TABLE 2. Characteristics of Subjects at Entry and at Year 5

	Subjects With Weight Gain		Subjects Without Weight Gain		Subjects With BP Elevation		Subjects Without BP Elevation	
	At Entry	At Year 5	At Entry	At Year 5	At Entry	At Year 5	At Entry	At Year 5
Subjects, n	59	59	101	101	41	41	119	119
Smoker/nonsmoker, n	15/44	10/49	22/79	13/88	13/28	8/33	24/95	15/104
Age, y	39±4	44±4‖	40±5	45±5‖	40±4	45±4‖	40±5	45±5‖
BMI, kg/m^2	22.2±1.8	24.6±2.0*§	22.9±1.7	22.4±1.9	22.6±1.6	24.3±2.1†§	22.7±1.7	22.8±1.9
Waist-to-hip ratio	0.92±0.11*	0.97±0.13*§	0.88±0.12	0.91±0.13	0.92±0.09†	0.94±0.11	0.89±0.11	0.92±0.12
Total fat mass, kg	14.1±2.1*	15.6±2.2*§	12.5±1.9	12.9±2.0	13.7±2.0†	15.1±2.0†§	12.8±2.0	13.4±2.2
Systolic BP, mm Hg	127±6	141±8*‖	128±6	131±7	132±7†	146±9‡‖	126±6	131±7
Diastolic BP, mm Hg	77±5*	80±5*	74±5	75±5	74±5	83±6†‖	76±4	75±6
Mean BP, mm Hg	94±5	101±6*§	93±5	94±6	93±6	104±6‡‖	92±6	94±6
Heart rate, bpm	70±5*	72±5	66±5	71±5§	71±4†	73±5	66±5	71±6§
Plasma norepinephrine, pmol/mL	1.18±0.11*	1.41±0.21*§	1.00±0.20	1.26±0.19	1.14±0.12†	1.43±0.24†§	1.01±0.16	1.27±0.18§

Data are mean±SD; n=160.
*P<0.05 vs subjects without weight gain; †P<0.05, ‡P<0.01 vs subjects without BP elevation; §P<0.05, ‖P<0.01 vs values at entry.

adrenoceptor polymorphism was 4.6%, and that of Arg64 of the β_3-adrenoceptor polymorphism was 17.4%, but all studied loci allele and genotype frequencies were in accordance with the Hardy-Weinberg equilibrium. The frequency distributions for homozygosity for the Arg16 and Gly16 alleles in this study were 28.1% and 22.5%. The frequency distributions for homozygosity for the Gln27 and Glu27 were 90.7% and 0.0%, and the frequency distributions for the Trp64 and Arg64 were 67.1% and 1.9%. The frequency distributions for homozygosity for the Glu27 and the Arg64 in our subjects were similar to those in previous studies in Japanese cohorts but lower than those found in studies in white subjects.[5,6,9,10,22,23] The frequency of Gly16 allele of the β_2-adrenoceptor gene was greater in subjects with weight gain than in those without weight gain. Additionally, the frequency of the Gly16 allele of the β_2-adrenergic receptor gene was significantly greater in subjects who showed a significant BP elevation over 5 years. The frequencies of the Glu27 and Trp64 alleles were higher in subjects with BP elevation than in those without BP elevation (Table 1).

Furthermore, to evaluate the relationships between the β-adrenoceptor alleles and weight gain–related BP elevation, we compared the frequencies of alleles between the groups with and without BP elevation in subjects who significantly gained body weight versus those without weight gain. In subjects who had a significant weight gain, those who also had a significant BP elevation carried a higher frequency of the Gly16 and Glu27 alleles compared with those without a significant BP elevation (χ^2=4.73, P=0.030; χ^2=6.35, P=0.012, respectively). In subjects who did not gain weight over the 5-year period, the allele frequencies in the 3 genotypes that were studied were similar in subjects with and without a BP elevation over time.

Table 2 shows the demographic characteristics of the 2 groups subdivided by significant weight gain (≥10%) over 5 years or BP elevation at entry and at year 5. At both periods, waist-to-hip ratio, total fat mass, heart rate, and plasma norepinephrine were higher in the group who had significant weight gain and in the group who had a significant rise in mean BP compared with those without weight gain or BP elevation. It is important to note that at entry, BMI and BP were similar between the groups with and without weight gain and BP elevation. Among the 41 subjects with a significant BP elevation, 32 of these individuals also had a significant weight gain.

Subjects were divided into the 2 subgroups in each studied genotype according to the dominant allele. Characteristics between those with and without the dominant allele are shown in Tables 3, 4, and 5. Total body fat mass and waist-to-hip ratio at entry in the subjects carrying the Gly16 allele and Glu allele of the β_2-adrenoceptor gene were greater than in the other genotypes (Tables 3 and 4). BMI and total body fat mass increased significantly in the subjects with the Gly16 allele and Glu27 allele of the β_2-adrenoceptor genes. Subjects who had the Gly16 and Glu27 of the β_2-adrenoceptor gene and the Trp64 of the β_3-adrenoceptor gene had significant increments in mean BP over the 5 years (Tables 3, 4, and 5).

As we have previously reported,[15,16,21] subjects with the most significant weight gain and BP elevation had the highest levels of plasma norepinephrine at entry compared with subjects without weight gain or BP elevation (Table 2). Plasma norepinephrine levels at both entry and year 5 were greater in subjects carrying Gly16 allele and Glu27 allele of the β_2-adrenoceptor genes than in the other genotypes (Tables 3 and 4). Plasma norepinephrine levels increased significantly over the 5-year period in those subjects with the abnormal β-adrenoceptor alleles. The same subjects also had higher plasma norepinephrine levels at entry.

Discussion

The present study shows that the Arg16Gly and the Gln27Glu of the β_2-adrenoceptor and the Trp64Arg of the β_3-adrenoceptor polymorphisms have a substantial influence on future gain in body weight or BP elevation in male subjects who were originally nonobese and normotensive. The subjects carrying the polymorphism for the Gly16, Glu27, and Trp64 alleles show higher frequency in those who had a

TABLE 3. Characteristics of Subjects According to Genotype of Arg16Gly at Entry and at 5 Years

	Without Gly16 Allele (Arg16Arg)		With Gly16 Allele (Arg16Gly+Gly16Gly)	
	At Entry	At Year 5	At Entry	At Year 5
Subjects, n	45	45	115	115
Smoker/nonsmoker, n (%)	10/35 (22.2/77.8)	7/38 (15.6/84.4)	27/88 (23.5/76.5)	16/99 (13.9/86.1)
BMI, kg/m^2	22.6±1.9	22.4±2.8	22.7±1.8	23.5±2.1*‡
Waist-to-hip ratio	0.87±0.10	0.88±0.11	0.91±0.10‡	0.95±0.12*‡
Total fat mass, kg	12.8±2.0	13.2±1.9	13.4±1.9‡	14.1±2.0*‡
Systolic BP, mm Hg	128±5	129±6	127±5	137±7†§
Diastolic BP, mm Hg	73±7	75±5	76±6	79±5‡
Mean BP, mm Hg	92±6	93±5	94±6	98±7*‡
Heart rate, bpm	65±6	69±5	69±6	72±6
Norepinephrine, pmol/mL	0.99±0.16	1.10±0.22	1.09±0.14‡	1.40±0.10†§

Data are mean±SD; n=160.
*P<0.05, †P<0.01 vs value at entry; ‡P<0.05, §P<0.01 vs subjects without Gly16 allele (Arg16Arg genotype).

significant weight gain or BP elevation over the 5-year study. Higher levels of plasma norepinephrine at entry were also seen in the groups with the Gly16 or Glu27 alleles. As we have shown in all studies, a heightened SNA (high mean plasma norepinephrine) predicted subsequent weight gain and BP elevation.[15,16,21] Now we show that the increased SNA is in part determined by the genetic influence of the β_2-adrenergic receptor systems.

Pathophysiological involvement of genetic abnormalities in the β_2-adrenergic receptor system in hypertension and obesity are well described.[5,6,24–26] Among β_2-adrenergic receptor polymorphisms, Arg16Gly and Gln27Glu are considered the most functionally important.[5,6,24–26] Gratze et al[27] found that young normotensive white men homozygous for the Gly16 allele of the β_2-adrenoceptor gene had higher BP and lower peripheral vasodilation after infusion of the β-blocker salbutamol. The Gly16 substitution exaggerates agonist-mediated receptor downregulation.[6,28] Our findings that the Gly16 allele is associated with weight gain and BP elevation associated with higher plasma norepinephrine levels are in accordance with these findings. The subjects who had weight gain–related BP elevation also had higher frequencies of the Gly16 and Glu27 alleles compared with those without BP elevation, suggesting that Gly16/Glu27 is related to obesity-related hypertension. On the other hand, the frequency associations of the Arg16 or Gly16 alleles of the Arg16Gly and the Gln27 or Glu27 alleles of the Gln27Glu with the onset of hypertension and obesity are more controversial.[6] The Glu27 receptor had been shown to be resistant to downregulation compared with Gln27 but when coexpressed with Arg16.[29] We were not able to observe any significant association of the Arg16 and Glu27 alleles with weight gain or BP elevation, probably because of the small sample size of the study.

The β_3-adrenergic receptor system is important in mediating the stimulation of lipolysis by catecholamines in white adipose cells in humans and in the development of obesity.[8–10] It is well documented that weight gain leads to BP elevation,[1,15,16] but there are few investigations about the genetic relations in the β_3-adrenoceptor such as polymor-

TABLE 4. Characteristics of Subjects According to Genotype of Gln27Glu at Entry and at 5 Years

	Without Glu27 Allele (Gln27Gln)		With Glu27 Allele (Gln27Glu)	
	At Entry	At Year 5	At Entry	At Year 5
Subjects, n	137	137	14	14
Smoker/nonsmoker, n (%)	34/103 (24.8/75.2)	22/115 (16.1/83.9)	3/11 (21.4/78.6)	1/13 (7.1/92.9)
BMI, kg/m^2	22.6±1.7	23.0±2.5	23.5±2.1	24.6±3.0*‡
Waist-to-hip ratio	0.89±0.10	0.92±0.11	0.92±0.11	0.99±0.10*‡
Total fat mass, kg	13.0±1.9	13.4±2.0	13.9±1.3‡	14.9±2.3*§
Systolic BP, mm Hg	127±5	135±5*	132±5	138±6*
Diastolic BP, mm Hg	75±5	76±6	77±6	83±5*§
Mean BP, mm Hg	93±5	94±5	95±5	101±6*§
Heart rate, bpm	67±5	71±6	69±5	70±6
Norepinephrine, pmol/mL	1.03±0.20	1.30±0.18*	1.29±0.14‡	1.42±0.19*‡

Data are mean±SD; n=151.
*P<0.05, †P<0.01 vs value at entry; ‡P<0.05, §P<0.01 vs subjects without Glu allele (Gln27Gln genotype).

TABLE 5. Characteristics of Subjects According to Genotype of Trp64Arg at Entry and at 5 Years

	With Trp64 Allele (Trp64Trp+Trp64Arg)		Without Trp64 Allele (Arg64Arg)	
Genotype	At Entry	At Year 5	At Entry	At Year 5
Subjects, n	155	155	3	3
Smoker/nonsmoker, n (%)	36/119 (23.2/76.8)	23/132 (14.6/85.4)	1/2 (33.3/66.7)	0/3 (0.0/100.0)
BMI, kg/m^2	23.1±1.7	23.2±2.7	22.8±0.5	24.0±0.6
Waist-to-hip ratio	0.90±0.06	0.93±0.08	0.90±0.09	0.94±0.10
Total fat mass, kg	13.2±1.8	13.9±2.0	13.1±2.0	13.5±2.0
Systolic BP, mm Hg	127±5	134±6†	126±6	128±7
Diastolic BP, mm Hg	75±5	78±5*	75±5	77±6
Mean BP, mm Hg	93±5	97±5†	92±5	94±6
Heart rate, bpm	68±5	71±6*	67±5	68±6
Norepinephrine, pmol/mL	1.06±0.20	1.31±0.14*	1.03±0.25	1.27±0.23

Data are mean±SD; n=158.
*$P<0.05$, †$P<0.01$ vs value at entry.

phisms in Trp64Arg and the association of these polymorphisms with hypertension in obesity.[30] Fujisawa et al[23] have shown in a Japanese population that the allele frequency of Arg64 in hypertensive subjects was similar to that in normotensive subjects. Other investigators have reported in a large Japanese cohort (n=3706) that the subjects with the Arg64/Arg64 genotype had a greater BMI and percent fat mass than those with the in Trp64/Trp64 genotype.[9] Conversely, we did not observe these associations in the genotype of the β_3-adrenoceptor in relation to weight gain–related BP elevation.

In the present study we used plasma norepinephrine levels as an index of SNA. Tuck,[31] Grassi and Esler,[32] and Rahn et al[33] observed that there are different results in SNA values in hypertensive patients depending on the method of SNA measurement, including regional norepinephrine spillover, muscle sympathetic nerve activity (microneurography), and plasma norepinephrine measurements. Spillover methods are considered the "gold standard" for SNA measurements, but in humans these are difficult and invasive measurements. Plasma norepinephrine levels are more practical for large population studies and represent several different processes (secretion, clearance, and reuptake).[3,15,16]

It is known that Asian people (Japanese) have a lower definition of obesity than the World Health Organization BMI cutoff point for obesity (≥30 kg/m^2),[13,14] which is controlled by genotypes. In a Japanese population, a strong association between visceral fat content and the metabolic syndrome has been reported, as seen even in subjects defined as nonobese by BMI but who were obese by CT.[34] In the present study the subjects who had the most significant weight gain and BP elevations also had a greater total body fat mass and waist-to-hip ratio plus higher plasma norepinephrine levels at entry, but BMI was not different between these entry groups. These findings suggest that abdominal obesity might be the link to heightened SNA, which is in part determined genetically by the abnormal β-adrenoceptor polymorphism. Alvarez et al[35,36] have reported that visceral obesity, but not subcutaneous obesity, is best associated with increased SNA. Grassi et al[37] have also found that central obesity is characterized by greater sympathetic activation and impaired baroreceptor sensitivity than peripherally obese or lean subjects.

In summary, these findings are from the first large cohort-based longitudinal study analyzing the effect of genetic variation in the β_2- and β_3-adrenoceptor genes over a fixed time period, showing their strong association with initiation of weight gain and BP elevation. SNA, as seen in plasma norepinephrine accompanying abdominal obesity, may be the major mediator of the β_2-adrenoceptor gene changes.

References

1. Hubert HB, Feinleib M, McNamara PM, Castelli WP. Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. *Circulation.* 1983;67:969–977.
2. Cui J, Hopper JL, Harrap SB. Genes and family environment explain correlations between blood pressure and body mass index. *Hypertension.* 2002;40:7–12.
3. Masuo K, Mikami H, Ogihara T, Tuck ML. Familial hypertension, insulin, sympathetic activity, and blood pressure elevation. *Hypertension.* 1998;32:96–100.
4. Masuo K, Mikami H, Ogihara T, Tuck ML. Familial obesity, sympathetic activation and blood pressure level. *Blood Pressure.* 2001;10:199–204.
5. Kato N, Sugiyama T, Morita H, Kurihara H, Sato T, Yamori Y, Yazaki Y. Association analysis of β2-adrenergic receptor polymorphisms with hypertension in Japanese. *Hypertension.* 2001;37:286–292.
6. Pereira AC, Floriano MS, Mota GF, Cunha RS, Herkenhoff FL, Mill JG, Krieger JE. β2 Adrenoceptor functional gene variants, obesity, and blood pressure level interactions in the general population. *Hypertension.* 2003;42(pt 2):685–692.
7. van Rossum CT, Hoebee B, Seidell JC, Bouchard C, van Baak MA, de Groot CP, Chagnon M, de Graaf C, Saris WH. Genetic factors as predictors of weight gain in young adult Dutch men and women. *Int J Obes Relat Metab Disord.* 2002;26:517–528.
8. Clement K, Vaisse C, Manning BS, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P, Strosberg AD. Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. *N Engl J Med.* 1995;333:352–354.
9. Oizumi T, Daimon M, Saitoh T, Kameda W, Yamaguchi H, Ohnuma H, Igarashi M, Eguchi H, Manaka H, Tominaga M, Kato T. Genotype Arg/Arg, but not Trp/Arg, of the Trp64Arg polymorphism of the β3-adrenergic receptor is associated with type 2 diabetes and obesity in a large Japanese sample. *Diabetes Care.* 2001;24:1579–1583.

10. Strazzullo P, Iacone R, Siani A, Cappuccio FP, Russo O, Barba G, Barbato A, D'Elia L, Trevisan M, Farinaro E. Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure: interaction with age: cross-sectional and longitudinal findings of the Olivetti Prospective Heart Study. *J Hypertens.* 2001;19:399–406.
11. Buettner R, Schaffler A, Arndt H, Rogler G, Nusser J, Zietz B, Enger I, Hugl S, Cuk A, Scholmerich J, Palitzsch KD. The Try64Arg polymorphism of the β3-adrenergic receptor gene is not associated with obesity or type 2 diabetes mellitus in a large population-based Caucasian cohort. *J Clin Endocrinol Metab.* 1998;83:2892–2897.
12. Gagnon J, Mauriege P, Roy S, Sjostrom D, Chagnon YC, Dionne FT, Oppert JM, Perusse L, Sjostrom L, Bouchard C. The Trp64Arg mutation of the β3 adrenergic receptor gene has no effect on obesity phenotypes in the Quebec Family Study and Swedish Obese Subjects cohorts. *J Clin Invest.* 1996;98:2086–2093.
13. WHO Expert Consultation. Appropriate body-mass index for Asian populations and its implications for policy and intervention strategies. *Lancet.* 2004;363:157–163.
14. Kanazawa M, Yoshiike N, Osaka T, Numba Y, Zimmet P, Inoue S. Criteria and classification of obesity in Japan and Asia-Oceania. *Asia Pac J Clin Nutr.* 2002;11(suppl 8):S732–S737.
15. Masuo K, Kawaguchi H, Mikami H, Ogihara T, Tuck ML. Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. *Hypertension.* 2003;42:474–480.
16. Masuo K, Mikami H, Ogihara T, Tuck ML. Weight gain–induced blood pressure elevation. *Hypertension.* 2000;35:1135–1140.
17. Willemsen JJ, Ross HA, Jacobs MC, Lenders JW, Thien T, Swinkels LM, Benraad TJ. Highly sensitive and specific HPLC with fluorometric detection for determination of plasma epinephrine and norepinephrine applied to kinetic studies in humans. *Clin Chem.* 1995;41:1455–1460.
18. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, Pesich R, Hebert J, Chen YD, Dzau VJ, Curb D, Olshen R, Risch N, Cox DR, Botstein D. High-throughput genotyping with single nucleotide polymorphisms. *Genome Res.* 2001;11:1262–1268.
19. Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, Walston J, Yoshinaga H, Kosaka K, Yamada N, Saito Y, Hagura R, Akanuma Y, Shuldiner A, Yazaki Y, Kadowaki T. A mutation in the β3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. *Biochem Biophys Res Commun.* 1995;215:555–560.
20. Walston J, Silver K, Bogardus C, Knowler WC, Celi FS, Austin S, Manning B, Strosberg AD, Stern MP, Raben N, Sorkin JD, Roth J, Shuldiner AR. Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β3-adrenergic-receptor gene. *N Engl J Med.* 1995;333:343–347.
21. Masuo K, Mikami H, Ogihara T, Tuck ML. Sympathetic nerve hyperactivity precedes hyperinsulinemia and blood pressure elevation in a young, nonobese Japanese population. *Am J Hypertens.* 1997;10:77–83.
22. Kawamura T, Egusa G, Fujikawa R, Okubo M. Gln27Glu variant of the beta2-adrenergic receptor gene is not associated with obesity and diabetes in Japanese-Americans. *Metabolism.* 2001;50:443–446.
23. Fujisawa T, Ikegami H, Yamato E, Hamada Y, Kamide K, Rakugi H, Higaki J, Murakami H, Shimamoto K, Ogihara T. trp64Arg mutation of beta3-adrenergic receptor in essential hypertension: insulin resistance and the adrenergic system. *Am J Hypertens.* 1997;10:101–105.
24. Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lannfelt L, Arner P. Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. *J Clin Invest.* 1997;100:3005–3013.
25. Castellano M, Rossi F, Giacche M, Perani C, Rivadossi F, Muiesan ML, Salvetti M, Beschi M, Rizzoni D, Agabiti-Rosei E. β2-Adrenergic receptor gene polymorphism, age, and cardiovascular phenotypes. *Hypertension.* 2003;41:361–367.
26. Timmermann B, Mo R, Luft FC, Gerdts E, Busjahn A, Omvik P, Li GH, Schuster H, Wienker TF, Hoehe MR, Lund-Johansen P. Beta2-adrenoceptor genetic variation is associated with genetic predisposition to essential hypertension: the Bergen Blood Pressure Study. *Kidney Int.* 1998;53:1455–1460.
27. Gratze G, Fortin J, Labugger R, Binder A, Kotanko P, Timmermann B, Luft FC, Hoehe MR, Skrabal F. β2-Adrenergic receptor variants affect resting blood pressure and agonist-induced vasodilation in young adult Caucasians. *Hypertension.* 1999;33:1425–1430.
28. Green SA, Turki J, Innis M, Liggett SB. Amino-terminal polymorphisms of the human β2-adrenergic receptor impart distinct agonist-promoted regulatory properties. *Biochemistry.* 1994;33:9414–9419.
29. Martinez FD, Graves PE, Baldini M, Solomon S, Erickson R. Association between genetic polymorphisms of the beta2-adrenoceptor and response to albuterol in children with and without a history of wheezing. *J Clin Invest.* 1997;100:3184–3188.
30. Melis MG, Secchi G, Brizzi P, Severino C, Maioli M, Tonolo G. The Trp64Arg beta3-adrenergic receptor amino acid variant confers increased sensitivity to the pressor effects of noradrenaline in Sardinian subjects. *Clin Sci (Lond).* 2002;103:398–402.
31. Tuck ML. The sympathetic nervous system in essential hypertension. *Am Heart J.* 1986;112:877–886.
32. Grassi G, Esler M. How to assess sympathetic activity in humans. *J Hypertens.* 1999;17:719–734.
33. Rahn KH, Barenbrock M, Hausberg M. The sympathetic nervous system in the pathogenesis of hypertension. *J Hypertens.* 1999;17(suppl 3):S11–S14.
34. Nagaretani H, Nakamura T, Funahashi T, Kotani K, Miyanaga M, Tokunaga K, Takahashi M, Nishizawa H, Kishida K, Kuriyama H, Hotta K, Yamashita S, Matsuzawa Y. Visceral fat is a major contributor for multiple risk factor clustering in Japanese men with impaired glucose tolerance. *Diabetes Care.* 2001;24:2127–2133.
35. Alvarez GE, Ballard TP, Beske SD, Davy KP. Subcutaneous obesity is not associated with sympathetic neural activation. *Am J Physiol.* 2004;287:H414–H418.
36. Alvarez GE, Beske SD, Ballard TP, Davy KP. Sympathetic neural activation in visceral obesity. *Circulation.* 2002;106:2533–2536.
37. Grassi G, Dell'Oro R, Facchini A, Quarti Trevano F, Bolla GB, Mancia G. Effect of central and peripheral body fat mass distribution on sympathetic and baroreflex function in obese normotensives. *J Hypertens.* 2004;22:2363–2369.

AJH 2005; 18:1009–1014

β_2-Adrenoceptor Polymorphisms Relate to Insulin Resistance and Sympathetic Overactivity as Early Markers of Metabolic Disease in Nonobese, Normotensive Individuals

Kazuko Masuo, Tomohiro Katsuya, Yuxiao Fu,
Hiromi Rakugi, Toshio Ogihara, and Michael L. Tuck

Background: The genes responsible for insulin resistance are also candidate genes for insulin resistance-related diseases, such as obesity and hypertension. Functional polymorphisms in the β_2- and β_3-adrenergic receptors have been reported to be associated with diabetes, hypertension, and obesity. To clarify the relevance of the β-adrenergic receptor polymorphisms to insulin resistance, we studied their association with polymorphisms of β_2 (Arg16Gly, Gln27Glu) and β_3 (Trp64Arg) adrenoceptor genes.

Methods: We studied 155 young, nonobese Japanese men using the homeostasis model assessment of insulin resistance (HOMA-IR) to divide individuals into insulin-sensitive and insulin-resistant groups. Insulin resistance in the participants was defined as HOMA-IR equal to or greater than the average plus 1 SD of 3.1. There were 69 men who were insulin resistant and 86 men who were insulin sensitive. Body mass index (BMI), blood pressure (BP), plasma glucose, insulin, leptin, norepinephrine (NE) levels, and the polymorphisms of Arg16Gly and Gln27Glu of the β_2- and Trp64Arg of the β_3-adrenoceptor polymorphisms were measured in all participants.

Results: The insulin-resistant group had higher frequency of the Gly16 allele of Arg16Gly compared with the insulin-sensitive group, whereas the frequencies of genotypes or alleles of Gln27Glu and Trp64Arg were similar. The insulin-resistant group had a higher mean HOMA-IR, fasting insulin, NE, and total fat mass compared with levels in the insulin-sensitive group, but the BMI and leptin levels were similar. The subjects carrying the Gly16 allele of the β_2-adrenoceptor gene had a higher mean HOMA-IR, fasting insulin, NE, body fat mass, and BP than those without the Gly16 allele.

Conclusions: The Gly16 mutation of the β_2-adrenoceptor gene is associated with increased insulin resistance, adiposity, and BP accompanied by higher plasma NE levels early in the metabolic disease in developing obesity. These findings show an important role of β_2-adrenoceptor gene polymorphisms in the association of insulin resistance in hypertension and obesity. Am J Hypertens 2005;18:1009–1014 © 2005 American Journal of Hypertension, Ltd.

Key Words: Insulin resistance, sympathetic nerve activity, β_2- and β_3-adrenoceptor polymorphisms, blood pressure, and obesity.

Obesity and hypertension are associated with metabolic disturbances such as insulin resistance, hyperinsulinemia, and dyslipidemia.[1,2] One pathophysiologic significance of early insulin resistance is that insulin has mitogenic properties that can potentiate vascular smooth-muscle growth, promoting structural changes in blood vessels and possibly contributing to atherosclerosis. Thus, insulin resistance may be an important etiologic factor in the cardiovascular risk seen in the development of obesity and hypertension.[1–3]

One major risk is that human obesity and hypertension have well defined genetic determinants such polymorphisms in the β_2- and β_3-adrenergic receptor.[4–12] We have

Received November 21, 2004. First decision December 29, 2004. Accepted January 12, 2005.

From the Human Neurotransmitter Laboratory (KM), Baker Heart Research Institute, Melbourne, Victoria, Australia; Department of Geriatric Medicine (KM, TK, YF, HR, TO), Osaka University Graduate School of Medicine, Suita, Osaka, Japan; and Endocrinology and Metabolism Division (MLT), Sepulveda Veterans Affairs Medical Center and David Geffen, University of California–Los Angeles School of Medicine, Los Angeles, California.

Address correspondence and reprint requests to Dr. Kazuko Masuo, Baker Heart Research Institute, PO Box 6492 St Kilda Road Central, Melbourne, Victoria 8008, Australia; e-mail: kmasuo@baker.edu.au

0895-7061/05/$30.00
doi:10.1016/j.amjhyper.2005.01.006

reported that insulin resistance and hyperinsulinemia are associated with heightened sympathetic nerve activity[13] and that heightened sympathetic nerve activity, as seen in elevated plasma NE, predicts insulin resistance, subsequent weight gain, and BP elevation.[14–16] In addition, normotensive and normal-weight individuals who have a positive family history of hypertension and obesity also have heightened sympathetic nerve activity.[17,18] These findings imply that sympathetic overactivity defined here as high plasma NE levels are associated with genetic determinants on the β-adrenergic receptor that may contribute to insulin resistance. The present study further examines the relationship between polymorphisms of the β-adrenergic receptors and progression in reduced insulin sensitivity in nonobese, normotensive Japanese men.

Methods

Subjects

A cohort of 1121 men working in Osaka, Japan, as part of their biannual medical evaluation were studied. Subjects were excluded who were >50 years of age, overweight (body mass index [BMI] 25 to 30 kg/m^2) or obese (BMI >30 kg/m^2), had diabetes mellitus (fasting glucose level>100 mg/dL), or hypertension (≥160/95 mm Hg). Additional exclusions were subjects who were taking medications for hypertension, hyperlipidemia, hyperuricemia, or other illness. After exclusion, 155 young men who were nonobese (BMI <25 kg/m^2) and nonhypertensive (BP <160/95 mm Hg) and who were not using any medications were recruited from the cohort. The subjects were subdivided into an insulin-sensitive group and an insulin-resistant group using the homeostasis model assessment of insulin resistance (HOMA-IR) and a cut-off limit of average + 1 SD (2.2 + 0.9) in participants. Because it is well known that recent alterations in plasma insulin, leptin, and NE levels are altered with weight changes, only those subjects who had steady body weight (weight had not changed significantly (<5%) over the past year) were enrolled in the present study.[15,16,19]

The protocol was approved by the Ethics Committee of Osaka University Graduate School of Medicine, Japan, and written informed consent was obtained from all of the subjects.

Measurements

After an overnight fast of 12 h, BMI, total body fat mass, BP, heart rate, and venous sampling for blood glucose, plasma norepinephrine (NE), insulin, leptin, and the extraction of genomic DNA from leukocytes were obtained after a 30-min rest period in the supine position. Lipids profiles (total cholesterol, triglyceride, HDL-cholesterol) and uric acid levels were also evaluated. Both BP and heart rate were measured three times in the supine position by an automated sphygmomanometer (TM-2713, A&D Co. Ltd., Tokyo, Japan), which had been standardized against a mercury sphygmomanometer. The percentage body fat mass was determined with impedance measurements (BF-102, Tanita, Japan), and total body fat mass (kg) was calculated according to the following formula: [percentage body fat mass (%)/100] × body weight (kg). Plasma NE was measured by high-performance liquid chromatography with a fluorometric method (intra-assay coefficient of variation [CV] = 2.1%; inter-assay CV = 3.6%; sensitivity = 0.06 to 120 nmol/L). Plasma immunoreactive insulin was measured by standard radioimmunoassay methods (insulin RIABEAD II, Dinabott; intra-assay CV = 1.9%; inter-assay CV = 2.2%; sensitivity = 0.75 to 300 μU/mL). Plasma leptin was measured by radioimmunoassay (human leptin RIA kit, Linco; intra-assay CV = 5.0%, interassay CV = 4.5%, and sensitivity = 0.03 to 6 nmol/L). The HOMA-IR was defined as the product of fasting plasma insulin (μU/mL) and glucose (mg/dL) divided by 405.[20]

Genotyping

Genotyping was performed by the TaqMan assay, as previously described.[21] Two polymorphisms (arginine/glycine substitution, Arg16Gly, and glutamine/glutamate substitution, Gln27Glu) of the β_2-adrenoceptors[6] and one polymorphism (tryptophan/arginine substitution, Trp64Arg) of the β_3-adrenergic receptor[11,12] were evaluated. For single-nucleotide polymorphisms of the β_2-adrenergic receptor gene, the probes and primers were as follows: for Arg16Gly, the probes were CGCATGGCTTCCATTGGGTGC and CGCATGGCTTCTATTGGGTGC, and the primers were GGAACGGCAGCGCCTTCT and CAGGACGATGAGAGACATGACGAT; for Gln27Glu, the probes were CTCGTCCCTTTCCTGCGTGACGT and CTCGTCCCTTTGCTGCGTGACGT, and the primers used in this assay were the same as those used for Arg16Gly. For the Trp64Arg single-nucleotide polymorphism in the β_3-adrenergic receptors, the probes were TCTCGGAGTCCAGGCGATGGCCA and CTCGGAGTCCGGGCGATGGCC, and the primers were GGAGGCAACCTGCTGGTCAT and CACGAACACGTTGGTCATGGT.

Statistical Analyses

Genotype frequencies and Hardy-Weinberg equilibrium were estimated with χ^2 test. Values are shown as mean ± SD. Differences among groups were examined by the paired or unpaired t test. Multiple regression linear analyses were applied to evaluate the relationship between HOMA-IR as a dependent variable and plasma NE, BMI, total body fat mass, and mean BP (systolic and diastolic BP) as independent variables. Values of $P < .05$ were considered significant.

Results

Prevalence of Insulin Resistance

A total of 69 subjects were insulin resistant and 86 subjects were insulin sensitive as defined by the HOMA-IR. The

insulin-resistant group had a significantly lower frequency of the Arg16/Arg16 genotype (χ^2= 12.38, P = .002) and a higher frequency of the Gly16 allele (χ^2= 5.53, P = .019) in analysis of the β_2-adrenoceptor gene compared with results in the insulin-sensitive group (Fig. 1). Frequencies of each genotype and allele of Gln27Glu and those of Trp64Arg were similar between the insulin-sensitive and insulin-resistant groups.

Profiles of Insulin-Resistant Subjects

The insulin-resistant group had higher HOMA-IR, fasting plasma insulin, NE, total body fat mass, uric acid, total cholesterol, triglyceride, and lower HDL-cholesterol levels, whereas BMI, BP levels, and leptin levels were similar in both groups (Table 1).

Profiles of the Subjects Carrying the Gly16 Allele of the β_2-Adrenoceptor

Insulin resistant subjects had a higher frequency of the Gly16 allele of the β_2-adrenoceptor gene, suggesting the Gly16 allele is related to insulin resistance. Thus, we compared the subjects with and without the Gly16 allele of the β_2-adrenoceptor gene regardless of the status of insulin sensitivity. The HOMA-IR, fasting plasmas insulin, NE, total body fat mass, serum uric acid levels, and systolic, diastolic, and mean BP levels were higher in the subjects with the Gly16 allele (the Arg16/Gly16 + Gly16/Gly16 genotype) compared with values in subjects without the Gly16 allele (the Arg16/Arg16 genotype) of the β_2-adrenoceptor gene (Table 2). When those subjects were subdivided by insulin sensitivity, only the insulin-resistant group with higher fasting plasma insulin ($P < .05$) and NE ($P < .05$) levels were found in the group with the Gly16 allele (Fig. 2).

Multiple Regression Linear Analyses

When HOMA-IR was used as a dependent variable, plasma NE (P = .012), total body fat mass (P = .016), and systolic (P = .034) and mean BP (P = .007) levels were significant determinant variables (R^2 = 0.379, F = 19.96, $P < .001$) in multiple regression linear analysis.

Discussion

To clarify the relationship of β-adrenoceptors polymorphisms, insulin resistance, and plasma NE levels as an index of the sympathetic nervous system activity, we studied profiles of hormones and relations of polymorphisms of β-adrenoceptor genes over time in healthy individuals. We found that the insulin-resistant subjects had higher frequencies of the Gly16 allele of the β_2-adrenoceptors, and that the subjects who carried the Gly16 allele had higher levels of fasting insulin (HOMA-IR), plasma NE, and uric acid. In addition, total body fat mass and BP levels were higher in the subjects with the Gly16 allele in nonobese, nonhypertensive men. These findings suggest 1) that insulin resistance could in part be determined by the genetic variant of the β_2-adrenoceptor gene, and 2) that the β_2-adrenoceptor polymorphism accompanying higher plasma NE levels could increase insulin resistance, adiposity (obesity), and BP elevation.

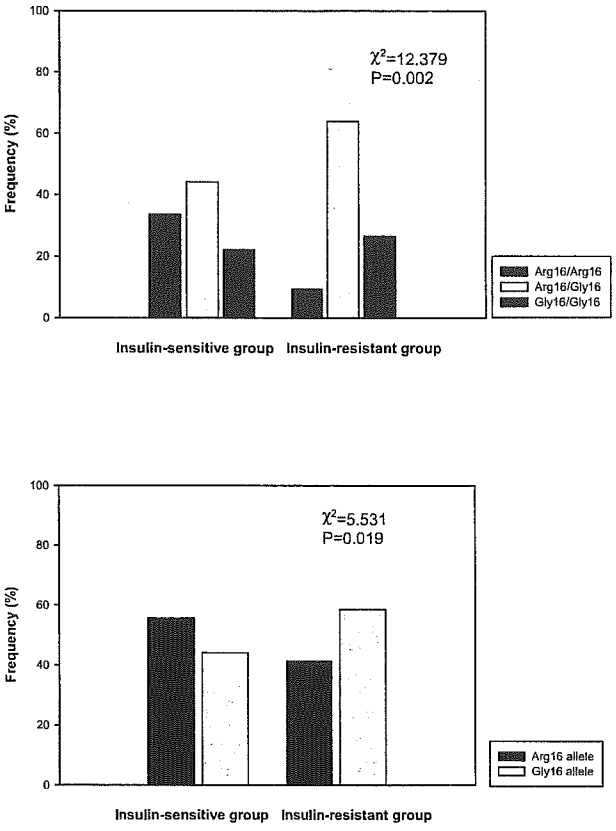

FIG. 1. Frequencies of the genotypes (**upper panel**) and the allele (**lower panel**) at Arg16Gly of the β_2-adrenoceptor gene.

In the present study, we used plasma NE levels as an index of sympathetic nerve activity. Tuck,[22] Grassi and Esler,[23] and Rahn et al[24] reported different results in sympathetic nervous system activity in hypertensive patients according to NE measurement methods, muscle sympathetic nerve activity using microneurography methods, plasma NE measurements, and regional spillover method.[25] Many investigators recommend the regional spillover method as a gold standard for sympathetic nerve activity, but these are difficult and invasive measurements. Plasma NE levels are much more practical for large populations and represent the result of several different processes such as secretion, clearance, and reuptake, especially in large population studies such as cross-sectional design studies[13,17] and in repeated measurements in longitudinal studies.[14–16]

β-Adrenoceptor Polymorphisms Versus Insulin Resistance

Significant evidence has been provided for a strong physiologic relationship between the β_2-adrenoceptor and β_3-adre-

Table 1. Comparisons of values between insulin-sensitive subjects and insulin-resistant subjects

Characteristic	Insulin-Sensitive Subjects	Insulin-Resistant Subjects
Number	86	69
Age (y)	37.0 ± 6.9	36.8 ± 7.8
Body mass index (kg/m^2)	21.6 ± 2.8	22.8 ± 2.8
Total body fat mass (kg)	14.4 ± 4.1	16.1 ± 3.9*
Waist-to-hip circumference ratio	0.90 ± 0.11	0.92 ± 0.13
Systolic blood pressure (mm Hg)	127 ± 12	129 ± 11
Diastolic blood pressure (mm Hg)	78 ± 11	79 ± 12
Mean blood pressure (mm Hg)	94 ± 10	96 ± 12
Heart rates (beats/min)	64 ± 3	65 ± 4
HOMA-IR	1.7 ± 0.9	4.2 ± 0.5†
Plasma insulin (μU/mL)	8.1 ± 2.7	17.1 ± 2.9†
Plasma norepinephrine (pmol/mL)	1.26 ± 0.29	1.74 ± 0.38*
Plasma leptin (ng/mL)	3.9 ± 2.0	4.1 ± 2.1
Blood glucose (mg/dL)	90.7 ± 5.8	93.5 ± 6.0
Total cholesterol (mg/dL)	200 ± 27	216 ± 22*
Triglyceride (mg/dL)	117 ± 35	173 ± 48‡
HDL-cholesterol (mg/dL)	58 ± 13	50 ± 12*
Uric acid (mg/dL)	5.3 ± 1.4	5.8 ± 1.2*

HOMA-IR = homeostasis model of insulin resistance.
* $P < .05$, † $P < .001$, ‡ $P < .01$ versus values in the insulin-sensitive subjects.

noceptor as seen in obesity,[6–9,11,26,27] hypertension,[6,10] and insulin resistance.[11,12] Among β_2- and β_3-adrenoceptor polymorphisms, amino acid substitutions, Arg16Gly and Gln27Glu of the β_2-adrenoceptor polymorphism, and Trp64Arg of the β_3-adrenoceptor polymorphism are also considered functionally important in understanding the genetic relationship among obesity, hypertension, and insulin resistance.

Gratze et al[28] found that young, normotensive, white male subjects homozygous for the Gly16 allele of the β_2-adrenoceptor gene had higher BP and lower peripheral vasodilation in response to the infusion of the β-blocker salbutamol. The β_2-adreoceptor is also expressed in pancreatic β-cells to modulate insulin secretion. Irakashi et al[29] suggested that the Arg16Gly variant of the β_2-adrenoceptor gene has an influence on insulin secretion. In the present study, the subjects with the Gly16 allele of the β_2-adreoceptor gene had higher plasma insulin and NE levels, suggesting that the Gly16 allele of the β_2-adrenoceptor gene is closely linked to insulin-resistant status

Table 2. Comparisons of values between subjects with and without Gly16 allele of the β_2- adrenoceptor gene

Characteristic	Subjects Without Gly16 Allele Arg16/Arg16	Subjects With Gly16 Allele (Arg16/Gly16 + Gly16/Gly16)
Number	45	110
Age (y)	36.2 ± 6.9	37.2 ± 6.5
Body mass index (kg/m^2)	21.6 ± 2.1	22.4 ± 2.5
Total body fat mass (kg)	14.6 ± 3.7	15.5 ± 3.9*
Waist to hip circumference ratio	0.90 ± 0.11	0.91 ± 0.13
Systolic blood pressure (mm Hg)	124 ± 12	129 ± 14*
Diastolic blood pressure (mm Hg)	76 ± 11	80 ± 12*
Mean blood pressure (mm Hg)	92 ± 12	96 ± 8*
Heart rates (beats/min)	63 ± 4	65 ± 3
HOMA-IR	2.5 ± 0.7	3.0 ± 0.5*
Plasma insulin (μU/mL)	10.2 ± 3.7	12.9 ± 2.2*
Plasma norepinephrine (pmol/mL)	1.28 ± 0.29	1.57 ± 0.38*
Plasma leptin (ng/mL)	3.8 ± 2.0	4.1 ± 2.1
Blood glucose (mg/dL)	91.0 ± 5.8	92.3 ± 6.0
Total cholesterol (mg/dL)	201 ± 30	210 ± 27
Triglyceride (mg/dL)	127 ± 43	148 ± 50
HDL-cholesterol (mg/dL)	57 ± 12	53 ± 13
Uric acid (mg/dL)	5.0 ± 1.4	5.7 ± 1.2*

Abbreviation as in Table 1.
* $P < .05$ versus values in the insulin-sensitive subjects.

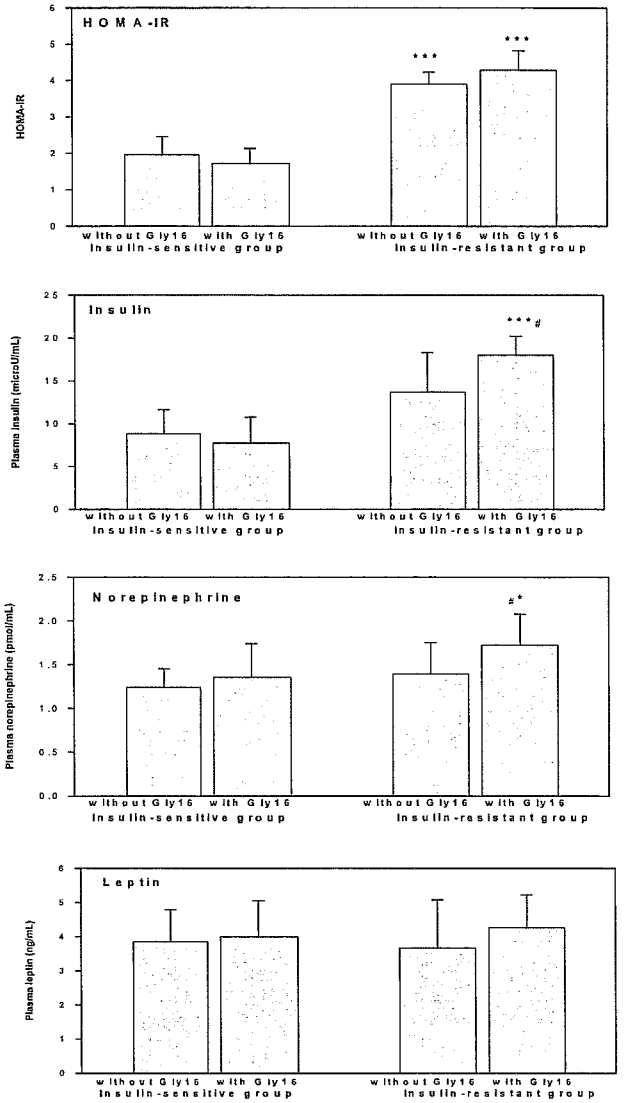

FIG. 2. The homeostasis model assessment of insulin resistance (HOMA-IR) (**top panel**), fasting plasma insulin levels (**second panel from top panel**), supine plasma norepinephrine levels (**second panel from bottom panel**), and plasma leptin levels (**bottom panel**) in the insulin-sensitive group and the insulin-resistant group according to the Gly16 allele of the β_2-adrenoceptor gene. $^*P < .05$, $^{***}P < .001$ versus values in the insulin-sensitive subjects. $^{\#}P < .05$ versus values in the subjects without Gly16 allele (carrying Arg16/Arg16 genotype) of the β_2-adrenoceptor gene.

associated with heightened sympathetic nerve activity shown as higher plasma NE levels and BP elevation. Thus, the Gly16 allele could lead to heightened sympathetic nerve activity, insulin resistance, and higher BP and adiposity and could predict these developments in nonobese, nonhypertensive individuals.

The Glu27/Glu27 genotype of the β_2-adrenoceptor gene has a well established association with obesity.[7] Subjects with Glu27 homozygotes have excess body fat and increased fat cell size compared with Gln homozygotes in a white population and also have abdominal obesity and insulin resistance.[26] We did not observe the association of the polymorphism at Gln27Glu of the β_2-adrenoceptor gene with insulin resistance, perhaps because of the very low frequency of the Glu27 allele. In a healthy Japanese population, distribution of the Glu27 allele of the β_2-adrenoceptor gene is different from that in individuals of non-Asian white ethnicity, as previously reported,[30] and the frequency of the Glu27 allele of the β_2-adrenoceptor gene is much lower.

Insulin Resistance Versus Sympathetic Overactivity

The group with the Gly16 allele of the β_2-adrenoceptor gene had a higher total body fat mass and BP levels, and our results in multiple regression analyses showed close relationships between HOMA-IR, plasma NE, total body fat mass, and mean BP. These findings demonstrate that the Gly16 allele that accompanies insulin resistance and heightened sympathetic nerve activity is associated with relatively greater adiposity and BP elevation. In addition, we have previously shown that insulin resistance is strongly related to heightened sympathetic nerve activity, BP elevation, and increased adiposity.[13–15,19] The present study was examined in a cross-sectional design. Hence, we could not discern the relations between genotype, BP elevation, and weight gain; however, we have reported in longitudinal studies that higher levels of plasma NE as a phenotype marker of sympathetic nerve activity predicts subsequent BP elevation and weight gain.[14–16] Taken together, our findings suggest the proposal that the adrenergic receptor defects lead to sympathetic nervous system overactivity that might play a role in the development of insulin resistance, hypertension, and obesity. In conclusion, a polymorphism at Arg16Gly of the β_2-adrenoceptor gene could be linked to insulin resistance and sympathetic nerve overactivity, as in this population of nonobese, nonhypertensive Japanese men.

References

1. Tuck ML: Glucose, insulin, and insulin resistance as biomedical predictors of hypertension. Am J Hypertens 1991;4:638S–641S.
2. Sowers JR: Obesity as a cardiovascular risk factor. Am J Med 2003;115(Suppl 8A):37S–41S.
3. Corry DB, Tuck ML: Glucose and insulin metabolism in hypertension. Am J Nephrol 1996;16:223–236.
4. Cui J, Hopper JL, Harrap SB: Genes and family environment explain correlations between blood pressure and body mass index. Hypertension 2002;40:7–12.
5. Dluhy RG: Screening for genetic causes of hypertension. Curr Hypertens Rep 2002;4:439–444.
6. Pereira A, Floriano MS, Mota GF, Cunha RS, Herkenhoff FL, Mill JG, Krieger JE: β_2 Adrenoceptor functional gene variants, obesity, and blood pressure level interactions in the general population. Hypertension 2003;42:685–692.
7. Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lannfelt L, Arner P: Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. J Clin Invest 1997;100:3005–3012.

8. Clement K, Vaisse C, Manning BS, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P, Strosberg AD: Genetic variation in the β_3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 1995; 333:352–354.
9. Yoshida T, Sakane N, Umekawa T, Sakai M, Takahashi T, Kondo M: Mutation of β_3-adrenergic receptor gene and responses to treatment of obesity. Lancet 1995;346:1433–1434.
10. Strazzullo P, Iacone R, Siani A, Cappuccio FP, Russo O, Barba G, Barbato A, D'Elia L, Trevisan M, Farinaro E: Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure interaction with age. Cross-sectional and longitudinal findings in the Olivetti Prospective Heart Study. J Hypertens 2001;19:399–406.
11. Kadowaki H, Yasuda K, Iwamoto S, Otabe S, Shimokawa K, Silver K, Walston J, Yoshinaga H, Kosaka K, Yamada N, Saito Y, Hagura R, Akanuma Y, Shuldiner A, Yazaki Y, Kadowaki T: A mutation in the β_3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 1995;215:555–560.
12. Walston J, Silver K, Bogardus C, Knowler WC, Celi FS, Austin S, Manning B, Strosberg AD, Stern MP, Raben N, Sorkin JD, Roth J, Shuldiner AR: Time of onset of non-insulin-dependent diabetes mellitus and genetic variation in the β_3-adrenergic-receptor gene. N Engl J Med 1995;333:343–347.
13. Masuo K, Mikami H, Ogihara T, Tuck ML: Prevalence of hyperinsulinemia in young, nonobese, Japanese men. J Hypertens 1997; 15:157–165.
14. Masuo K, Mikami H, Ogihara T, Tuck ML: Sympathetic nerve hyperactivity precedes hyperinsulinemia and blood pressure elevation in a young, nonobese Japanese population. Am J Hypertens 1997;10:77–83.
15. Masuo K, Mikami H, Ogihara T, Tuck ML: Weight gain-induced blood pressure elevation. Hypertension 2000;35:1135–1140.
16. Masuo K, Kawaguchi H, Mikami H, Ogihara T, Tuck ML: Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. Hypertension 2003; 42:474–480.
17. Masuo K, Mikami H, Ogihara T, Tuck ML: Familial hypertension, insulin, sympathetic activity, and blood pressure elevation. Hypertension 1998;32:96–100.
18. Masuo K, Mikami H, Ogihara T, Tuck ML: Familial obesity, sympathetic activation and blood pressure level. Blood Press 2001; 10:199–204.
19. Masuo K, Mikami H, Ogihara T, Tuck ML: Weight reduction and pharmacological treatment in obesity hypertensives. Am J Hypertens 2001;14:530–538.
20. Kanauchi M, Yamano S, Kanauchi K, Saito Y: Homeostasis model assessment of insulin resistance, quantitive insulin sensitivity check index, and oral glucose insulin sensitivity index in nonobese, nondiabetic subjects with high-normal blood pressure. J Clin Endocrinol Metab 2003;88:3444–3446.
21. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, Pesich R, Hebert J, Chen YD, Dzau VJ, Curb D, Olshen R, Risch N, Cox DR, Botstein D: High-throughput genotyping with single nucleotide polymorphisms. Genome Res 2001;11:1262–1268.
22. Tuck ML: The sympathetic nervous system in essential hypertension. Am Heart J 1988;112:877–886.
23. Grassi G, Esler M: How to assess sympathetic activity in humans. J Hypertens 1999;17:719–734.
24. Rahn KH, Barenbrock M, Hausberg M: The sympathetic nervous system in the pathogenesis of hypertension. J Hypertens 1999; 17(Suppl 3):S11–S14.
25. Kopin IJ, Rundqvist B, Friberg P, Lenders J, Goldstein DS, Eisenhofer G: Different relationships of spillover to release of norepinephrine in human heart, kidney, and forearm. Am J Physiol 1998; 27:R165–R173.
26. Hellstrom L, Large V, Reynisdottir S, Wahrenberg H, Arner P: The different effects of Gln27Glu β_2-adrenoceptor gene polymorphism on obesity in males and in females. J Intern Med 1999;245:253–259.
27. Clement K, Manning BS, Basdevant A, Strosberg AD, Guy-Grand B, Froguel P: Gender effect of the Trp64Arg mutation in the β_3 adrenergic receptor gene on weight gain in morbid obesity. Diabetes Metab 1997;23:424–427.
28. Gratze G, Fortin J, Labugger R, Binder A, Kotanko P, Timmermann B, Luft FC, Hoehe MR, Skarbal F: β_2-adrenergic receptor variants affect resting blood pressure and agonist-induced vasodilation in young adult Caucasians. Hypertension 1999;33:1425–1430.
29. Ikarashi T, Hanyu O, Maruyama S, Souda S, Kobayashi C, Abe E, Ukisu J, Naganuma K, Suzuki A, Toya M, Kaneko S, Suzuki K, Nakagawa O, Aizawa Y: Genotype Gly/Gly of the Arg16Gly polymorphism of the beta2-adrenergic receptor is associated with elevated fasting serum insulin concentrations, but not with acute insulin response to glucose in type 2 diabetic patients. Diabetes Res Clin Pract 2004;63:11–18.
30. Kawamura T, Egusa G, Fujikawa R, Okubo M: Gln27Glu variant of the beta2-adrenergic receptor gene is not associated with obesity and diabetes in Japanese-Americans. Metabolism 2001;50:443–446.

AJH 2005; 18:1508–1516

Rebound Weight Gain as Associated With High Plasma Norepinephrine Levels That Are Mediated Through Polymorphisms in the β2-Adrenoceptor

Kazuko Masuo, Tomohiro Katsuya, Hideki Kawaguchi, Yuxiao Fu, Hiromi Rakugi, Toshio Ogihara, and Michael L. Tuck

Background: A successful weight loss program is essential treatment for obesity-related diseases, but it is well known that the majority of individuals do not succeed in weight loss maintenance. The present study evaluates hormonal mechanisms and the relationship of β2-adrenoceptor polymorphisms involved in individuals who regain weight after initially successful weight loss.

Methods: Overweight Japanese men (n = 154) were enrolled in a 24-month weight loss program. Body mass index (BMI), total body fat mass, plasma norepinephrine (NE) and leptin levels, and β2-adrenoceptor polymorphisms (Arg16Gly, Gln27Glu) were measured every 6 months for the 24-month period. Maintenance of weight loss was defined as significant weight loss (≥10% reduction) from entry weight at 6 months and maintenance of the weight loss for an additional 18 months. Rebound weight gain was defined as significant weight loss at 6 months but subsequent regain of body weight during the next 18 months.

Results: The results showed that 37 subjects maintained weight loss during 24 months, whereas 36 subjects had rebound weight gain. The BMI at entry and calorie intake and physical activity at each period were similar between the two groups. Subjects who maintained weight loss had at entry a significantly lower fat mass and plasma NE levels compared to those with rebound weight gain. Body fat mass, NE, and leptin levels at entry predicted the degree of change in body weight during the 24-month study period. Subjects with rebound weight gain had a significantly higher frequency of the Gly16 allele for the β2-adrenoceptor polymorphism compared to subjects who had a 24-month maintenance of weight loss. Subjects carrying the Gly16 allele also had significantly higher plasma NE, leptin, and body fat mass levels and a greater waist-to-hip ratio both at entry and throughout the study.

Conclusions: A high initial degree of body fat mass and high plasma NE levels as determined by the Gly16 allele for the β2-adrenoceptor polymorphisms predict those individuals who will have rebound weight gain after their initial successful weight loss. Am J Hypertens 2005;18:1508–1516 © 2005 American Journal of Hypertension, Ltd.

Key Words: Rebound weight gain, weight loss resistance, sympathetic nerve activity, leptin, obesity, β2-adrenoceptor polymorphisms.

Weight loss and maintenance of weight loss are the most effective nonpharmacologic treatments for correction of cardiovascular and metabolic risk factors in obese patients.[1–7] However, few obese people succeed in sustained weight loss, and long-term results of weight loss programs are disappointing with a substantial proportion of people regaining most of the weight initially lost.

See related article on page 1517

There is strong evidence suggesting that human obesity has both genetic and environmental determinants.[8,9] Investigations have reported associations of polymorphisms of the β2- and β3-adrenoceptors in obesity,[10–15] and regulation of thermogenesis is mainly attributed to β2- and β3-adrenergic receptor activity. Increased energy expenditure and increased resting metabolic rate are predictive

Received April 5, 2005. First decision May 4, 2005. Accepted May 12, 2005.

From the Human Neurotransmitter Laboratory, Baker Heart Research Institute (KM), Melbourne, Victoria, Australia; Department of Geriatric Medicine, Osaka University Graduate School of Medicine (KM, TK, HK, YF, HR, TO), Suita City, Osaka, Japan; and Endocrinology and Metabolism Division, Sepulveda VA Medical Center and the David Geffen, UCLA School of Medicine (MLT), Los Angeles, California.

Address correspondence and reprint requests to Dr. Kazuko Masuo, Baker Heart Research Institute, PO Box 6492 St Kilda Road Central, Melbourne, Victoria 8008, Australia; e-mail: kmasuo@baker.edu.au

0895-7061/05/$30.00
doi:10.1016/j.amjhyper.2005.05.006

of weight loss, and the sympathetic nervous system plays a key role in regulating energy balance through stimulation of thermogenesis. Effects on rates of thermogenesis are also influenced by genetic factors. Few studies have taken into account success in maintenance of weight loss, resistance to weight loss, and rebound weight gain as part of hormonal changes associated with changes in body weight or the polymorphisms of the β-adrenoceptor genes that occur with weight change. We examined weight loss in relation to changes in body fat mass, plasma norepinephrine (NE), leptin, and insulin. In addition, we compared polymorphisms of β2- and β3-adrenoceptor genes in subjects who maintained weight loss during 24 months to those who regain body weight (rebound weight) in a protocol of a defined, constant dietary intake and exercise program.

Methods

Subjects

The weight loss program enrolled 154 overweight (25 kg/m^2 ≤body mass index [BMI] <30 kg/m^2) men, consisting of 89 overweight normotensive men (blood pressure [BP] <140/90 mm Hg) and 65 overweight, untreated mildly hypertensive men (140/90 mm Hg ≤BP <160/95 mm Hg). None of the subjects had diabetes (fasting blood glucose level >100 mg/dL) or other illness including psychological or emotional problems.[16] No subject was taking antihypertensive agents or other medications. Furthermore, no subject had any symptoms of obstructive sleep apnea (ie, breathing pauses every night or almost every night) or extremely loud habitual snoring or sleepiness during the daytime.[17,18] Only subjects whose body weight had not changed for at least the past 2 years (weight change <5%) provided in their biannual medical evaluation records were enrolled in the present study.[4,19] The subjects enrolled in this weight loss program were emotionally stable,[16] and had a similar socioeconomic status. The protocol was approved by the Ethics Committee of Osaka University Graduate School of Medicine, Japan, and written informed consent was obtained from all the subjects.

Study Design

The weight loss program consisted of a low caloric diet (1600 kcal/d, 55% of calories from carbohydrate, 30% from protein, and 15% from fat) and a low sodium diet (7g NaCl per day) and aerobic exercise of more than 1 h daily (eg, walking, jogging, or gym exercise). The subjects attended a 1-h private teaching and counseling session each week for 4 weeks, followed by biweekly 1-h sessions for 23 additional months. All sessions were led by experts in nutrition and exercise counseling. Calorie intake was calculated based on the subjects meal diary, which was assisted by nutritionists. The physical activity was quantified and recorded by the use of step-counters used on a daily basis. Diet and exercise compliance were monitored according to the subjects' own records every 2 weeks and were recorded at private counseling sessions. Compliance to diet and exercise was considered excellent and consistent based on those records.

Methods

After an overnight fast of 12 h and 30 min rest in the supine position, height, body weight, BMI, percentage total body fat mass, BP, heart rate, and venous blood sampling for measurements of blood glucose, plasma NE, leptin, insulin, and the extraction of genomic DNA from leukocytes were obtained. Samples were taken at entry and at 6, 12, and 24 months during the study. The BP and heart rate were measured more than three times in the supine position by an automated sphygmomanometer (TM-2713, A&D, Tokyo, Japan) using an adjusted cuff size based on arm circumference. Recorded BP levels and heart rates were averaged. The percentage body fat mass was determined by impedance measurements (BF-102, Tanita, Tokyo, Japan). Total body fat mass (in kilograms) was calculated according to the following formula: [percentage body fat mass (%)/100] × body weight (kg). Plasma NE was measured after separation by high-performance liquid chromatography using the fluorometric method as previously described in detail,[19] and plasma immunoreactive insulin was measured by a standard radioimmunoassay method as described in detail (insulin RIABEAD II, Dinabott, Tokyo, Japan).[19] Plasma leptin was measured by radioimmunoassay[19] (human leptin RIA kit, Linco, St. Charles, MO, USA). The homeostasis model assessment of insulin resistance (HOMA-IR) was defined as the product of fasting plasma insulin (in microunits per milliliter) and glucose (in milligrams per deciliter) divided by 405.[20]

Genotyping

Genotyping was performed by the TaqMan assay, as previously detailed (Applied Biosystems, Foster City, CA, USA).[21] Two polymorphisms (Arg16Gly, Gln27Glu) of the β2-adrenoceptors[13,14] and one polymorphism (Trp64Arg) of the β3-adrenoceptor[22] were evaluated. For single-nucleotide polymorphisms (SNPs) in the β2-adrenoceptor genes, the probes and primers were as follows: for Arg16Gly, the probes were CGCATGGCTTCCATTGGGTGC and CGCATGGCTTCTATTGGGTGC, and the primers were GGAACGGCAGCGCCTTCT and CAGGACGATGAGAGACATGACGAT; and for Gln27Glu, the probes were CTCGTCCCTTTCCTGCGTGACGT and CTCGTCCCTTTGCTGCGTGACGT (the primers used in this assay were the same as those used for Arg16Gly). For the Trp64Arg SNP in the β3- adrenoceptor, the probes were TCTCGGAGTCCAGGCGATGGCCA and CTCGGAGTCCGGGCGATGGCC, and the primers were GGAGGCAACCTGCTGGTCAT and CACGAACACGTTGGTCATGGT.

Table 1. Characteristics in the four study groups according to responses to weight loss

	Weight Loss Maintenance	Rebound	Slow Weight Loss	Weight Loss Resistance
Subjects (*n*)	37	36	60	21
Age at entry (yr)	35 ± 6	37 ± 6	37 ± 6	37 ± 8
Height (m)	1.73 ± 0.05	1.74 ± 0.04	1.74 ± 0.05	1.72 ± 0.05
BMI (kg/m^2)				
Entry	27.1 ± 1.9	27.7 ± 1.6	27.3 ± 1.7	27.4 ± 2.0
6 months	22.8 ± 1.4†**	22.8 ± 0.8†‖**	25.4 ± 2.0‡	26.6 ± 2.2§
12 months	22.6 ± 2.3†**	23.4 ± 2.2*#	24.9 ± 1.9	26.1 ± 2.9‡
18 months	22.4 ± 2.0†‡**	25.1 ± 2.2#	24.1 ± 2.0*#	26.0 ± 2.3‖#
24 months	22.1 ± 1.5†§**	26.4 ± 2.1‖	23.8 ± 1.9*‡**	26.1 ± 2.2‖#
Total body fat mass (kg)				
Entry	21.5 ± 5.4†‡‖	25.6 ± 4.9*	25.9 ± 6.1	27.8 ± 4.2‡
6 months	17.8 ± 4.7†‡‖#	21.0 ± 3.8*#	22.3 ± 5.2	24.3 ± 4.1‡
12 months	16.5 ± 4.1†‡‖#	21.5 ± 4.1#	20.7 ± 4.9#	22.8 ± 3.8#
18 months	15.6 ± 3.8†§**	22.2 ± 4.7‖#	18.7 ± 5.1*‡**	21.9 ± 4.1‖**
24 months	15.2 ± 4.3†§**	22.5 ± 5.0‖#	17.8 ± 4.9*‡**	21.2 ± 4.7‖**
Waist-to-hip ratio				
Entry	1.16 ± 0.10*‖	1.20 ± 0.11	1.23 ± 0.15	1.23 ± 0.15
6 months	0.98 ± 0.09*‖#	1.03 ± 0.10*‖#	1.15 ± 0.14*‡	1.22 ± 0.09‡‖
12 months	0.95 ± 0.10*‡#	1.05 ± 0.09*#	1.05 ± 0.11*#	1.17 ± 0.13‡‖
18 months	0.92 ± 0.09†§**	1.15 ± 0.20‖	1.03 ± 0.13*‡#	1.12 ± 0.11‖#
24 months	0.90 ± 0.12†§**	1.12 ± 0.14‖	0.98 ± 0.12*‡**	1.16 ± 0.13‖#
Systolic BP (mm Hg)				
Entry	136 ± 12	134 ± 10	137 ± 10	133 ± 12
6 months	135 ± 10	134 ± 9	134 ± 9	134 ± 8
12 months	133 ± 9	136 ± 7	134 ± 8	135 ± 8
18 months	128 ± 10*‡	135 ± 8	132 ± 9	134 ± 6
24 months	128 ± 9#	133 ± 8	130 ± 10#	130 ± 11
Diastolic BP (mm Hg)				
Entry	78 ± 10	77 ± 9	79 ± 9	74 ± 11
6 months	77 ± 9	76 ± 7	76 ± 8	73 ± 7
12 months	76 ± 8	77 ± 6	75 ± 8	73 ± 8
18 months	74 ± 9	77 ± 7	74 ± 9	76 ± 7
24 months	73 ± 8#	76 ± 7	72 ± 10#	72 ± 11
Mean BP (mm Hg)				
Entry	98 ± 12	96 ± 10	98 ± 9	94 ± 14
6 months	97 ± 10	96 ± 7	96 ± 9	94 ± 7
12 months	95 ± 9	97 ± 6	95 ± 8	94 ± 8
18 months	92 ± 10	96 ± 8	94 ± 9	95 ± 6
24 months	92 ± 9#	95 ± 8	91 ± 11#	93 ± 13
Heart rate (beats/min)				
Entry	68 ± 7	70 ± 8	68 ± 8	71 ± 7
6 months	66 ± 6	68 ± 7	67 ± 7	71 ± 6
12 months	65 ± 7	69 ± 7	66 ± 8	69 ± 6
18 months	64 ± 5‡	70 ± 8	66 ± 7	68 ± 7
24 months	62 ± 6‡#	68 ± 7	65 ± 7	67 ± 5
Plasma norepinephrine (pmol/mL)				
Entry	1.53 ± 0.32†‡‖	1.87 ± 0.37*‖	2.25 ± 0.31*‡	2.59 ± 0.53‡‖
6 months	1.31 ± 0.30†¶	1.49 ± 0.41†#	2.01 ± 0.33‡	2.22 ± 0.51§
12 months	1.17 ± 0.31†‡¶#	1.63 ± 0.44*	1.78 ± 0.35#	2.07 ± 0.47‡
18 months	1.11 ± 0.32†‖#	1.40 ± 0.51*#	1.54 ± 0.31**	2.02 ± 0.46‡‖#
24 months	1.09 ± 0.28†‡‖**	1.32 ± 0.37*#	1.41 ± 0.37**	1.87 ± 0.48‡‖**
Plasma leptin (ng/mL)				
Entry	7.9 ± 2.8*‖	8.9 ± 2.9*‖	10.1 ± 3.0‡	11.7 ± 2.7‡
6 months	5.8 ± 2.5†#	6.2 ± 2.4†#	7.8 ± 2.9*	10.2 ± 3.0§‖
12 months	4.5 ± 1.9†‖#	5.8 ± 2.3*#	6.8 ± 2.7*#	8.5 ± 2.4‡‖
18 months	4.0 ± 1.7†‡**	6.1 ± 2.6*#	6.0 ± 2.8*#	8.2 ± 3.0‡‖#
24 months	3.7 ± 1.8†‡**	6.7 ± 2.5‖#	5.1 ± 1.7*‡**	7.1 ± 2.9‖#
HOMA-IR				
Entry	2.3 ± 0.5	2.5 ± 0.5	2.5 ± 0.6	2.7 ± 0.6
6 months	2.0 ± 0.3*	2.0 ± 0.4*#	2.3 ± 0.5	2.5 ± 0.5‡
12 months	1.7 ± 0.5‖	2.0 ± 0.5	2.1 ± 0.4	2.2 ± 0.6
18 months	1.7 ± 0.5*#	2.1 ± 0.6	2.0 ± 0.5#	2.2 ± 0.5
24 months	1.4 ± 0.6*‡‖**	2.1 ± 0.5	1.8 ± 0.5**	2.2 ± 0.4#

Table 1. *Continued*

	Weight Loss Maintenance	Rebound	Slow Weight Loss	Weight Loss Resistance
Caloric intake (×1,000kcal/d)				
Entry	2.3 ± 0.6	2.4 ± 0.5	2.4 ± 0.5	2.3 ± 0.4
6 months	1.5 ± 0.4**	1.5 ± 0.3**	1.6 ± 0.3**	1.6 ± 0.2**
12 months	1.4 ± 0.5**	1.5 ± 0.4**	1.5 ± 0.3**	1.5 ± 0.3**
18 months	1.5 ± 0.4**	1.5 ± 0.5**	1.6 ± 0.5**	1.6 ± 0.2**
24 months	1.5 ± 0.3**	1.5 ± 0.4**	1.5 ± 0.4**	1.5 ± 0.2**
Physical activity (×1,000steps/d)				
Entry	9.7 ± 4.3	10.1 ± 4.8	9.6 ± 3.7	9.8 ± 2.9
6 months	21.5 ± 3.8**	22.3 ± 4.1**	20.9 ± 2.9**	20.5 ± 2.1**
12 months	22.5 ± 2.9**	19.5 ± 4.5#	20.4 ± 3.9**	20.1 ± 2.5**
18 months	21.7 ± 2.3**	20.1 ± 3.2**	19.8 ± 3.4**	20.9 ± 2.5**
24 months	20.7 ± 3.7**	19.7 ± 4.1**	22.3 ± 4.5**	20.7 ± 3.0**

Weight loss resistance indicates the subjects who fail to lose weight significantly during 24 months.
BMI = body mass index; BP = blood pressure.
* $P < .05$, † $P < .01$ compared with values in weight loss resistant subjects; ‡ $P < .05$, § $P < .01$ compared with rebound subjects; ‖ $P < .05$, ¶ $P < .01$ compared with values in slow weight loss subjects; # $P < .05$, ** $P < .01$ compared with values at baseline. Data are mean ± SD. $n = 154$.

Statistical Analyses

Genotype frequencies and the Hardy-Weinberg equilibrium were estimated with χ^2 test. Values are shown as mean ± SD. All data analyses were performed with SPSS 8.0 for Windows program (Chicago, IL, USA). Changes in measured parameters within each group and differences among groups were examined by two-way analysis of variance. When these differences were significant, the Dunnett test was used to determine whether the differences of the mean measured variables at 6, 12, and 24 months were significant within the groups and among the groups compared from baseline. Multiple linear regression analyses were used to examine relations among variables using changes in body weight or in mean BP versus changes in hormonal measurements during weight and BP changes.

Results

Prevalence of Weight Loss Maintenance, Rebound Weight Gain, and Weight Loss Resistance

When significant weight loss was defined as a 10% or more reduction in BMI from baseline, 73 subjects succeeded in achieving weight loss at 6 months. Maintenance of weight loss was noted in 37 subjects and rebound weight gain was found in 36 subjects. Sixty other subjects, who did not have a significant weight loss at 6 months, actually succeeded in significant weight loss at 24 months (slow weight loss group). Thus, a total of 97 subjects succeeded in significant weight loss at 24 months. Fifty-seven subjects failed to have significant weight loss at 24 months, 36 subjects had rebound weight gain, and 21 subjects failed to lose weight during the entire 24-month period. Thus, there were four study groups: subjects who failed to lose weight during 24 months represented the weight loss resistant group ($n = 21$); subjects with maintenance of weight loss represented the weight loss maintenance group ($n = 37$); subjects with weight regain represented the weight rebound group ($n = 36$); and those who failed to lose weight at 6 month but succeeded to lose weight at 24 months represented the slow weight loss group ($n = 60$).

Calorie Intake and Physical Activity

Diet compliance (calorie intake) and physical activity (steps per day) were not significantly different among the four groups (Table 1). Behavior (alcohol intake, cigarette smoking) and socioeconomic status was similar among the four study groups throughout the study. Thus, compliance was considered very good for the 24-month period.

Frequencies of β2- and β3-Adrenergic Receptor Polymorphism

Table 2 shows the frequencies of the genotypes and the alleles of β2- and β3-adrenoceptor genes in the four study groups according to the response in weight loss. The weight loss resistant group, the rebound weight gain group, and the slow weight loss group had a significantly higher frequency of the Gly16 allele of the Arg16Gly of the β2-adrenoceptor compared to the weight loss maintenance group ($\chi^2 = 5.76$, $P = .016$; $\chi^2 = 5.38$, $P = .020$; $\chi^2 = 6.11$, $P = .013$, respectively). The weight loss resistant and slow weight loss groups (both groups failed to lose weight at 6 months) had a higher frequencies of the Glu27 allele of the Gln27Glu of the β2-adrenoceptor compared to a combined group with weight loss maintenance and rebound weight gain group (both groups succeeded in significant weight loss at 6 months) ($\chi^2 = 6.16$, $P = .013$; $\chi^2 = 6.22$, $P = .013$, respectively) (Table 2). The frequency distribution of the Glu27 allele of Gln27Glu was 9.4% and that of the Arg64 allele of Trp64Arg

Table 2. Frequencies of the genotype and the allele of the β2- and β3-adrenoceptor polymorphisms in the four study groups according to the response in weight loss

Groups	Genotype (%)			χ^2 Test Among Four Genotype	Allele (%)		χ^2 Test Among Two Alleles
Arg16Gly of β2-adrenoceptor	Arg16/Arg16	Arg16/Gly16	Gly16/Gly16		Arg16	Gly16	
Weight loss maintenance group	18 (48.6%)	14 (37.8%)	5 (13.5%)		50 (67.6%)	24 (32.4%)	
Rebound weight gain group	8 (22.2%)	18 (50.0%)	10 (27.8%)	$\chi^2 = 13.88$,	34 (47.2%)	38 (52.8%)	$\chi^2 = 11.36$,
Slow weight loss group	13 (21.7%)	32 (53.3%)	15 (25.0%)	$P = .031$	58 (48.3%)	62 (51.7%)	$P = .010$
Weight loss resistant group	3 (14.3%)	12 (57.1%)	6 (28.6%)		18 (42.9%)	24 (57.1%)	
Gln27Glu of β2-adrenoceptor	Gln27/Gln27	Gln27/Glu27	Glu27/Glu27		Gln27	Glu27	
Weight loss maintenance group	33 (91.7%)	4 (10.8%)	0 (0.0%)		70 (94.6%)	4 (5.4%)	
Rebound weight gain group	34 (94.4%)	2 (5.6%)	0 (0.0%)	$\chi^2 = ---$,	70 (97.2%)	2 (2.8%)	$\chi^2 = 9.86$,
Slow weight loss group	44 (73.3%)	16 (26.7%)	0 (0.0%)	$P = ---$	104 (86.7%)	16 (13.3%)	$P = .020$
Weight loss resistant group	14 (66.7%)	7 (33.3%)	0 (0.0%)		35 (83.3%)	7 (16.7%)	
Trp64Arg of β3-adrenoceptor	Trp64/Trp64	Trp64/Arg64	Arg64/Arg64		Trp64	Arg64	
Weight loss maintenance group	26 (70.2%)	10 (27.0%)	1 (2.7%)		62 (83.8%)	12 (16.2%)	
Rebound weight gain group	28 (77.8%)	8 (22.2%)	0 (0.0%)	$\chi^2 = 7.91$,	64 (88.9%)	8 (11.1%)	$\chi^2 = 5.43$,
Slow weight loss group	32 (53.3%)	27 (45.0%)	1 (1.7%)	$P = .245$	91 (75.8%)	29 (24.2%)	$P = .143$
Weight loss resistant group	13 (61.9%)	8 (38.1%)	0 (0.0%)		34 (81.0%)	8 (19.0%)	

The definitions for the 4 study groups according to the response in weight loss are referred to in the Results, Prevalence of weight loss maintenance, rebound weight gain, and weight loss resistance section.

was 18.5%. The frequency distributions for alleles in our subjects were similar to those in previous studies in Japanese cohorts, but lower than studies in whites.[23,24]

Physical Measurements

The mean age, BMI, BP levels, and heart rates at entry were similar among the four groups (Table 1). However, the entry measurements for total body fat mass and waist-to-hip ratio were significantly lower in the weight loss maintenance group versus the other three groups (weight loss resistant, slow weight loss, or rebound weight gain). At 6 months, the weight loss maintenance group had significantly greater weight loss, body fat loss, and a decrease in the waist-to-hip ratio compared to the weight loss resistant and slow weight loss groups. The BP reductions at 24 months were significantly greater in the weight loss maintenance and slow weight loss groups compared to the weight loss resistant group and the rebound weight gain group (Table 1). Only in the weight loss maintenance group did the heart rates decline at 24 months.

The subjects carrying the Gly16 allele had greater total body fat mass and waist-to-hip ratios at entry and throughout the study (Table 3), and the subjects carrying the Glu27 allele had greater total body fat mass (Table 4).

In all subjects, weight loss and mean BP reduction during 24 months were 8.9 ± 4.4 kg (10.8% ± 5.3%) and 4.5 ± 3.1 mm Hg (4.7% ± 3.2%). Mean BP reductions per amount of weight lost were similar among the four study groups (0.4 ± 0.2 mm Hg/kg in the weight loss maintenance group; 0.3 ± 0.1 mm Hg/kg in the rebound weight gain group; 0.5 ± 0.2 mm Hg/kg in the weight loss resistant group; and 0.5 ± 0.3 mm Hg/kg in the slow weight loss group).

Hormone Levels

Plasma NE and leptin levels, and HOMA-IR decreased with weight loss in the four study groups (Table 1). The most significant finding was that plasma NE and leptin levels were substantially greater in the weight loss resistant group compared to the weight loss maintenance group at entry and throughout the study. In the rebound weight gain group, plasma NE level was significantly greater than in the weight loss maintenance group. The slow weight loss group also had higher plasma NE and leptin levels at entry compared to the groups who succeeded in a significant weight loss at 6 months (weight loss maintenance and rebound weight gain groups), but lower values than the weight loss resistant group (Table 1). Plasma NE and leptin levels in the subjects carrying the Gly16 and Glu27 alleles were higher at entry and throughout the study compared to those without the Gly16 or Glu27 allele. The HOMA-IR in the subjects with the Gly16 allele was higher throughout the study, as previously we reported,[25] whereas that in the subjects with the Glu27 allele was similar (Tables 3 and 4).

Table 3. Characteristics of subjects according the genotype of the Gly16 at entry and during a weight loss program

Genotype	Without Gly16 Allele (Arg16Arg)			With Gly16 Allele (Arg16Gly + Gly16Gly)		
	At Entry	At 6 Months	At 24 Months	At Entry	At 6 Months	At 24 Months
Subjects (*n*)	42	42	42	112	112	112
Age (yr)	36 ± 7	37 ± 7§	38 ± 7§	37 ± 6	37 ± 6§	39 ± 6§
BMI (kg/m^2)	27.3 ± 2.0	23.8 ± 2.1	23.6 ± 2.0†§	27.4 ± 1.8	24.5 ± 2.3§	24.6 ± 1.9§
Total body fat mass (kg)	24.0 ± 5.4*	20.2 ± 4.8*‡	17.6 ± 4.5*§	25.5 ± 4.8	21.7 ± 4.3‡	19.2 ± 5.1§
Waist-to-hip ratio	1.16 ± 0.12*	1.05 ± 0.10‡	0.96 ± 0.13*§	1.24 ± 0.10	1.09 ± 0.12‡	1.04 ± 0.14§
Systolic BP (mm Hg)	132 ± 9	130 ± 10	126 ± 9‡	133 ± 10	132 ± 10	131 9
Diastolic BP (mm Hg)	79 ± 9	79 ± 8	75 ± 6‡	79 ± 10	78 ± 9	75 7‡
Mean BP (mm Hg)	97 ± 10	96 ± 10	92 ± 7‡	97 ± 11	96 ± 9	94 ± 9
Heart rate (beats/min)	69 ± 9	67 ± 7	64 ± 7‡	69 ± 7	68 ± 7	66 ± 7
Norepinephrine (pmol/mL)	1.85 ± 0.39*	1.56 ± 0.33*‡	1.26 ± 0.37*§	2.11 ± 0.35	1.80 ± 0.36‡	1.41 ± 0.40§
Leptin (ng/mL)	8.6 ± 2.9*	6.6 ± 2.7*‡	4.9 ± 2.1*§	9.3 ± 3.0	7.3 ± 2.8‡	5.7 ± 2.9‡
HOMA-IR	2.2 ± 0.7*	2.1 ± 0.4	1.8 ± 0.6‡	2.6 ± 0.6	2.3 ± 0.5	2.0 ± 0.5‡

Data are mean ± SD.
BMI = body mass index; BP = blood pressure; HOMA-IR = the homeostasis model assessment of insulin resistance.
* $P < .05$, † $P < .01$ compared with values in subjects with the Gly16 allele; ‡ $P < .05$, § $P < .01$ compared with values at entry.

Relationship With Weight Loss and BP Reduction

Using linear regression analysis, plasma NE levels at entry and at 24 months correlated significantly with mean BP ($r = 0.54$, $P < .001$, $r = 0.42$, $P < .001$, respectively), heart rate ($r = 0.27$, $P < .05$, $r = 0.21$, P = not significant, respectively), BMI ($r = 0.28$, $P < .05$, $r = 0.25$, $P < .05$, respectively), total body fat mass ($r = 0.36$, $P < .001$, $r = 0.35$, $P < .001$, respectively), and plasma leptin level ($r = 0.42$, $P < .001$, $r = 0.37$, $P < .001$, respectively). Changes in heart rate for 24 months did not correlate with changes in plasma NE.

In multiple linear regression analysis, total body fat mass ($P = .043$), plasma NE ($P = .016$) and leptin levels ($P = .020$), but not heart rate, at entry were significant determinant factors for absolute weight changes for 24 months ($R^2 = 0.337$, F = 3.56, $P = .010$). Mean BP ($P = .050$), total body fat mass ($P = .041$), and plasma NE level ($P = .042$) at entry were significant determinant factors for absolute changes in mean BP for 24 months ($R^2 = 0.301$, F = 2.45, $P = .047$). Changes in total body fat mass ($P = .019$), waist-to-hip ratio ($P = .034$), plasma NE ($P = .033$) and leptin levels ($P = .022$) for 2 years were significant determinant factors for absolute changes in mean BP ($R^2 = 0.381$, F = 5.03, $P = .007$).

Discussion

The present study shows that the initial levels of total body fat mass, plasma NE and leptin levels, and the frequency of the Gly16 allele of the Arg16Gly of the β2-adrenoceptor polymorphism are significantly higher in people resistant to weight loss and those who have rebound weight gain compared to those with successful weight loss maintenance. Thus, measurement of these parameters might predict those subjects who will fail to lose weight in both the short and long term or who will regain weight after an initial success in weight loss as determined in a dietary and exercise weight loss program. In addition, the frequency of the Glu27 allele of the β2-adrenoceptor is higher in subjects who are weight loss resistant and in those with slow weight loss, in people who fail to lose weight in the short term (6 months), compared to those with weight loss maintenance or rebound weight gain, who lose weight in the short term. These findings indicate that sympathetic overactivity as reflected by high plasma NE levels associated with the Gly16 and Glu27 polymorphisms might be linked to mechanisms that explain weight loss resistance and rebound weight gain despite adherence to long-term diet and exercise programs.

A number of studies have demonstrated several BP-lowering mechanisms accompanying weight loss.[6,7] In our weight loss program, average percent reduction in body weight and mean BP for 24 months in all subjects were 10.8% and 4.7%, respectively. These results revealed similar values to those levels in the meta-analysis by Neter et

Table 4. Characteristics of subjects according the genotype of the Glu27 at entry and during a weight loss program

Genotype	Without Glu27 Allele (Gln27Gln)			With Glu Allele (Gln27Glu)		
	At Entry	At 6 Months	At 24 Months	At Entry	At 6 Months	At 24 Months
Subjects (*n*)	125	125	125	29	29	29
Age (yr)	36 ± 6	37 ± 6§	38 ± 6§	37 ± 7	37 ± 7§	39 ± 7§
BMI (kg/m^2)	27.4 ± 1.7	24.0 ± 2.1§	24.1 ± 2.0§	27.3 ± 1.8	24.9 ± 2.2‡	24.5 ± 2.1§
Total body fat mass (kg)	24.5 ± 5.7*	20.5 ± 4.5*§	18.0 ± 4.3*§	25.9 ± 6.0	22.4 ± 3.8‡	20.1 ± 4.7§
Waist-to-hip ratio	1.19 ± 0.13	1.09 ± 0.10	0.98 ± 0.12§	1.22 ± 0.11	1.13 ± 0.09	1.03 ± 0.13‡
Systolic BP (mm Hg)	133 ± 9	131 ± 10	125 ± 9*‡	134 ± 10	133 ± 9	131 ± 10
Diastolic BP (mm Hg)	79 ± 9	79 ± 9	75 ± 9‡	79 ± 9	78 ± 7	75 ± 9
Mean BP (mm Hg)	97 ± 10	96 ± 9	91 ± 8*‡	97 ± 11	96 ± 7	94 ± 10
Heart rate (beats/min)	69 ± 8	67 ± 7	65 ± 7	69 ± 7	68 ± 6	65 ± 7
Norepinephrine (pmol/mL)	1.94 ± 0.33*	1.67 ± 0.41*‡	1.29 ± 0.34*§	2.20 ± 0.33	1.92 ± 0.45	1.51 ± 0.42‡
Leptin (ng/mL)	7.1 ± 2.8*	6.9 ± 2.8*	5.1 ± 2.7*‡	9.5 ± 3.1	8.0 ± 3.0‡	5.8 ± 2.8‡
HOMA-IR	2.4 ± 0.4	2.1 ± 0.7	2.0 ± 0.6	2.5 ± 0.6	2.3 ± 0.6	2.0 ± 0.5

Data are mean ± SD.
Abbreviations as in Table 3.
* $P < .05$, † $P < .01$ compared with values in subjects with the Glu27 allele; ‡ $P < .05$, § $P < .01$ compared with values at entry.

al.[7] And, normalization of BP often occurs before obese subjects reach their ideal weight. Therefore, overweight and obese hypertensive patients should be encouraged to lose even a modest amount of weight as it has pronounced beneficial effects on BP levels and other risk factors.

It is established that weight loss is accompanied by reductions in sympathetic nerve activity (SNA), insulin resistance, plasma leptin levels, and BP levels.[1–3,5] However, few investigations have examined how the sympathetic nervous system, insulin resistance, and leptin level are involved in weight loss resistance and rebound weight gain.[26] More than 20 years ago, Tuck et al found significant reductions in SNA and BP during rapid weight loss and weight loss using a very low calorie diet.[2,3,5] In the present study, we note that plasma NE, leptin, and the HOMA-IR levels track with weight changes and in addition that plasma NE and leptin levels at entry are determinant factors for predicting changes in body weight during a weight loss program, thus further demonstrating that SNA (plasma NE levels) and plasma leptin levels are major control factors for changes in body weight.[5,19,27]

In the present study, we used plasma NE levels as an index of SNA. Tuck,[28] Grassi and Esler,[29] and Rahn et al[30] reviewed that there are different results in SNA values in hypertensive patients depending on the method of SNA measurement including: regional NE spillover, muscle sympathetic nerve activity (microneurography), and plasma NE measurements. Spillover methods are considered as the gold standard for SNA measurements, but in humans these are difficult and invasive measurements. Furthermore , Rumantir et al[31] reported different values for regional sympathetic nerve activity between the kidneys and heart in obesity-related hypertensive subjects. Plasma NE levels are more practical for large population studies, [5,15,19,25] but represent several different process (secretion, clearance, and reuptake of NE) making it difficult to determine whether the defect is overproduction or decreased metabolism.

Pathophysiological involvement of genetic abnormalities in the β2- and β3-adrenoceptor system in obesity are well described.[10–15,22] Among β2- and β3-adrenoceptor polymorphisms, amino acid substitutions, Arg16Gly and Gln27Glu of the β2-adrenoceptor and Trp64Arg of the β3-adrenoceptor polymorphism are considered functionally important in the control of body weight.[10–15,22] In the present study, the weight loss maintenance group have a lower frequency of the Gly16 and Glu27 alleles of the β2-adrenoceptor and lower plasma NE levels, suggesting that the Gly16 and Glu27 alleles are related to a blunted β2-adrenoceptor activity and resultant sympathetic overactivity as shown by higher plasma NE levels.[15] Furthermore, the slow weight loss and weight loss resistance groups in our study during a 24-month period have a higher frequency of the Glu27 allele and higher plasma NE levels compared to the groups who succeed in significant weight loss in the short term. We have reported that the individuals carrying the Gly16 and Glu27 alleles have greater weight gain and BP elevations.[15] Taken together,

one could propose that the characteristics of the Gly16 and Glu27 alleles of the β2-adrenoceptor polymorphisms during weight gain may stabilize body weight even with on-going caloric restriction and exercise causing resistance to weight loss.

Kaye et al[32] found a strong relationship between heart rate and the level of cardiac sympathetic nerve activity measured by the spillover method. Our results show that changes in plasma NE do not correlate with changes in heart rate, whereas heart rate correlates with plasma NE at entry. These findings indicate that the limitation that plasma NE level does not always precisely reflect the response of regional (heart) sympathetic nerve activity to weight change, but we could speculate that the subjects carrying the Gly16 or Glu27 alleles who have less reductions in heart rate might have an impaired response of cardiac sympathetic nerve activity to weight loss through the blunted β2-adrenoceptor sensitivity and resultant cardiac risk through resistance to weight loss. However, further studies are needed to evaluate the differences in the sympathetic-mediated thermogenesis in the subjects carrying the β2-adrenoceptor polymorphisms.

In conclusion, greater adiposity and sympathetic overactivity (high plasma NE levels) might predict those obese individuals who have complete resistance to lose weight during the 24-month period and those who will have rebound weight gain after a successful initial weight loss. The sympathetic overactivity in those subjects who have rebound weight gain and in those who have resistance to weight loss may be associated with the polymorphisms in the Gly16 and Glu27 alleles of the β2-adrenoceptor.

References

1. Tuck ML, Sowers J, Dornfeld L, Kledzik G, Maxwell M: The effect of weight reduction on blood pressure, plasma renin activity, and plasma aldosterone levels in obese patients. N Engl J Med 1981; 304:930–933.
2. Tuck ML, Sowers J, Dornfeld L, Whitfield L, Maxwell M: Reductions in plasma catecholamines and blood pressure during weight loss in obese subjects. Acta Endocrinol (Copenh) 1983;102:252–257.
3. Sowers JR, Whitfield LA, Catania RA, Stern N, Tuck ML, Dornfeld L, Maxwell M: Role of the sympathetic nervous system in blood pressure maintenance in obesity. J Clin Endocrinol Metab 1982;54: 1181–1186.
4. Mertens IL, Van Gaal LF: Overweight, obesity, and blood pressure: the effects of modest weight reduction. Obes Res 2000;8:270–278.
5. Masuo K, Mikami H, Ogihara T, Tuck ML: Weight reduction and pharmacologic treatment in obese hypertensives. Am J Hypertens 2001;14:530–538.
6. Hubert HB, Feinleib M, McNamara PM, Castelli WP: Obesity as an independent risk factor for cardiovascular disease: a 26-year follow-up of participants in the Framingham Heart Study. Circulation 1983;67:969–977.
7. Neter JE, Stam BE, Kok FJ, Grobbee DE, Geleijnse JM: Influence of weight reduction on blood pressure: a meta-analysis of randomized controlled trials. Hypertension 2003;42:878–884.
8. Cui J, Hopper JL, Harrap SB: Genes and family environment explain correlations between blood pressure and body mass index. Hypertension 2002;40:7–12.
9. Hainer V, Stunkard A, Kunesova M, Parizkova J, Stich V, Allison DB: A twin study of weight loss and metabolic efficiency. Int J Obes Relat Metab Disord 2001;25:533–537.
10. Clement K, Vaisse C, Manning BS, Basdevant A, Guy-Grand B, Ruiz J, Silver KD, Shuldiner AR, Froguel P, Strosberg AD: Genetic variation in the β3-adrenergic receptor and an increased capacity to gain weight in patients with morbid obesity. N Engl J Med 1995; 333:352–354.
11. Yoshida T, Sakane N, Umekawa T, Sakai M, Takahashi T, Kondo M: Mutation of β3-adrenergic-receptor gene and response to treatment of obesity. Lancet 1995;346:1433–1434.
12. Strazzullo P, Iacone R, Siani A, Cappuccio FP, Russo O, Barba G, Barbato A, D'Elia L, Trevisan M, Farinaro E: Relationship of the Trp64Arg polymorphism of the beta3-adrenoceptor gene to central adiposity and high blood pressure interaction with age. Cross-sectional and longitudinal findings in the Olivetti Prospective Heart Study. J Hypertens 2001;19:399–406.
13. Pereira AC, Floriano MS, Mota GF, Cunha RS, Herkenhoff FL, Mill JG, Krieger JE: β2 Adrenoceptor functional gene variants, obesity, and blood pressure level interactions in the general population. Hypertension 2003:42:685–692.
14. Large V, Hellstrom L, Reynisdottir S, Lonnqvist F, Eriksson P, Lannfelt L, Arner P: Human beta-2 adrenoceptor gene polymorphisms are highly frequent in obesity and associate with altered adipocyte beta-2 adrenoceptor function. J Clin Invest 1997;100:3005–3013.
15. Masuo K, Katsuya T, Fu Y, Rakugi H, Ogihara T, Tuck ML: β2- and β3-Adrenergic receptor polymorphisms are related to the onset of weight gain and blood pressure elevation over 5 years. Circulation 2005;111:3429–3434.
16. Hasler G, Pine DS, Gamma A, Milos G, Ajdacic V, Eich D, Rossler W, Angst J: The associations between psycopathology and being overweight: a 20-year prospective study. Psychol Med 2004;34:1047–1057.
17. Narkiewicz K, van de Borne PJH, Cooley RL, Dyken ME, Somers VK: Sympathetic activity in obese subjects with and without obstructive sleep apnea. Circulation 1998;98:772–776.
18. Young T, Palta M, Dempsey J, Skatrud J, Weber S, Badr S: The occurrence of sleep-disordered breathing among middle-aged adults. N Engl J Med 1993;328:1230–1235.
19. Masuo K, Mikami H, Ogihara T, Tuck ML: Weight gain-induced blood pressure elevation. Hypertension 2000;35:1135–1140.
20. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC: Homeostasis model assessment: insulin resistance and beta-cell function from plasma glucose and insulin concentrations in man. Diabetologia 1985;28:412–419.
21. Ranade K, Chang MS, Ting CT, Pei D, Hsiao CF, Olivier M, Pesich R, Hebert J, Chen YD, Dzau VJ, Curb D, Olshen R, Risch N, Cox DR, Botstein D: High-throughput genotyping with single nucleotide polymorphisms. Genome Res 2001;11:1262–1268.
22. Kadowaki H, Yasuda K, Iwamoto K, Otabe S, Shimokawa K, Silver K, Walston J, Yoshinaga H, Kosaka K, Yamada N, Saito Y, Hagura R, Akanuma Y, Shuldiner A, Yazaki Y, Kadowaki T: A mutation in the β3-adrenergic receptor gene is associated with obesity and hyperinsulinemia in Japanese subjects. Biochem Biophys Res Commun 1995;215:555–560.
23. Kato N, Sugiyama T, Morita H, Kurihara H, Sato T, Yamori Y, Yazaki Y: Association analysis of β2-adrenergic receptor polymorphisms with hypertension in Japanese. Hypertension 2001;37:286–292.
24. Oizumi T, Daimon M, Saitoh T, Kameda W, Yamaguchi H, Ohnuma H, Igarashi M, Eguchi H, Manaka H, Tominaga M, Kato T: Funagata Diabetes Study. Genotype Arg/Arg, but not Trp/Arg, of the Trp64Arg polymorphism of the β3-adrenergic receptor is associated with type 2 diabetes and obesity in a large Japanese sample. Diabetes Care 2001;24:1579–1583.
25. Masuo K, Katsuya T, Fu Y, Rakugi H, Ogihara T, Tuck ML: β2-adrenoceptor polymorphisms relate to insulin resistance and sympathetic overactivity as early markers of metabolic disease in nonobese, normotensive individuals. Am J Hypertens 2005; 18:1009–1019.

26. Itoh K, Imai K, Masuda T, Abe S, Tanaka M, Koga R, Itoh H, Nakamura M: Association between blood pressure and insulin resistance in obese females during weight loss and weight rebound phenomenon. Hypertens Res 2001;24:481–487.
27. Masuo K, Kawaguchi H, Mikami H, Ogihara T, Tuck ML: Serum uric acid and plasma norepinephrine concentrations predict subsequent weight gain and blood pressure elevation. Hypertension 2003;42:474–480.
28. Tuck ML: The sympathetic nervous system in essential hypertension. Am Heart J 1986;112:877–886.
29. Grassi G, Esler M: How to assess sympathetic activity in humans. J Hypertens 1999;17:719–734.
30. Rahn KH, Barenbrock M, Hausberg M: The sympathetic nervous system in the pathogenesis of hypertension. J Hypertens 1999; 17(Suppl 3):S11–S14.
31. Rumantir MS, Vaz M, Jennings GL, Collier G, Kaye DM, Seals DR, Wiesner GH, Brunner-La Rocca HP, Esler MD: Neural mechanisms in human obesity-related hypertension. J Hypertens 1999;17:1125–1133.
32. Kaye DM, Smirk B, Finch S, Williams C, Esler MD: Interaction between cardiac sympathetic drive and heart rate in heart failure: modulation by adrenergic receptor genotype. J Am Coll Cardol 2004;44:2008–2015.

Association of Hypoadiponectinemia With Smoking Habit in Men

Yoshio Iwashima, Tomohiro Katsuya, Kazuhiko Ishikawa, Iwao Kida, Mitsuru Ohishi, Takeshi Horio, Noriyuki Ouchi, Koji Ohashi, Shinji Kihara, Tohru Funahashi, Hiromi Rakugi, Toshio Ogihara

Abstract—Adiponectin is emerging as an important molecule in obesity, the metabolic syndrome, and cardiovascular disease. On the other hand, smoking habit is well known to be related to cardiovascular disease and hypertension. To examine the association between adiponectin concentration and smoking habit, we performed an epidemiological survey and an acute exposure test in humans and an experiment in adipocytes to elucidate the mechanism underlying the association between adiponectin and smoking. In the epidemiological study, we enrolled a total of 331 male subjects to examine chronic smoking exposure. Plasma adiponectin was significantly lower ($P=0.01$) in current smokers (5.3 ± 0.3 μg/mL) than in never-smokers (6.5 ± 0.4 μg/mL). A significant association between smoking and low adiponectin level was also confirmed in multiple regression analysis including age, body mass index, hypertension, diabetes, hyperlipidemia, and creatinine clearance (never-smokers 6.5 ± 0.4 μg/mL; past smokers 5.6 ± 0.3 μg/mL; current smokers 5.2 ± 0.4 μg/mL; $F=4.52$; $P=0.01$). To examine the acute effect of smoking on adiponectin concentration for 12 hours, we measured plasma adiponectin level in 5 male never-smokers before smoking and 3, 6, and 12 hours after smoking, with the result that adiponectin showed a significant decrease after smoking (12 hours; $-14.5\pm0.6\%$; $P<0.01$). In cultured mouse 3T3-L1 adipocytes, H_2O_2 and nicotine reduced the mRNA expression and secretion of adiponectin in a dose-dependent manner. Smoking habit is associated with adiponectin concentration in men, and its suppressive effect is mediated in part through direct inhibition of smoking on adiponectin expression in adipocytes. (***Hypertension.*** **2005;45:1094-1100.)**

Key Words: smoking ■ oxidative stress ■ risk factors ■ lipids ■ lipoprotein ■ metabolism

Cigarette smoking exacts a continuing toll on public health and is an established risk factor for hypertension and cardiovascular disease, and nonsmoking is a leading preventive strategy against coronary artery disease. Furthermore, cigarette smoking and its cessation are reported to alter lipid metabolism.[1–3] It is well established that smoking stimulates lipolysis in vivo. The lipolytic effect of smoking has been attributed to the nicotine component being mediated via release of catecholamines.[3] Nicotine, a major component of cigarette smoke, promotes inflammation[4] and progression of atherosclerotic lesions.[5,6] Furthermore, nicotine also has a direct effect on human adipose tissue.[7–9] On the other hand, oxidative stress has been shown to be a key phenomenon involved in the effects of smoking. Cigarette smoke contains a large amount of free radicals, which degrade NO released from the endothelium and also produce highly reactive intermediates, resulting in endothelial injury. Oxidative stress can damage many cell components, such as DNA, lipid membranes, and proteins, and lead to apoptosis and cell damage.[10,11]

Adiponectin, an adipose tissue–specific collagen-like factor, is abundantly present in plasma and possesses antiatherogenic properties. Adiponectin is emerging as an important molecule in obesity,[12] the metabolic syndrome,[13–15] cardiovascular disease,[16] lipid metabolism,[15] and hypertension.[17,18] In addition, adiponectin concentration is correlated independently with the vasodilator response to reactive hyperemia, and its concentration could be an independent parameter of endothelial function.[19] Endothelial dysfunction, an early marker of atherosclerosis, has been observed in chronic smokers as well as after acute cigarette smoking.[20,21] These results suggest that adiponectin may be a mediator between smoking and several diseases such as hypertension and coronary artery disease. Furthermore, smoking may directly regulate adiponectin concentration via lipolysis.

Although Miyazaki et al[22] reported that in subjects with coronary artery disease, smoking status was associated with reduced adiponectin concentration, using a small number of subjects, the association between plasma adiponectin and smoking status was evaluated without adjusting for con-

Received February 24, 2005; first decision March 19, 2005; revision accepted April 5, 2005.

From the Departments of Geriatric Medicine (Y.I., T.K., K.I., I.K., M.O., H.R., T.O.) and Internal Medicine and Molecular Science (N.O., K.O., S.K., T.F.), Osaka University Graduate School of Medicine, Japan; and Division of Hypertension and Nephrology (T.H.), Department of Medicine, National Cardiovascular Center, Osaka, Japan.

Correspondence to Tomohiro Katsuya, MD, PhD, Department of Geriatric Medicine, Osaka University Graduate School of Medicine, 2-2 Yamadaoka, Suita 565-0871, Japan. E-mail katsuya@geriat.med.osaka-u.ac.jp

Hypertension **is available at http://www.hypertensionaha.org** **DOI: 10.1161/01.HYP.0000169444.05588.4c**