Palano F, Tocci G, Baldini R, Volpe M. Antihypertensive treatment and development of heart failure in hypertension: a Bayesian network meta-analysis of studies in patients with hypertension and high cardiovascular risk [published online November 8, 2010]. *Arch Intern Med.* 2011;171 (5):384-394.
2. Ogihara T, Nakao K, Fukui T, et al; Candesartan Antihypertensive Survival Evaluation in Japan Trial Group. Effects of candesartan compared with amlodipine in hypertensive patients with high cardiovascular risks: candesartan antihypertensive survival evaluation in Japan trial. *Hypertension.* 2008;51(2):393-398.

Corrected on March 30, 2011

3. Sawada T, Yamada H, Dahlöf B, Matsubara H; KYOTO HEART Study Group. Effects of valsartan on morbidity and mortality in uncontrolled hypertensive patients with high cardiovascular risks: KYOTO HEART Study. *Eur Heart J*. 2009;30(20):2461-2469.
4. Kasanuki H, Hagiwara N, Hosoda S, et al; HIJ-CREATE Investigators. Angiotensin II receptor blocker-based vs. non-angiotensin II receptor blocker-based therapy in patients with angiographically documented coronary artery disease and hypertension: the Heart Institute of Japan Candesartan Randomized Trial for Evaluation in Coronary Artery Disease (HIJ-CREATE). *Eur Heart J*. 2009;30(10):1203-1212.
5. Verdecchia P, Angeli F, Cavallini C, et al. Blood pressure reduction and renin-angiotensin system inhibition for prevention of congestive heart failure: a meta-analysis. *Eur Heart J*. 2009;30(6):679-688.

Network Meta-analysis of Heart Failure Prevention by Antihypertensive Drugs

We agree with the conclusions of the "network meta-analysis" of heart failure prevention by antihypertensive drugs, which used a Bayesian technique and 26 clinical trials.[1] We have reported similar results supporting the superiority of a diuretic using both the network meta-analytic technique of Lumley,[2] and a Bayesian technique, which included data from all 47 or 53 of the then published hypertension clinical trials.[3,4] We noted the great preponderance of evidence favoring chlorthalidone, which accounts for more than 90% of their heart failure cases. Although a network meta-analysis in 2004 suggested no significant difference between chlorthalidone vs another diuretic as initial therapy to prevent heart failure,[5] data from all 61 hypertension trials involving 355 225 subjects (from the first Veterans Administration trial[6] to the very recent Valsartan Amlodipine Randomized Trial[7]), using the therapeutic categories of Sciarretta et al[1] suggests a major difference in precision among diuretics (**Figure**). "Other diuretics" has the smallest number of heart failure cases of any treatment, nearly 4-times smaller than that for chlorthalidone. The *P* value for chlorthalidone is nearly 5 orders of magnitude smaller than that of "other diuretics." Our results were robust to many sensitivity analyses, such as when combining "conventional therapy" and "β-blocker," including only studies that randomized subjects to initial therapy, omitting data from studies done in nonhypertensive patients, and omitting data from the Antihypertensive and Lipid-Lowering to prevent Heart Attack Trial (ALLHAT). We add to the conclusions of Sciaretta et al[1] that the clinical trial evidence is strongest, by far, for chlorthalidone as the most effective antihypertensive agent to prevent heart failure.

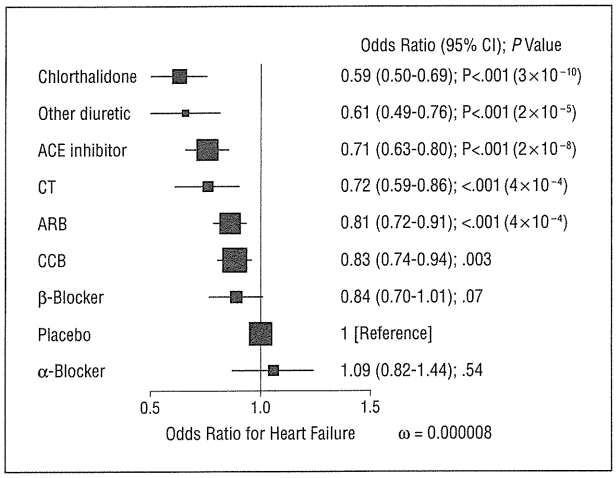

Figure. Odds ratios (95% confidence intervals [CIs]) for incident heart failure associated with different types of antihypertensive drugs in clinical trials, determined by the network meta-analytic technique of Lumley.[2] ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CCB, calcium channel blocker; CT, conventional therapy (diuretic and/or β-blocker); ω, incoherence.

William J. Elliott, MD, PhD
Sanjib Basu, PhD
Peter M. Meyer, PhD

Author Affiliations: Division of Pharmacology, Pacific Northwest University of Health Sciences, Yakima, Washington (Dr Elliott); Department of Preventive Medicine, RUSH Medical College, Chicago, Illinois (Drs Basu and Meyer). **Correspondence:** Dr Elliott, Division of Pharmacology, Pacific Northwest University of Health Sciences, 200 University Pkwy, Yakima, WA 98901 (welliott@pnwu.org).
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1. Sciarretta S, Palano F, Tocci G, Baldini R, Volpe M. Antihypertensive treatment and development of heart failure in hypertension: a Bayesian network meta-analysis of studies in patients with hypertension and high cardiovascular risk [published online November 8, 2010]. *Arch Intern Med*. 2011;171(5):384-394.
2. Lumley T. Network meta-analysis for indirect treatment comparisons. *Stat Med*. 2002;21(16):2313-2324.
3. Elliott WJ, Meyer PM. Antihypertensive drug therapy and incident heart failure in clinical trials in hypertension: an updated network meta-analysis [abstract]. *J Clin Hypertens (Greenwich)*. 2007;9:A210.
4. Elliott WJ, Basu S, Meyer PM. Initial antihypertensive drugs for heart failure prevention: Network and Bayesian meta-analyses of clinical trial data [abstract]. *Circulation*. 2008;118(suppl 2):S886.
5. Psaty BM, Lumley T, Furberg CD. Meta-analysis of health outcomes of chlorthalidone-based vs nonchlorthalidone-based low-dose diuretic therapies. *JAMA*. 2004;292(1):43-44.
6. Veterans Administration Cooperative Study Group on Antihypertensive Agents. Effects of treatment on morbidity in hypertension. Results in patients with diastolic blood pressures averaging 115 through 129 mm Hg. *JAMA*. 1967;202(11):1028-1034.
7. Narumi H, Takano H, Shindo S, et al; Valsartan Amlodipine Randomized Trial Investigators. Effects of valsartan and amlodipine on cardiorenal protection in Japanese hypertensive patients: the Valsartan Amlodipine Randomized Trial. *Hypertens Res*. 2011;34(1):62-69.

In reply

We thank Nakao and colleagues for their letter. A selection bias in meta-analyses is mainly due to errors in the search strategy to screen the trials to be considered. These errors are related to an arbitrary selection of the trials or to the choice of factious search and eligibility criteria to select them. As a result, some studies are excluded from the analysis although they should be considered.

To avoid a selection bias, in our article[1] we predefined and clearly reported the process for study selection, in keeping with the PRISMA check list. Our search keywords were generic and impartial and could not systematically exclude trials with specific characteristics. The meta-analysis by Verdecchia et al,[2] which evaluated our same outcome (ie, heart failure [HF]), was only used as a control.

In a recent comprehensive meta-analysis, Bangalore et al[3] evaluated all the trials assessing an antihypertensive therapy published between 1950 and 2010. All the trials con-

Corrected on March 30, 2011

3. Denys K, Cankurtaran M, Janssens W, Petrovic M. Metabolic syndrome in the elderly: an overview of the evidence. *Acta Clin Belg.* 2009;64(1):23-34.
4. Arnold AM, Psaty BM, Kuller LH, et al. Incidence of cardiovascular disease in older Americans: the cardiovascular health study. *J Am Geriatr Soc.* 2005; 53(2):211-218.
5. Pinsky JL, Jette AM, Branch LG, Kannel WB, Feinleib M. The Framingham Disability Study: relationship of various coronary heart disease manifestations to disability in older persons living in the community. *Am J Public Health.* 1990; 80(11):1363-1367.
6. Rejeski WJ, Marsh AP, Chmelo E, Rejeski JJ. Obesity, intentional weight loss and physical disability in older adults. *Obes Rev.* 2010;11(9):671-685.
7. Manton KG, Vaupel JW. Survival after the age of 80 in the United States, Sweden, France, England, and Japan. *N Engl J Med.* 1995;333(18):1232-1235.
8. Franklin BA, Hall L, Timmis GC. Contemporary cardiac rehabilitation services. *Am J Cardiol.* 1997;79(8):1075-1077.
9. Gordon NF, English CD, Contractor AS, et al. Effectiveness of three models for comprehensive cardiovascular disease risk reduction. *Am J Cardiol.* 2002; 89(11):1263-1268.
10. Eves ND, Plotnikoff RC. Resistance training and type 2 diabetes: considerations for implementation at the population level. *Diabetes Care.* 2006;29(8):1933-1941.
11. Corti MC, Guralnik JM, Salive ME, Sorkin JD. Serum albumin level and physical disability as predictors of mortality in older persons. *JAMA.* 1994;272(13): 1036-1042.
12. Hirvensalo M, Rantanen T, Heikkinen E. Mobility difficulties and physical activity as predictors of mortality and loss of independence in the community-living older population. *J Am Geriatr Soc.* 2000;48(5):493-498.
13. Lampinen P, Heikkinen E. Reduced mobility and physical activity as predictors of depressive symptoms among community-dwelling older adults: an eight-year follow-up study. *Aging Clin Exp Res.* 2003;15(3):205-211.
14. Newman AB, Simonsick EM, Naydeck BL, et al. Association of long-distance corridor walk performance with mortality, cardiovascular disease, mobility limitation, and disability. *JAMA.* 2006;295(17):2018-2026.
15. Guralnik JM, LaCroix AZ, Abbott RD, et al. Maintaining mobility in late life, I: demographic characteristics and chronic conditions. *Am J Epidemiol.* 1993;137(8): 845-857.
16. Cleeman JI, Grundy SM, Becker D, et al; Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults. Executive Summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). *JAMA.* 2001;285(19):2486-2497.
17. Simonsick EM, Montgomery PS, Newman AB, Bauer DC, Harris T. Measuring fitness in healthy older adults: the Health ABC Long Distance Corridor Walk. *J Am Geriatr Soc.* 2001;49(11):1544-1548.
18. Pettee Gabriel KK, Rankin RL, Lee C, Charlton ME, Swan PD, Ainsworth BE. Test-retest reliability and validity of the 400-meter walk test in healthy, middle-aged women. *J Phys Act Health.* 2010;7(5):649-657.
19. Ayabe M, Brubaker PH, Mori Y, et al. Self-monitoring moderate-vigorous physical activity versus steps/day is more effective in chronic disease exercise programs. *J Cardiopulm Rehabil Prev.* 2010;30(2):111-115.
20. Ayabe M, Brubaker PH, Dobrosielski D, et al. The physical activity patterns of cardiac rehabilitation program participants. *J Cardiopulm Rehabil.* 2004;24 (2):80-86.
21. Rejeski WJ, Brawley LR, Ambrosius WT, et al. Older adults with chronic disease: benefits of group-mediated counseling in the promotion of physically active lifestyles. *Health Psychol.* 2003;22(4):414-423.
22. Borg G. *Perceived Exertion and Pain Scales.* Champaign, IL: Human Kinetics; 1998.
23. US Department of Agriculture Web site. http://www.mypyramid.gov. Accessed October 13, 2010.
24. Clark MM, Abrams DB, Niaura RS, Eaton CA, Rossi JS. Self-efficacy in weight management. *J Consult Clin Psychol.* 1991;59(5):739-744.
25. Perri MG, Nezu AM, McKelvey WF, Shermer RL, Renjilian DA, Viegener BJ. Relapse prevention training and problem-solving therapy in the long-term management of obesity. *J Consult Clin Psychol.* 2001;69(4):722-726.
26. Knowler WC, Barrett-Connor E, Fowler SE, et al; Diabetes Prevention Program Research Group. Reduction in the incidence of type 2 diabetes with lifestyle intervention or metformin. *N Engl J Med.* 2002;346(6):393-403.
27. Pi-Sunyer X, Blackburn G, Brancati FL, et al; Look AHEAD Research Group. Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial. *Diabetes Care.* 2007; 30(6):1374-1383.
28. Kwon S, Perera S, Pahor M, et al. What is a meaningful change in physical performance? findings from a clinical trial in older adults (the LIFE-P study). *J Nutr Health Aging.* 2009;13(6):538-544.
29. Pahor M, Blair SN, Espeland M, et al; LIFE Study Investigators. Effects of a physical activity intervention on measures of physical performance: results of the lifestyle interventions and independence for Elders Pilot (LIFE-P) study. *J Gerontol A Biol Sci Med Sci.* 2006;61(11):1157-1165.
30. Ettinger WH Jr, Burns R, Messier SP, et al. A randomized trial comparing aerobic exercise and resistance exercise with a health education program in older adults with knee osteoarthritis: the Fitness Arthritis and Seniors Trial (FAST). *JAMA.* 1997;277(1):25-31.
31. Berry MJ, Rejeski WJ, Adair NE, Ettinger WH, Zaccaro DJ, Sevick MA. A randomized, controlled trial comparing long-term and short-term exercise in patients with chronic obstructive pulmonary disease. *J Cardiopulm Rehabil.* 2003; 23(1):60-68.
32. Messier SP, Loeser RF, Miller GD, et al. Exercise and dietary weight loss in overweight and obese older adults with knee osteoarthritis: the Arthritis, Diet, and Activity Promotion Trial. *Arthritis Rheum.* 2004;50(5):1501-1510.
33. Villareal DT, Apovian CM, Kushner RF, Klein S; American Society for Nutrition; NAASO, the Obesity Society. Obesity in older adults: technical review and position statement of the American Society for Nutrition and NAASO, the Obesity Society. *Obes Res.* 2005;13(11):1849-1863.

Correction

Error in Correspondence. In the Letter to the Editor titled "Considering Selection Bias When Developing a Search Strategy" by Nakao et al, published in the March 14, 2011, issue of the *Archives* (2011;171[5]: 471-472), an incorrect e-mail address appeared in the Correspondence section. The correct e-mail address is as follows: kenji.ueshima@at3.ecs.kyoto-u.ac.jp.

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ORIGINAL ARTICLE
Hypertension and Circulatory Control

Influence of Coronary Risk Factors on Coronary Events in Japanese High-Risk Hypertensive Patients

– Primary and Secondary Prevention of Ischemic Heart Disease in a Subanalysis of the Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) Trial –

Kenji Ueshima, MD, PhD; Koji Oba, BSc; Shinji Yasuno, MD, PhD; Akira Fujimoto, BSc; Shiro Tanaka, PhD; Toshio Ogihara, MD, PhD; Takao Saruta, MD, PhD; Kazuwa Nakao, MD, PhD

Background: The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial was conducted to compare the effects of candesartan and amlodipine on cardiovascular events in Japanese high-risk hypertensive patients. The aim of the present subanalysis was to evaluate the influence of coronary risk factors on coronary events in these patients as an observational study irrespective of allocated drugs.

Methods and Results: The adjusted hazard ratios (HRs) of the association of baseline risk factors including gender, age, allocated drugs, body mass index, systolic/diastolic blood pressure (SBP/DBP), diabetes mellitus (DM), hyperlipidemia (HL), smoking, left ventricular hypertrophy, previous ischemic heart disease (IHD), previous cerebrovascular events, and chronic kidney disease (CKD) with coronary events in 4,703 patients who were enrolled in the CASE-J trial, were examined. The coronary events occurred in 83 patients, and were significantly associated with previous IHD, DM, male sex, CKD, and low DBP. Significant predictors were previous IHD (HR, 3.89), DM (HR, 3.10), male sex (HR, 1.81), CKD (HR, 1.60), and low DBP (HR, 1.36), respectively. In 4,107 patients without previous IHD, DM (HR, 4.88), HL (HR, 2.67), and DBP (HR, 1.39) were significantly associated with the risk of coronary events, while male sex (HR, 3.03), CKD (HR, 2.44), and DM (HR, 2.15) were in 596 patients with previous IHD.

Conclusions: DM is the important factor in both primary and secondary prevention of coronary events. Comprehensive risk management including surveillance of DM, CKD and HL is needed for preventing coronary events, in addition to blood pressure control. (*Circ J* 2011; **75:** 2411–2416)

Key Words: Coronary event; Coronary risk factor; Hypertension; Japanese; Prevention

Although the current incidence of acute myocardial infarction in Japan is still lower than that in North America and Europe,[1] a recent report indicated that there has been a steady trend of increasing incidence of acute myocardial infarction during the past 30 years in the Japanese population.[2] We should be deeply concerned about this increase of coronary events in Japan with regard to westernized lifestyle and aging of the population. Hypertension has been one of the major risk factors for cardiovascular (CV) events, and CV risks are well known to cluster in hypertensive patients.[3,4] It is important to consider coronary risk factors in hypertensive patients when we try to reduce the incidence of ischemic heart disease (IHD) in terms of primary and secondary prevention.

Editorial p 2316

The Candesartan Antihypertensive Survival Evaluation in Japan (CASE-J) trial was conducted to compare the effects of the angiotensin II receptor blocker candesartan and the calcium channel blocker amlodipine on the incidence of CV events in Japanese high-risk hypertensive patients.[5,6] The CASE-J trial found that candesartan and amlodipine equally suppressed total CV mortality and morbidity in high-risk hy-

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EBM Research Center (K.U., K.O., S.Y., A.F., K.N.), Department of Medicine and Clinical Science (K.N.), Kyoto University Graduate School of Medicine, Kyoto; Translational Research Center, Kyoto University Hospital, Kyoto (S.T.); Osaka General Medical Center, Osaka (T.O.); and Keio University, Tokyo (T.S.), Japan

Mailing address: Kenji Ueshima, MD, PhD, EBM Research Center, Kyoto University Graduate School of Medicine, Yoshida-Konoechou, Sakyo-ku, Kyoto 606-8501, Japan. E-mail: kenji.ueshima@at3.ecs.kyoto-u.ac.jp

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Table 1. Selected Coronary Risk Factors and Definitions for Baseline Characteristics

Gender
Age
Allocated drugs: candesartan or amlodipine
BMI at baseline
SBP at baseline
DBP at baseline
Smoking (including previous history of smoking)
DM: at least one of the following factors: fasting blood glucose ≥126 mg/dl, causal blood glucose ≥200 mg/dl, HbA_{1c} ≥6.5%, 2-h blood glucose on 75-g OGTT ≥200 mg/dl, or current treatment with hypoglycemic agent
HL: current treatment with anti-lipidemic agent
Cerebrovascular disease: history of cerebral hemorrhage, cerebral infarction, or transient ischemic attack until 6 months prior to the screening
LVH: thickness of the posterior wall of left ventricle or thickness of the wall of interventricular septum ≥12 mm on echocardiography or Sv1+Rv5 ≥35 mm on electrocardiography
IHD: angina pectoris, and a past history (≥6 months before giving informed consent) of myocardial infarction
CKD: proteinuria ≥+1 or renal impairment (estimated glomerular filtration rate <60 $ml \cdot min^{-1} \cdot 1.73 m^{-2}$ by a predictive equation) within 3 months at the time of giving informed consent
Anti-hypertensive medication prior to screening

BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; DM, diabetes mellitus; OGTT, oral glucose tolerance test; HL, hyperlipidemia; LVH, left ventricular hypertrophy; IHD, ischemic heart disease; CKD, chronic kidney disease.

Table 2. Baseline Subject Characteristics

	All patients (n=4,703)	Previous IHD (+) (n=596)	Previous IHD (–) (n=4,107)
Gender, male (%)	2,597 (55.2)	397 (66.6)	2,200 (53.6)
Age (years), mean±SD	63.9±10.5	67.2±8.7	63.4±10.7
Allocated drugs, candesartan (%)	2,354 (50.1)	298 (50.0)	2,056 (50.1)
BMI, mean±SD	24.6±3.7	24.3±3.3	24.6±3.7
SBP, mean±SD	162.9±14.2	157.5±12.3	163.6±14.3
DBP, mean±SD	91.7±11.2	86.9±9.1	92.4±11.3
Smoking (no) (%)	3,205 (68.1)	346 (58.1)	2,859 (69.5)
DM (yes) (%)	2,018 (42.9)	203 (34.1)	1,815 (44.2)
HL (yes) (%)	2,078 (67.3)	325 (54.5)	1,753 (42.7)
LVH (yes) (%)	1,612 (34.3)	178 (29.9)	1,434 (34.9)
Previous IHD (yes) (%)	596 (12.7)	596 (100)	0 (0)
Previous stroke (yes) (%)	473 (10.1)	41 (6.9)	432 (10.5)
CKD (yes) (%)	2,571 (54.7)	296 (49.7)	2,275 (55.4)
Previous AHD (yes) (%)	3,165 (67.3)	512 (85.9)	2,653 (64.6)

AHD, anti-hypertensive drug. Other abbreviations see in Table 1.

pertensive patients under strict blood pressure (BP) control.

Although some Japanese cohort studies have been performed to clarify the coronary risk factors, this trial was an observational study irrespective of allocated drugs, and the purpose of the present subanalysis was to clarify the adverse effects of coronary risks in Japanese high-risk hypertensive patients. Although prior cohort studies focused on the general population, the present study focused on those subjects taking anti-hypertensive drugs. Therefore, the influence of risk factors on coronary events under control could be clarified.

Methods

Study Design

The CASE-J trial was a prospective, multicenter, randomized, open-label, active-controlled, 2-arm parallel-group comparison study to evaluate the efficacy of candesartan and amlodipine for reducing the incidence of CV events in high-risk hypertensive patients.[5,6] The rationale and complete design of the CASE-J trial have been previously reported.[5] Briefly, 4,728 patients with high-risk hypertension were randomly assigned to either a candesartan- or amlodipine-based treatment regimen. High risk was defined as the presence of any one of the following: (1) severe hypertension (systolic BP/diastolic BP [SBP/DBP] ≥180/110 mmHg; (2) type 2 diabetes mellitus (DM); (3) history of stroke or transient ischemic attack >6 months prior to screening; (4) left ventricular hypertrophy (LVH; SV1+RV5 ≥3.5 mV on electrocardiography [ECG] and/or LV wall thickness ≥12 mm on echocardiography), angina pectoris, or a history of myocardial infarction >6 months prior to screening; (5) proteinuria or serum creatinine concentration ≥1.3 mg/dl; and (6) arteriosclerotic peripheral artery obstruction.[5] The target BP was determined according to the guideline proposed by the Japanese Society of Hypertension.[7] Finally, 4,703 randomly assigned patients were included in the analysis.

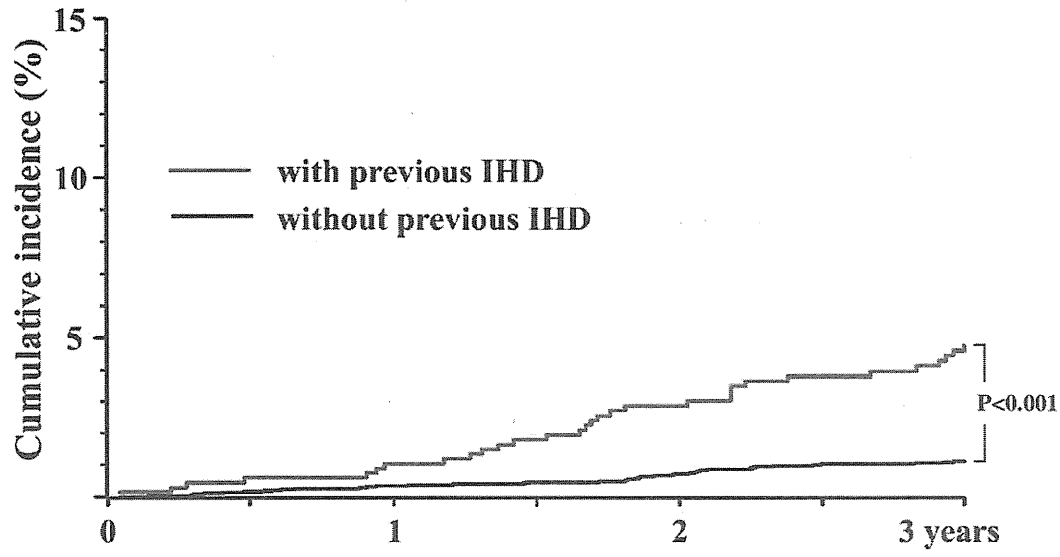

Figure. Cumulative incidence of the primary composite endpoint adjusted for baseline characteristics in patients without ischemic heart disease (IHD) at baseline and with IHD at baseline.

Outcome Measurements

In the CASE-J trial, the primary endpoint was the first fatal/non-fatal CV event (a composite of cardiac events including sudden death, angina pectoris, acute myocardial infarction, or heart failure; cerebrovascular events including stroke or transient ischemic attack; renal events including serum creatinine concentration ≥4.0 mg/dl, doubling of the serum creatinine concentration, or end-stage renal disease; and vascular events including dissecting aortic aneurysm or arteriosclerotic occlusion of a peripheral artery).[6] In the present subanalysis, we focused on the incidence of coronary events. Thus, the endpoint of this subanalysis was coronary events, and they were defined as a composite of sudden death (unexpected death happening within 24 h without external causes), angina pectoris (nitrate-sensitive chest pain and ischemic ECG changes during chest pain or stress testing), and acute myocardial infarction (nitrate-resistant chest pain, elevated myocardial specific enzymes, and typical ECG signs). Event evaluation was performed independently by the Event Evaluation Committee, which was blinded to the assigned treatment groups and adjudicated according to the prespecified protocol criteria as described here.[6]

Baseline Characteristics

Background coronary risk factors such as gender, age, allocated drugs, body mass index (BMI), SBP, DBP, smoking, type 2 DM, hyperlipidemia (HL), LVH, history of previous IHD, history of cerebrovascular disease, chronic kidney disease (CKD), and anti-hypertensive medication prior to the screening were analyzed (Table 1). Baseline characteristics of enrolled patients are listed in Table 2. Moreover, we focused on the presence or absence of previous IHD including angina pectoris, and a past history (≥6 months before giving informed consent) of myocardial infarction, which were 1 of the inclusion criteria in the CASE-J trial. Enrolled patients were divided into 4,107 patients without previous IHD and 596 patients with previous IHD. In the present study, risk factors for the first coronary event were assessed in patients without previous IHD and those for the recurrence of coronary events were done in patients with previous IHD. Their baseline characteristics are also given in Table 2. When we analyzed the data of patients with or without coronary risk factors as an observational study irrespective of allocated drugs, there were statistical differences between these 2 groups. Analyses were then adjusted by baseline characteristics as described in the following section.

Statistical Analysis

Data are expressed as mean±SD or proportions. Risk-adjusted cumulative incidence of coronary events was calculated using the corrected group prognosis method,[8] with adjustment for baseline characteristics, including history of prior anti-hypertensive treatment, allocated drugs, age, sex, BMI, type 2 DM, history of cerebrovascular disease, history of IHD, renal dysfunction, history of vascular disease, SBP and DBP at baseline. The hazard ratio (HR) and 95% confidence intervals (95%CIs) were estimated using Cox regression analysis. We also used multiple Cox regression analysis to examine the association between rate of coronary events and coronary risk factors (gender, age, allocated drugs, BMI, SBP, DBP, smoking, DM, HL, LVH, previous IHD, previous cerebrovascular disease, CKD, and previous anti-hypertensive medication). All statistical tests were 2-sided with an alpha level of 0.05, and were performed using SAS version 9.1 (SAS Institute, Cary, NC, USA).

Results

Changes in BP

Blood pressure was generally controlled to <140/80 mmHg in both groups. Mean SBP/DBP was 162.9±14.2/91.7±11.2 mmHg at baseline and 135.4±11.9/77.0±9.2 mmHg after 3 years. While the mean SBP/DBP in patients without previous IHD was 163.6±14.3/92.4±11.3 mmHg at baseline and

Table 3. Prognostic Value of Coronary Risk Factors in All Enrolled Patients

	HR (95%CI)	P value
Male (vs. female)*	1.81 (1.06–3.10)	0.031
Age: 10-year increase	1.09 (0.83–1.44)	0.525
Amlodipine (vs. candesartan)	1.32 (0.85–2.04)	0.214
BMI: 1-kg/m² increase	1.01 (0.83–1.44)	0.525
SBP (at entry): 10-mmHg increase	1.14 (0.96–1.37)	0.145
DBP (at entry): 10-mmHg decrease*	1.36 (1.09–1.68)	0.006
Smoking: no (vs. yes)	1.02 (0.62–1.69)	0.945
DM: no (vs. yes)*	3.10 (1.90–5.04)	<0.001
HL: no (vs. yes)	1.36 (0.86–2.14)	0.186
LVH: no (vs. yes)	1.26 (0.80–1.99)	0.319
Previous IHD: no (vs. yes)*	3.89 (2.40–6.31)	<0.001
Previous stroke: no (vs. yes)	0.78 (0.34–1.82)	0.569
CKD: no (vs. yes)*	1.60 (1.01–2.54)	0.046
Previous AHD: no (vs. yes)	1.54 (0.85–2.81)	0.159

HR, hazard ratio; CI, confidence interval. Other abbreviations see in Tables 1,2.
*P<0.05.

Table 4. Prognostic Value of Coronary Risk Factors in Patients Without Previous IHD (Primary Prevention)

	HR (95%CI)	P value
Male (vs. female)	1.44 (0.73–2.84)	0.294
Age: 10-year increase	1.18 (0.83–1.64)	0.364
Amlodipine (vs. candesartan)	1.07 (0.61–1.87)	0.811
BMI: 1-kg/m² increase	0.99 (0.91–1.07)	0.810
SBP (at entry): 10-mmHg increase	1.18 (0.94–1.54)	0.160
DBP (at entry): 10-mmHg decrease	1.39 (1.05–2.18)	0.020
Smoking: no (vs. yes)	0.70 (0.35–1.37)	0.296
DM: no (vs. yes)	4.88 (2.35–10.16)	<0.001
HL: no (vs. yes)	2.67 (1.47–4.85)	0.001
LVH: no (vs. yes)	1.21 (0.65–2.24)	0.555
Previous stroke: no (vs. yes)	1.03 (0.40–2.63)	0.957
CKD: no (vs. yes)	1.21 (0.67–2.19)	0.521
Previous AHD: no (vs. yes)	1.19 (0.62–2.28)	0.595

Abbreviations see in Tables 1–3.
*P<0.05.

Table 5. Prognostic Value of Coronary Risk Factors in Patients With Previous IHD (Secondary Prevention)

	HR (95%CI)	P value
Male (vs. female)*	3.05 (1.15–14.63)	0.025
Age: 10-year increase	1.00 (0.63–1.28)	0.992
Amlodipine (vs. candesartan)	1.77 (0.87–3.63)	0.117
BMI: 1-kg/m² increase	1.03 (0.91–1.15)	0.673
SBP (at entry): 10-mmHg increase	1.16 (0.84–1.57)	0.360
DBP (at entry): 10-mmHg decrease	1.22 (0.82–1.81)	0.323
Smoking: no (vs. yes)	1.54 (0.72–3.45)	0.268
DM: no (vs. yes)*	2.15 (1.06–4.38)	0.035
HL: no (vs. yes)	0.54 (0.26–1.12)	0.098
LVH: no (vs. yes)	1.39 (0.69–2.81)	0.360
Previous stroke: no (vs. yes)	0.34 (0.05–2.54)	0.290
CKD: no (vs. yes)*	2.44 (1.16–5.13)	0.018
Previous AHD: no (vs. yes)	5.13 (0.68–38.49)	0.112

Abbreviations see in Tables 1–3.
*P<0.05.

135.7±12.0/77.3±9.3 mmHg after 3 years, the mean SBP/DBP in patients with previous IHD was 157.5±12.3/86.9±9.1 mmHg at baseline and 133.2±11.4/75.1±8.2 mmHg after 3 years. But both SBP and DBP in patients with previous IHD were slightly but significantly lower than in those without previous IHD at baseline and after 3 years (P<0.001, respectively).

Prognostic Value of Coronary Risk Factors for Coronary Event Rate

During 3.2±0.9 years of follow-up (5th–95th percentile interval, 1.0–4.2), coronary events occurred in 50 patients without previous IHD (1.2%; 15 sudden death, 11 angina pectoris, 24 myocardial infarction) at baseline for a rate of 3.7 per 1000 patient-years and in 33 patients with previous IHD (5.5%; 11 sudden death, 11 angina pectoris, 11 myocardial infarction) at baseline for a rate of 16.9 per 1,000 patient-years (adjusted HR, 3.89; 95%CI: 2.40–6.31; P<0.001; Figure). We also evaluated the prognostic value of the coronary risk factors in all enrolled patients. As shown in Table 3, the onset of coronary events was significantly associated with previous IHD (adjusted HR, 3.89), DM (adjusted HR, 3.10; 95%CI: 1.90–5.04; P<0.001), male sex (adjusted HR, 1.81; 95%CI: 1.06–3.10; P=0.031), CKD (adjusted HR, 1.60; 95%CI: 1.01–2.54; P=0.046), and DBP (10-mmHg decrease, adjusted HR, 1.36; 95%CI: 1.09–1.68; P=0.006).

In addition, the prognostic value of the coronary risk factors for each event category was evaluated in patients without previous IHD (primary prevention) and in patients with previous IHD (secondary prevention), respectively. As shown in Table 4, the onset of coronary events in patients without previous IHD was significantly associated with DM (adjusted HR, 4.88; 95%CI: 2.35–10.16; P<0.001), HL (adjusted HR, 2.67; 95%CI: 1.47–4.85; P=0.001), and DBP (10 mmHg decrease, adjusted HR, 1.39; 95%CI: 1.05–2.18; P=0.02). In patients with previous IHD, the incidence of coronary events was significantly associated with male sex (adjusted HR, 3.05; 95%CI: 1.15–14.63; P=0.025), CKD (adjusted HR, 2.44; 95%CI: 1.16–5.13; P=0.018), and DM (adjusted HR, 2.15; 95%CI: 1.06–4.38; P=0.035; Table 5). Although a significant difference was observed in the risk factors between primary prevention and secondary prevention, interactions between the coronary risk factors and previous IHD were not significant except for HL. Notably, DM was the common predictor of coronary events both in primary and secondary prevention.

Discussion

Increases in BP loads induce myocardial remodeling, such as cardiac hypertrophy, myocardial fibrosis and coronary endothelial damage. Progression of myocardial remodeling and coronary atherosclerosis leads to coronary artery disease, heart failure, arrhythmia and sudden death. In men, morbidity and mortality rates due to coronary artery disease increase by approximately 15% with a 10-mmHg increase in SBP.[9] Conventional anti-hypertensive drug therapy primarily using diuretics and β-blockers, however, does not markedly reduce the incidence of coronary artery disease, whereas it markedly decreases the incidence of stroke.[10] Coronary risk factors other than hypertension may have a markedly different impact on the occurrence of coronary artery disease.[11] Accordingly, the aim of the present study was to clarify the influence of coronary risk factors on coronary events in Japanese high-risk hypertensive patients as a subanalysis of the CASE-J trial. The follow-up rate and coronary event evaluation are markedly reliable from this cohort study. Furthermore, although some

Japanese cohort studies were performed to clarify coronary risk factors,[12–16] this subanalysis focused on the subjects under anti-hypertensive treatment. Therefore, the adverse effects of coronary risk factors under BP control could be evaluated.

First, we demonstrated that a history of previous IHD was the strongest predictor for coronary events in all enrolled patients. Because the prognosis of patients with previous IHD is worse, a sufficient reduction of BP in these patients is important to decrease CV events and mortality.[6,17,18] In the Japan Lipid Intervention Trial, the incidence of coronary events in patients with a history of IHD was 5-fold higher than in patients without this.[19] In addition, Iso reported that the percentage of preventable IHD was 34% in men and 17% in women for control of hypertension.[8] A total of 80–90% of hypertensive patients in their 30s and 40s, however, are untreated in Japan.[9] For reduction of coronary events, population-wide health education is needed for prevention of high BP through lifestyle modifications.

With regard to primary prevention, we also found that DM, HL, and DBP decrease at baseline were significant predictors of occurrence of CV events. DM is well known to be 1 of the most important causes of CV events. Berman et al identified a significant impact of DM on cardiac risk and found that diabetic women have a significantly greater risk of cardiac death than other patients.[20] Haffner et al found that diabetic patients without previous myocardial infarction in a Finnish population-based study had as high a risk of myocardial infarction as non-diabetic patients with previous myocardial infarction.[21] In Japanese people, Nishimura et a; also demonstrated that the CV event rates were similar among non-diabetic patients with prior myocardial infarction and diabetic patients without prior myocardial infarction.[22] It is also well established that HL is a major cause of IHD. According to a Japanese autopsy study, the myocardial infarction size among urban men with HL was large, as also observed in Western populations.[23] Mabuchi et al found that patients with a total cholesterol concentration ≥240 mg/dl developed coronary heart disease more often than patients with total cholesterol <240 mg/dl.[19] The serum low-density lipoprotein cholesterol concentration positively correlated with incidence of the coronary events. The percentage of preventable coronary heart disease was 5% in men and 9% in women for control of DM, and 5% in men and 8% in women for control of HL.[8] In the present study, the lower DBP was extracted from the database as a significant predictor of coronary events. Several observational studies have provided evidence suggesting that pulse pressure was a better predictor of CV events than mean pressure.[24–26] In the Multiple Risk Factor Intervention Trial, the greatest risk was observed in subjects with the highest SBP (≥160 mmHg) and lowest DBP (<70 mmHg) at enrollment. Furthermore, the 2-year probability of a major CV endpoint increased with lower DBP at any given level of SBP.[26] Because patients at high risk of coronary events had increased arterial stiffness, lower DBP was identified as a significant marker of coronary events. We also reported that a strong association with risk of new-onset DM was seen in patients with higher pulse pressures, arising mainly due to the lower DBP.[27]

With regard to secondary prevention, we demonstrated that male sex, CKD, and DM were significant predictors for coronary events. Hypertension causes functional or structural changes to varying degrees in the kidney from an early stage, and renal dysfunction may also cause hypertension. Renal dysfunction and proteinuria are known to be risk factors for end-stage renal failure,[28,29] but they have also recently been found to be strong risk factors for CV disease.[16,30,31] The concept of CKD was introduced for preventing the occurrence of CV disease as well as renal insufficiency by the early detection and treatment of renal diseases.[32] Therefore, strict management of BP as well as treatment of the underlying renal disease is important.

Prevention and treatment for glucose normalization is important in not only primary prevention for coronary events but also in secondary prevention. Elevated blood glucose levels on admission are associated with increased mortality in patients with acute myocardial infarction. In the J-ACCESS study, patients with suspected or confirmed IHDs were classified into 4 groups according to DM and prior myocardial infarction. The prognosis was worst among diabetic patients with prior myocardial infarction.[22] Because the risks of CV events in patients with DM, who achieved SBP/DBP <130/75–79 mmHg, were still significantly higher than in those without DM, strict BP control is needed to reduce CV events in hypertensive patients with DM.[33] In the present subanalysis, previous IHD including angina pectoris and myocardial infarction was one of the inclusion criteria in the CASE-J trial, and there were several differences in baseline characteristics between previous IHD (–) and previous IHD (+), as shown in Table 2. Therefore, when we evaluated the data of patients with baseline coronary risk factors, the analyses had to be adjusted by the baseline characteristics because of their statistical differences. Interestingly, DM was the common predictor of coronary events both in primary and secondary prevention.

Several limitations in the present study should be noted. This was a post-hoc analysis. The number of coronary events may not be sufficient to analyze the influence of the coronary risk factors on these events. Furthermore, a 3.2-year mean follow-up period may not be sufficient to evaluate the relationship between underlying risks and the incidence of coronary events. The CASE-J trial was extended for 3 years from 2006, it was named the CASE-J Ex and it was an observational study.[34] The CASE-J Ex may resolve this issue. Moreover, a difference in the significant risk factors between primary and secondary prevention was observed. Moreover, because DM at baseline was diagnosed by each participant doctor, we did not evaluate specific blood sugar or HbA_{1c}. Thus, information on the relationship between blood sugar or HbA_{1c} and the risk of coronary events is not available. Finally, because HL was diagnosed only on taking medications for HL, there is a possibility that the influence of HL on coronary events was underestimated.

In conclusion, previous IHD, DM, male sex, CKD, and DBP decrease are significant risk factors in all enrolled patients to the CASE-J trial. Because DM, HL and DBP decrease are risk factors in patients without previous IHD, lowering blood sugar and cholesterol are still important measures in terms of primary prevention. In addition, because male sex, CKD and DM are risk factors in patients with previous IHD, CKD and DM are important issues in terms of secondary prevention. Although there are some differences in the coronary risk factors between primary and secondary prevention in Japanese high-risk hypertensive patients, DM is the important factor in both primary and secondary prevention. Furthermore, comprehensive risk management including surveillance of DM, CKD and HL is needed for preventing coronary events, in addition to BP control. General and particular care of lifestyle-related disease leads to prevention of IHD.

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Disclosures

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References

1. Sekikawa A, Ueshima H, Kadowaki T, El-Saed A, Okamura T, Takamiya T, et al. Less subclinical atherosclerosis in Japanese men in Japan than in White men in the United States in the post-World War II birth cohort. *Am J Epidemiol* 2007; **165:** 617–624.
2. Takii T, Yasuda S, Takahashi J, Ito K, Shiba N, Shirato K, et al. Trends in acute myocardial infarction incidence and mortality over 30 years in Japan: Report from the MIYAGI-AMI Registry Study. *Circ J* 2010; **74:** 93–100.
3. Nakamura Y, Saitoh S, Takagi S, Ohnishi H, Chiba Y, Kato N, et al. Impact of abnormal glucose tolerance, hypertension, and other risk factors on coronary heart disease. *Circ J* 2007; **71:** 20–25.
4. Kohro T, Hayashi D, Okada Y, Yamazaki T, Nagai R; JCAD Investigators. Demographics and changes in medical/interventional treatment of coronary artery disease patients over a 3.5-year period in Japan: The Japanese Coronary Artery Disease Study: Trend examination. *Circ J* 2008; **72:** 1397–1402.
5. Fukui T, Rahman M, Hayashi K, Takeda K, Higaki J, Sato T, et al. Candesartan Antihypertensive Survival Evaluation in JAPAN (CASE-J) trial of cardiovascular events in high-risk hypertensive patients: Rationale, design, and methods. *Hypertens Res* 2003; **26:** 979–990.
6. Ogihara T, Nakao K, Fukui T, Fukiyama K, Ueshima K, Oba K, et al; Candesartan Antihypertensive Survival Evaluation in Japan trial Group. Effects of candesartan compared with amlodipine in hypertensive patients with high cardiovascular risks: Candesartan antihypertensive survival evaluation in Japan trial. *Hypertension* 2008; **51:** 393–398.
7. Japanese Society of Hypertension Guidelines Subcommittee for the Management of Hypertension. Guidelines for the management of hypertension for general practitioners. *Hypertens Res* 2001; **24:** 613–634.
8. Iso H. Changes in coronary heart disease risk among Japanese. *Circulation* 2008; **118:** 2725–2729.
9. The Japanese Society of Hypertension Guidelines for the Management of Hypertension (JSH 2009). Epidemiology of hypertension. *Hypertens Res* 2009; **32:** 6–10.
10. Moser M, Hebert P, Hennekens CH. An overview of the meta-analyses of the hypertension treatment trials. *Arch Intern Med* 1991; **151:** 1277–1279.
11. Ueshima K, Yasuno S, Oba K, Fujimoto A, Ogihara T, Saruta T, et al. The effects of cardiac complications on cardiovascular events in Japanese high-risk hypertensive patients: A subanalysis of CASE-J trial. *Circ J* 2009; **73:** 1080–1085.
12. Katsuki S. Epidemiological and clinicopathological study on cerebrovascular disease in Japan. *Prog Brain Res* 1966; **21:** 64–89.
13. Ueda K, Fujii I, Kawano H, Hasuo Y, Yanai T, Kiyohara Y, et al. Severe disability related to cerebral stroke: Incidence and risk factors observed in a Japanese community, Hisayama. *J Am Geriatr Soc* 1987; **35:** 616–622.
14. Obara F, Saitoh S, Takagi S, Shimamoto K. Influence of hypertension on the incidence of cardiovascular disease in two rural communities in Japan: The Tanno-Sobetsu study. *Hypertens Res* 2007; **30:** 677–682.
15. Ueshima H, Choudhury SR, Okayama A, Hayakawa T, Kita Y, Kadowaki T, et al. Cigarette smoking as a risk factor for stroke death in Japan: NIPPON DATA80. *Stroke* 2004; **35:** 1836–1841.
16. Nakamura K, Okamura T, Hayakawa T, Kadowaki T, Kita Y, Ohnishi H, et al; The NIPPON DATA90 Research Group. Chronic kidney disease is a risk factor for cardiovascular death in a community-based population in Japan: NIPPON DATA90. *Circ J* 2006; **70:** 954–959.
17. Staessen JA, Wang JG, Thijs L. Cardiovascular protection and blood pressure reduction: A meta-analysis. *Lancet* 2001; **358:** 1305–1315.
18. ALLHAT Officers and Coordinators for the ALLHAT Collaborative Research Group. The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial. Major outcomes in high-risk hypertensive patients randomized to angiotensin-converting enzyme inhibitor or calcium channel blocker vs diuretic: The Antihypertensive and Lipid-Lowering Treatment to Prevent Heart Attack Trial (ALLHAT). *JAMA* 2002; **288:** 2981–2997.
19. Mabuchi H, Kita T, Matsuzaki M, Matsuzawa Y, Nakaya N, Oikawa S, et al; J-LIT Study Group. Japan Lipid Intervention Trial. Large scale cohort study of the relationship between serum cholesterol concentration and coronary events with low-dose simvastatin therapy in Japanese patients with hypercholesterolemia and coronary heart disease: Secondary prevention cohort study of the Japan Lipid Intervention Trial (J-LIT). *Circ J* 2002; **66:** 1096–1100.
20. Berman DS, Kang X, Hayes SW, Friedman JD, Cohen I, Abidov A, et al. Adenosine myocardial perfusion single-photon emission computed tomography in women compared with men: Impact of diabetes mellitus on incremental prognostic value and effect on patient management. *J Am Coll Cardiol* 2003; **41:** 1125–1133.
21. Haffner SM, Lehto S, Ronnemaa T, Pyorala K, Laakso M. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med* 1998; **339:** 229–334.
22. Nishimura T, Nakajima K, Kusuoka H, Yamashina A, Nishimura S. Prognostic study of risk stratification among Japanese patients with ischemic heart disease using gated myocardial perfusion SPECT: J-ACCESS study. *Eur J Nucl Med Mol Imaging* 2008; **35:** 319–328.
23. Konishi M, Iso H, Iida M, Naito Y, Sato S, Komachi Y, et al. Trends for coronary heart disease and its risk factors in Japan: Epidemiologic and pathologic studies. *Jpn Circ J* 1990; **54:** 428–435.
24. Rutan GH, Kuller LH, Neaton JD, Wentworth DN, McDonald RH, McFate Smith W. Mortality associated with diastolic hypertension and isolated systolic hypertension among men screened for the Multiple Risk Factor Intervention Trial. *Circulation* 1988; **77:** 504–514.
25. Benetos A, Safar M, Rudnichi A, Smulyan H, Richard JL, Ducimetieère P, et al. Pulse pressure: A predictor of long-term cardiovascular mortality in a French male population. *Hypertension* 1997; **30:** 1410–1415.
26. Neaton JD, Wentworth D; for the Multiple Risk Factor Intervention Trial Research Group. Serum cholesterol, blood pressure, cigarette smoking, and death from coronary heart disease: Overall findings and differences by age for 316,099 white men. *Arch Intern Med* 1992; **152:** 56–64.
27. Yasuno S, Ueshima K, Oba K, Fujimoto A, Hirata M, Ogihara T, et al. Is pulse pressure a predictor of new-onset diabetes in high-risk hypertensive patients? A subanalysis of the candesartan antihypertensive survival evaluation in Japan (CASE-J) trial. *Diabetes Care* 2010; **33:** 1122–1127.
28. The GISEN Group (Gruppo Italiano di Studi Epidemiologici in Nefrologia). Randomised placebo-controlled trial of effect of ramipril on decline in glomerular filtration rate and risk of terminal renal failure in proteinuric, non-diabetic nephropathy. *Lancet* 1997; **349:** 1857–1863.
29. Go AS, Chertow GM, Fan D, McCulloch CE, Hsu CY. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. *N Engl J Med* 2004; **351:** 1296–1305.
30. Ninomiya T, Kiyohara Y, Kubo M, Tanizaki Y, Doi Y, Okubo K, et al. Chronic kidney disease and cardiovascular disease in a general Japanese population: The Hisayama Study. *Kidney Int* 2005; **68:** 228–236.
31. Irie F, Iso H, Sairenchi T, Fukasawa N, Yamagishi K, Ikehara S, et al. The relationships of proteinuria, serum creatinine, glomerular filtration rate with cardiovascular disease mortality in Japanese general population. *Kidney Int* 2006; **69:** 1264–1271.
32. Levey AS, Coresh J, Balk E, Kausz AT, Levin A, Steffes MW, et al; National Kidney Foundation. National Kidney Foundation practice guidelines for chronic kidney disease: Evaluation, classification, and stratification. *Ann Intern Med* 2003; **139:** 137–147.
33. Ogihara T, Saruta T, Rakugi H, Fujimoto A, Ueshima K, Yasuno S, et al. Relationship between the achieved blood pressure and the incidence of cardiovascular events in Japanese hypertensive patients with complications: A sub-analysis of the CASE-J trial. *Hypertens Res* 2009; **32:** 248–254.
34. Ueshima K, Oba K, Yasuno S, Fujimoto A, Sato Y, Fukiyama K, et al. Long-term effects of candesartan and amlodipine on cardiovascular mortality and morbidity in Japanese high-risk hypertensive patients: Rationale, design, and characteristics of Candesartan Antihypertensive Survival Evaluation in Japan Extension (CASE-J Ex). *Contemp Clin Trials* 2009; **30:** 97–101.

Significance of Adrenocorticotropin Stimulation Test in the Diagnosis of an Aldosterone-Producing Adenoma

Takuhiro Sonoyama, Masakatsu Sone, Kazutoshi Miyashita, Naohisa Tamura, Kenichi Yamahara, Kwijun Park, Naofumi Oyamada, Daisuke Taura, Megumi Inuzuka, Katsutoshi Kojima, Kyoko Honda, Yasutomo Fukunaga, Naotetsu Kanamoto, Masako Miura, Akihiro Yasoda, Hiroshi Arai, Hiroshi Itoh, and Kazuwa Nakao

Department of Medicine and Clinical Science, Kyoto University Graduate School of Medicine, Sakyo-ku, Kyoto 606-8507, Japan

Context: Adrenal venous sampling is the "gold standard" test in the diagnosis of an aldosterone-producing adenoma (APA) among patients with primary aldosteronism (PA) but is available only in specialized medical centers. Meanwhile, an APA is reported to be generally more sensitive to ACTH than idiopathic hyperaldosteronism.

Objective: The aim was to evaluate the diagnostic accuracy of the ACTH stimulation test in the diagnosis of an APA among those with suspicion of PA.

Patients and Setting: Fifty-nine patients admitted to Kyoto University Hospital on suspicion of PA were included in the study.

Interventions: ACTH stimulation tests with 1-mg dexamethasone suppression were performed.

Main Outcome Measure: Plasma aldosterone concentrations (PAC) were examined every 30 min after ACTH stimulation. Receiver-operated characteristics curve analysis was used to evaluate the diagnostic accuracy.

Results: PAC after ACTH stimulations were significantly higher in patients with an APA than in patients with idiopathic hyperaldosteronism or non-PA. Receiver-operated characteristics curve analyses showed that the PAC after ACTH stimulation was effective for the diagnosis of an APA among patients suspected of PA. The diagnostic accuracy was highest at 90 min after ACTH injection, with the optimal cutoff value greater than 37.9 ng/dl corresponding with sensitivity and specificity of 91.3 and 80.6% for the diagnosis of an APA.

Conclusions: Our study indicates that the ACTH stimulation test is useful in the diagnosis of an APA among patients suspected of PA. This test can be used to select patients who are highly suspected of an APA and definitely require adrenal venous sampling. (*J Clin Endocrinol Metab* 96: 2771–2778, 2011)

Primary aldosteronism (PA) is a major cause of secondary hypertension. The incidence of PA in hypertensive patients was thought to be less than 1%, but recent studies have shown that it is far more common than previously perceived (1–6). The diagnosis of PA should not be missed because it has been reported that patients with PA exhibit higher rates of cardiovascular and renal complications compared with those with essential hypertension

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Abbreviations: A/C, Aldosterone to cortisol ratio; AII, angiotensin II; AII-R, AII-responsive; AII-U, AII-unresponsive; APA, aldosterone-producing adenoma; ARR, aldosterone renin ratio; AUC, area under the curve; AVS, adrenal venous sampling; CLR, contralateral ratio; CT, computed tomography; IHA, idiopathic hyperaldosteronism; LR, lateralization ratio; PA, primary aldosteronism; PAC, plasma aldosterone concentration; PRA, plasma renin activity; ROC, receiver-operated characteristic.

(7–10). The ratio of plasma aldosterone concentration (PAC) over plasma renin activity (PRA) [aldosterone renin ratio (ARR)] is usually used to screen for PA (11, 12). A positive ARR should always be confirmed by a follow-up test, such as captopril challenge test (11, 13), saline infusion test (11, 14), oral sodium-loading test (11, 15), and fludrocortisone suppression test (11, 16).

If a diagnosis of PA is made, the subtype should be identified. PA has two major subtypes; aldosterone-producing adenoma (APA) and idiopathic hyperaldosteronism (IHA) (17). In patients with an APA, hypersecretion of aldosterone is usually from one adrenal gland, whereas in those with IHA, hypersecretion of aldosterone is from both adrenal glands. Although rarer forms of PA exist, APA and IHA account for more than 95% of all PA cases. The differential diagnosis of these subtypes is crucial because an APA can be cured surgically, whereas bilateral IHA should be treated medically.

Imaging tests, such as computed tomography (CT) are initially used to classify the subtype and exclude a carcinoma (11). However, CT findings are often misleading (18, 19). One reason is that APA are frequently less than 1 cm in diameter, a size that is sometimes difficult to detect by CT scanning. Another reason is that a mass found on CT is sometimes nonfunctioning.

Adrenal venous sampling (AVS) is the current "gold standard" test to distinguish between unilateral and bilateral PA (11, 19). The procedure is recommended before surgery for patients with PA to avoid the risk of unilateral adrenalectomy of the wrong side (11, 12). However, it is not widely available because AVS requires an experienced angiographer who can locate the small right adrenal vein and take blood samples from adrenal veins (20). Also, AVS is an invasive procedure, somewhat risky, and costly (20). Therefore, AVS is largely limited to specialized major tertiary centers. Adrenal scintigraphy with ^{131}I-6β-iodomethyl-19-norcholesterol under dexamethasone suppression is another method used in the differential diagnosis of an APA or IHA (21). However, the sensitivity of this test largely depends on the size of the tumor because tracer uptake is poor in an APA less than 1 cm in diameter (21, 22). Like AVS, this test is not widely available. Therefore, it would be better if there was an easy method to select patients who are highly suspicious of an APA and definitely need AVS.

In prior years, the posture stimulation test was used to distinguish between an APA and IHA (23, 24). This test was based on the observation that the PAC in IHA patients increased when they changed from a supine to a standing position, owing to enhanced sensitivity of the adrenal zona glomerulosa to changes in angiotensin II (AII) (25, 26). Conversely, APA were considered to be unresponsive to AII stimulation. Later reports, however, showed that AII-responsive (AII-R) APA account for a considerable portion of APA (27–29). AII-R APA are difficult to distinguish from IHA by posture stimulation test because, in the cases of AII-R APA, the PAC responds as well to upright posture as IHA (27). In fact, data show that the posture stimulation test had a relatively low diagnostic accuracy in the differential diagnosis between an APA and IHA (30, 31). Meanwhile, PAC of both AII-unresponsive (AII-U) APA and AII-R APA cases were reported to be more responsive to ACTH stimulation than those of IHA cases (28), although there are reports of ACTH-responsive IHA cases (32).

The present study was performed to test the hypothesis that this difference in sensitivity to ACTH could be useful in the diagnosis of an APA among patients suspected of PA. To address this hypothesis, we tested the efficiency of the ACTH stimulation test under 1-mg dexamethasone suppression.

Patients and Methods

We retrospectively analyzed the medical records of patients with suspected PA who were admitted to the Department of Endocrinology and Metabolism of Kyoto University Hospital, Kyoto, Japan, over the past 7 yr. The study was approved by the Kyoto University Graduate School and Faculty of Medicine Ethics Committee and conducted in accordance with the principles of the Declaration of Helsinki. Patients with an ARR over 20 ng/dl per ng/ml · h who were admitted to our hospital were initially included (12). All antihypertensive drugs except calcium channel blockers and α-blockers were stopped at least 2 wk before hospitalization. Patients with hypokalemia (*i.e.* serum potassium levels <3.5 mEq/liter) were allowed to take oral potassium supplementation.

Blood pressures were measured in a quiet and warm room with patients in the seated position with the arm held at heart level. The blood pressures described in Table 1 were those obtained the morning after hospitalization. All tests were performed during morning hours in a quiet room. PRA and PAC were measured in blood samples obtained after 30 min of rest in a supine position in the morning. We used the captopril challenge test to confirm diagnosis of PA in this study. An ARR of at least 20 ng/dl per ng/ml · h at 60 min after administration of 50 mg of captopril was considered positive for PA; a post-captopril ARR below 20 ng/dl per ng/ml · h indicated non-PA (12).

Patients with confirmed PA underwent subtype diagnosis before surgery. Adrenal CT scanning was done for initial localization. The definitive tests for subtype diagnosis were AVS. AVS was performed by expert radiologists with ACTH stimulation as we previously described (33). Adrenal vein cannulation was considered successful if the adrenal vein/inferior vena cava cortisol gradient (selectivity index) was greater than 3.0. We considered lateralization when the aldosterone to cortisol ratio (A/C) from one adrenal gland was at least three times the ratio from the other adrenal gland [lateralization ratio (LR)] and the A/C in the contralateral adrenal vein was lower than the A/C in the vena cava [contralateral ratio (CLR)] (19). When the CLR was greater than 1.0 and the LR was no greater than 3.0 in a patient with con-

TABLE 1. Baseline characteristics of the patients of each group

	IHA group	*P* value IHA *vs.* APA	APA group	*P* value APA *vs.* non-PA	Non-PA group[a]
n	16		23		20
Age (yr)	57.8 ± 3.0	ns	48.4 ± 3.1	ns	51.7 ± 2.2
Sex (males:females)	7:9		12:11		13:7
Basal PAC (pg/ml)	166.1 ± 21.1	$P < 0.05$	368.2 ± 60.9	$P < 0.005$	150.2 ± 12.4
Basal PRA (ng/ml · h)	0.39 ± 0.10	ns	0.31 ± 0.07	$P < 0.001$	0.95 ± 0.18
U-Aldo (μg/d)	10.2 ± 1.1	$P < 0.01$	19.7 ± 2.6	$P < 0.01$	11.6 ± 1.0
Serum K (mEq/liter)	3.67 ± 0.09	ns	3.39 ± 0.11	$P < 0.001$	3.83 ± 0.07
Systolic BP (mm Hg)[b]	127.5 ± 4.9	ns	135.7 ± 3.7	$P < 0.05$	125.7 ± 3.5
Diastolic BP (mm Hg)[a]	78.1 ± 3.0	ns	87.7 ± 2.6	ns	83.2 ± 2.9

Data are expressed as means ± SEM. U-Aldo, Urinary aldosterone; K, potassium; BP, blood pressure; ns, not significant.

[a] Non-PA group in this study indicates a group of patients who were positive for the screening test of PA (*i.e.* an ARR ≥20 ng/dl per ng/ml · h) but were negative for captopril challenge test.

[b] 80% of the non-PA group, 87.5% of the IHA group, and 87.0% of the APA group were taking antihypertensive agents.

firmed PA, bilateral aldosterone secretion was considered. As shown in *Exclusion criteria* below, those with an ambiguous AVS outcome (*i.e.* LR >3.0 and CLR >1.0, or LR ≤3.0 and CLR ≤1.0) were excluded from this study.

Diagnosis of an APA required the following: 1) diagnosis of PA by the captopril challenge test; 2) lateralization of aldosterone secretion at AVS; and 3) CT evidence of adrenal mass and/or pathological evidence of adrenal adenoma in the adrenal gland with aldosterone hypersecretion. Patients with confirmed PA for whom bilateral aldosterone hypersecretion was confirmed by AVS were diagnosed with IHA.

Exclusion criteria

The following patients were excluded from this study: confirmed PA with no acceptable subtype diagnosis; unsuccessful AVS; ambiguous AVS outcomes (*i.e.* LR >3.0 and CLR >1.0, or LR ≤3.0 and CLR ≤1); a negative post-captopril ARR with a pathologically confirmed APA; and autonomous cortisol secretion (*i.e.* plasma cortisol level ≥3.0 μg/dl after overnight 1-mg dexamethasone suppression).

ACTH stimulation test under 1-mg dexamethasone suppression

ACTH stimulation tests under 1-mg dexamethasone suppression were performed on all patients. Dexamethasone was administered at 2300 h the night before ACTH injection to avoid the effect of endogenous ACTH. At 0800 h the following morning, 0.25 mg (25 IU) of synthetic ACTH (cosyntropin) was injected, and blood samples were taken every 30 min until 120 min after the injection. We examined the PAC values before and after ACTH injection (PAC_{0min}, PAC_{30min}, PAC_{60min}, PAC_{90min}, and PAC_{120min}) and the fold increase from PAC_{0min} after ACTH injection (PAC_{30min}/PAC_{0min}, PAC_{60min}/PAC_{0min}, PAC_{90min}/PAC_{0min}, and PAC_{120min}/PAC_{0min}).

Statistical analysis

All the data are expressed as mean ± SEM. We used one-way ANOVA followed by *post hoc* Tukey's multiple comparison test to compare means between groups. A P value <0.05 was considered to be statistically significant. The diagnostic accuracy of different time points used to analyze outcomes of the ACTH stimulation test was assessed with a receiver-operated characteristics (ROC) curve and the area under the ROC curve (AUC). When the variable under study could not be distinguished between groups, the AUC was 0.5 (*e.g.* the ROC curve coincided with the diagonal). When there were no overlapping values between the groups, the AUC equaled 1 and the ROC curve reached the upper left corner of the plot. The optimal cutoff point of each time point (*i.e.* the best combination of sensitivity and the lowest false-positive rate) was set at the closest point to the upper left corner of the ROC curve plot.

Results

We analyzed 80 consecutive patients with a positive screening test of an ARR greater than 20 ng/dl per ng/ml · h who were admitted to our hospital for the diagnosis of PA. Of these patients, 20 were diagnosed with non-PA (the non-PA group; *i.e.* screening-positive and confirmation-negative group) by captopril challenge test. The following patients were excluded from the study: three patients with confirmed PA who did not undergo AVS; seven patients with unsuccessful AVS; four with ambiguous AVS results; four with a negative post-captopril ARR with a pathologically confirmed APA; and three patients with autonomous cortisol secretion. The remaining patients were classified as belonging to either the IHA or APA group, according to criteria defined in *Patients and Methods*.

A total of 59 patients were included in this study: 20 in the non-PA group (*i.e.* screening-positive and confirmation-negative group), 16 in the IHA group, and 23 in APA group (Fig. 1). All but three APA patients underwent laparoscopic adrenalectomy, and all those who underwent surgery had pathologically confirmed adrenal adenoma.

Baseline characteristics

Baseline characteristics of the IHA, APA, and non-PA groups are shown in Table 1. Patients with IHA and

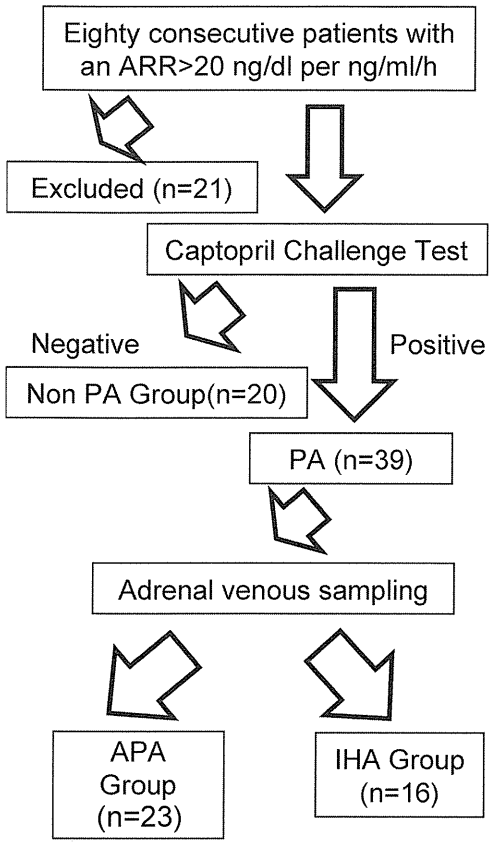

FIG. 1. Trial design.

APA had significantly lower basal PRA levels than those of non-PA ($P < 0.05$ and $P < 0.001$, respectively). Basal PRA levels did not differ significantly between patients in the IHA and APA groups. Basal PAC levels were significantly higher in the APA group compared with the IHA and non-PA groups ($P < 0.005$ and $P < 0.001$, respectively). The APA group also had significantly higher urinary aldosterone levels than the IHA and non-PA groups ($P < 0.01$ and $P < 0.01$, respectively). Serum K levels were significantly lower in the APA *vs.* the non-PA group ($P < 0.001$); 87.5% of the IHA group, 87.0% of the APA, and 80% of the non-PA group were taking antihypertensive agents. In the IHA group, 31.3% of patients were taking oral potassium supplementation; the figures in the APA and non-PA groups were 73.9 and 15.0%, respectively.

ACTH stimulation test under dexamethasone suppression

We analyzed the results of the ACTH stimulation tests under 1-mg dexamethasone suppression in all 59 patients to investigate its accuracy in the differential diagnosis of IHA, APA, and non-PA groups. The mean PAC values of the IHA, APA, and non-PA groups before ACTH injection (PAC_{0min}) were 11.6, 17.1, and 11.1 ng/dl, respectively. The PAC_{0min} value in the APA group tended to be higher than those in the IHA and non-PA groups, but the differences were not significant. PAC values rose in all groups after ACTH stimulation. In the IHA group, the mean PAC_{30min}, PAC_{60min}, PAC_{90min}, and PAC_{120min} values were 33.0, 34.6, 31.9, and 27.8 ng/dl, respectively (Fig. 2A). In the APA group, the mean PAC_{30min}, PAC_{60min}, PAC_{90min}, and PAC_{120min}values were 64.7, 80.1, 75.2, and 62.6 ng/dl, respectively (Fig. 2A). In the non-PA group, the mean PAC_{30min}, PAC_{60min}, PAC_{90min}, and PAC_{120min} values were 32.4, 34.4, 32.2, and 27.0 ng/dl, respectively. The fold increase from PAC_{0min} after ACTH injection (PAC_{30min}/PAC_{0min}, PAC_{60min}/PAC_{0min}, PAC_{90min}/PAC_{0min}, and PAC_{120min}/PAC_{0min}) in the IHA and APA groups are shown in Fig. 2B. After ACTH stimulation, the PAC values of the APA group were significantly higher than those in the IHA and non-PA groups at all data points. Figure 3 shows the plots of PAC values of each group before and after ACTH stimulation.

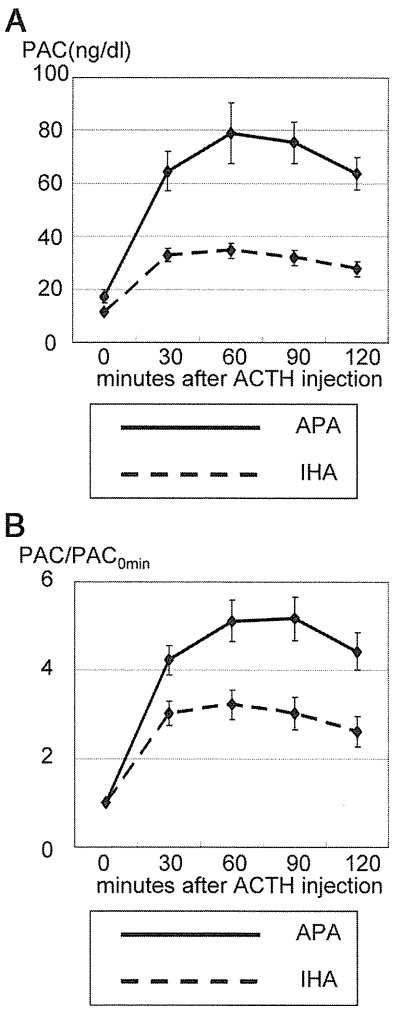

FIG. 2. PAC values (A) and PAC_{0min}-corrected PAC values (B), before (0 min) and after (30, 60, 90, 120 min) ACTH injections of IHA group (*dashed line*), and APA group (*continuous line*). Data are shown as means ± SEM.

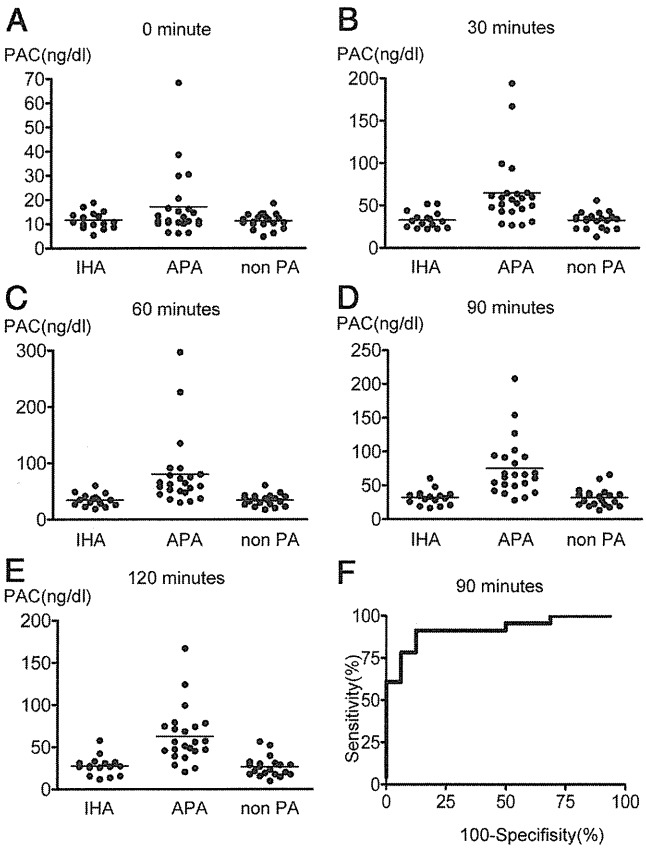

FIG. 3. A–E, Scattergram of PAC values of non-PA, IHA, and APA group before (A), and 30 min (B), 60 min (C), 90 min (D), and 120 min (E) after the ACTH injection. F, ROC curve of PAC values 90 min after the ACTH injection in the differential diagnosis between IHA and APA.

Diagnostic accuracy of ACTH stimulation test in the diagnosis of an APA

We first analyzed the diagnostic accuracy of ACTH stimulation test in the differential diagnosis between IHA and an APA. Table 2 shows the results of ROC curve analysis of PAC values for each time point after ACTH stimulation. At all time points, the AUC was higher than that under the diagonal, indicating that the PAC values for 30, 60, 90, and 120 min after ACTH stimulation were all useful in the differential diagnosis between the APA and IHA groups. The AUC was highest at 90 min after ACTH stimulation, and the optimal cutoff value of the PAC for the diagnosis of an APA was greater than 37.9 ng/dl. These results corresponded with sensitivity and specificity of 91.3 and 87.5%, respectively.

Next, we analyzed the diagnostic accuracy of the ACTH stimulation test in the diagnosis of an APA among IHA, APA, and non-PA patients. Table 2 shows the results of the ROC curve analysis for PAC values at each time point after ACTH stimulation. At every time point, the AUC was higher than that under the diagonal, indicating that the PAC values 30, 60, 90, and 120 min after ACTH stimulation were all useful in the diagnosis of an APA. The AUC was highest at 90 min after ACTH stimulation, and the optimal cutoff value of the PAC for the diagnosis of an APA was greater than 37.9 ng/dl. These data correspond with sensitivity and specificity of 91.3 and 80.6%, respectively.

To investigate whether the fold increase from PAC_{0min} after ACTH stimulation could improve the diagnostic accuracy over the raw PAC value, we measured the AUC under ROC curve of the fold increase from PAC_{0min} after ACTH stimulation. We found that the fold increase from PAC_{0min} worsens the diagnostic accuracy at any time point after ACTH stimulation (data not shown).

Discussion

The high prevalence of PA among hypertensive patients and the potential need for surgery in those with an APA

TABLE 2. Results of the ROC curve analysis of PAC values before and after ACTH stimulation

Time point	AUC (95% CI)	Optimal cutoff (ng/dl)	Sensitivity at optimal cutoff (%)	Specificity at optimal cutoff (%)
Diagnosis of APA group between APA and IHA groups				
Before	0.622 (0.443 to 0.801)	>11.0	65.2	56.3
30 min	0.851 (0.731 to 0.970)	>41.5	82.6	81.3
60 min	0.889 (0.788 to 0.989)	>47.3	78.3	87.5
90 min	0.921 (0.836 to 1.007)	>37.9	91.3	87.5
120 min	0.883 (0.771 to 0.995)	>35.3	87.0	87.5
Diagnosis of APA group among IHA, APA, and non-PA groups				
Before	0.623 (0.472 to 0.775)	>12.9	47.8	69.4
30 min	0.855 (0.745 to 0.965)	>42.2	82.6	86.1
60 min	0.885 (0.794 to 0.976)	>43.6	82.6	86.1
90 min	0.913 (0.838 to 0.988)	>37.9	91.3	80.6
120 min	0.889 (0.800 to 0.978)	>35.3	87.0	86.1

CI, Confidence interval.

make effective detection of PA an important issue. Subtype diagnosis of PA requires a costly procedure that is not always accessible. Taking into consideration the high prevalence of PA among hypertensive patients, it is practically impossible to perform confirmatory subclass diagnostic procedures, such as AVS, for all the patients with confirmed PA because these procedures are currently limited to large medical centers. Therefore, a simpler and more accessible procedure is required to select patients who are highly suspicious of an APA and definitely need confirmatory subclass diagnostic procedures.

The posture stimulation test, which was formerly used to distinguish between an APA and IHA (23, 24), proved to have a low diagnostic accuracy (30, 31), probably because of the considerable percentage of AII-R APA, which are indistinguishable from IHA by the test, among all APA (27).

Meanwhile, PAC in the cases of AII-R APA were reported to also be responsive to ACTH stimulation (28). Stowasser *et al.* (28) found that the PAC of AII-R APA patients during ACTH infusion were similar to, or even higher than, those of AII-U APA patients, although the PAC of IHA patients during ACTH infusion were significantly lower than those of AII-R APA and AII-U APA patients. This report suggests that ACTH stimulation is useful in the differential diagnosis between an APA and IHA because PAC of both AII-R APA and APA-U APA patients are more responsive to ACTH than in IHA patients.

The idea of the ACTH stimulation test under dexamethasone suppression was first published by Kem *et al.* (34) in 1978. In that report, there was a difference in PAC in response to ACTH between APA and IHA patients under certain circumstances. However, the diagnostic accuracy of this test in differentiating an APA and IHA was not evaluated. In our study, we demonstrated that the ACTH stimulation test under dexamethasone suppression is highly sensitive for distinguishing patients with an APA from those with IHA or non-PA.

Our study is based on the prior observation that an APA is generally more sensitive to ACTH than IHA or essential hypertension, probably owing to the expression of ACTH receptors on an APA (35). Similar to APA cases, several reports show that in some cases of IHA, PAC respond well to ACTH stimulation (32). This study included such cases. In two patients in the IHA group, PAC values at 90 min after ACTH stimulation were higher than 37.9 ng/dl. However, as shown in our ROC curve analyses, we could identify patients highly suspected of having an APA among those suspected of PA with high sensitivity. Although this test cannot be used for final diagnosis of an APA, it can be used to screen patients who are highly suspected of having an APA and definitely need AVS.

In the present study, we hypothesized that 1-mg dexamethasone suppression would result in a greater decrease in PAC_{0min} in the ACTH-sensitive APA group compared with IHA and non-PA patients and that the fold increase from PAC_{0min} after ACTH stimulation would provide more accurate diagnostic criteria than raw PAC values. However, to our surprise, the latter proved more useful according to the ROC curve analysis. This is partly because the PAC_{0min} values were higher in APA patients than non-PA and IHA patients despite the dexamethasone suppression. Further studies are needed to clarify whether dexamethasone suppression is necessary in this ACTH stimulation test.

In this study, we made the decision of the lateralization of aldosterone hypersecretion with LR and CLR in AVS after ACTH stimulation. However, when performing AVS, we always obtain blood samples from both adrenal veins and inferior vena cava before and after ACTH stimulation to avoid sampling errors. Applying the same criterion for lateralization as AVS with ACTH stimulation (LR >3 and CLR ≤ 1 for unilateral aldosterone hypersecretion; and LR ≤ 3 and CLR >1 for bilateral aldosterone hypersecretion) to AVS without ACTH stimulation (36), two of 23 patients in the APA group were diagnosed with bilateral aldosterone hypersecretion by AVS without ACTH stimulation, and two of 16 patients in the IHA group were diagnosed with unilateral aldosterone hypersecretion by AVS without ACTH stimulation. The PAC of

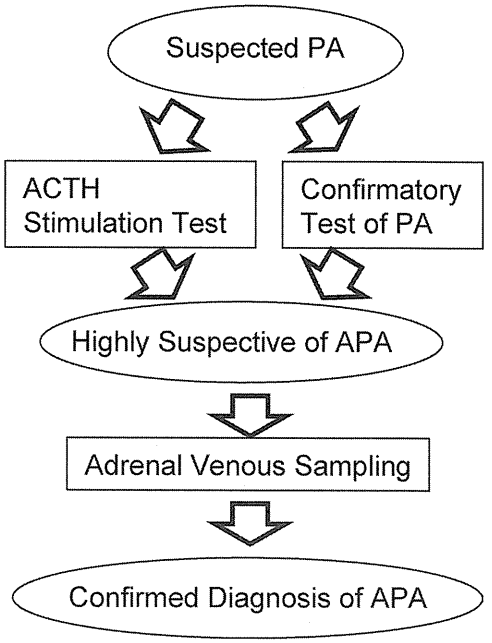

FIG. 4. A diagnostic flowchart that we suggest in the diagnosis of an APA.