

Leucine 7 to Proline 7 Polymorphism in the Preproneuropeptide Y Is Associated With Proteinuria, Coronary Heart Disease, and Glycemic Control in Type 1 Diabetic Patients

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OBJECTIVE — Neuropeptide Y is a potent vasoconstrictor thought to enhance the development of atherosclerosis. The leucine 7 to proline 7 (Leu7Pro) polymorphism, located in the signal peptide part of the human preproneuropeptide Y, has been associated with serum lipid levels, intima-media thickness of the common carotid arteries, and diabetic retinopathy in type 2 diabetic patients. Therefore, we investigated the impact of the Leu7Pro polymorphism on diabetic nephropathy, cardiovascular risk factors, and cardiovascular disease in type 1 diabetic patients.

RESEARCH DESIGN AND METHODS — A total of 996 patients from the Finnish Diabetic Nephropathy study were studied in a case-control, cross-sectional study. The carrier frequency of the Pro7 substitution was 13% in the entire study population.

RESULTS — The Pro7 substitution was more common in patients with proteinuria than in those with a normal albumin excretion rate (16 vs. 11%, $P < 0.05$). Patients with the Pro7 allele had worse glycemic control (HbA_{1c} 8.8 vs. 8.5%, $P < 0.005$), more coronary heart disease (CHD) (14 vs. 8%, $P < 0.05$), and higher serum triglycerides (1.65 vs. 1.35 mmol/l, $P < 0.005$) than patients with the wild-type genotype. There were no differences in the plasma neuropeptide Y levels between the patients with Pro7 compared with those with the wild-type genotype. The Leu7Pro polymorphism was independently associated with HbA_{1c} ($P < 0.001$), proteinuria ($P < 0.01$), and CHD ($P < 0.01$) in multiple regression analyses.

CONCLUSIONS — We conclude that the Leu7Pro polymorphism may contribute to the genetic susceptibility to diabetic nephropathy and CHD in type 1 diabetic patients, possibly by influencing glycemic control and triglycerides.

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D iabetic nephropathy is associated with a high risk for coronary heart disease (CHD) and early mortality. Although risk factors such as poor gly-

mic control, hypertension, and dyslipidemia contribute to nephropathy, they cannot entirely explain why it develops in only a subset of patients. Evidence sug-

gests that a genetic predisposition is involved in the pathogenesis of diabetic nephropathy, but the genetic factors are still unknown (1).

Neuropeptide Y (NPY) is a 36–amino acid polypeptide mainly present as an intact peptide in the central and peripheral nervous system, several peripheral organs, and plasma. NPY has been shown to regulate renal blood flow and to alter renal hormone levels such as renin (2). At least two of five known NPY receptors are expressed in the kidney. Stimulation of these receptors has resulted in a decrease in glomerular filtration rate, aldosterone concentration, plasma renin activity, and increase in sodium excretion (3). Furthermore, since interventions that block the renin-angiotensin-aldosterone system alter the renal function in diabetic nephropathy (4), it could be hypothesized that NPY is involved in the pathogenesis of the disease.

The gene that codes NPY is located on chromosome 7 p15.1. A functional leucine 7 to proline 7 (Leu7Pro) polymorphism located in the signal peptide region of the human preproneuropeptide Y (prepro-NPY) was recently observed. This signal peptide region, which is cleaved off during the peptide maturation process, is important for the proper folding and packaging of the NPY peptide, and a substitution can result in a loss of directing activity of the signal sequence (5). This Leu7Pro polymorphism has been associated with variation in blood pressure (6), serum total and LDL cholesterol (7), serum triglycerides (8), and accelerated progression of carotid atherosclerosis in both obese healthy subjects (6) and patients with type 2 diabetes (9). Type 2 diabetic patients with the Pro7 substitution had a higher prevalence of diabetic retinopathy (10). Whether the Leu7Pro polymorphism is associated with diabetic nephropathy is not known. Therefore, the

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Abbreviations: AER, albumin excretion rate; CHD, coronary heart disease; ESRD, end-stage renal disease; Leu7Pro, leucine 7 to proline 7; NPY, neuropeptide Y; prepro-NPY, preproneuropeptide Y; RIA, radioimmunoassay.

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present study was undertaken in order to evaluate whether the Leu7Pro polymorphism of the prepro-NPY is associated with diabetic nephropathy, cardiovascular risk factors, or cardiovascular disease in a cohort of Finnish type 1 diabetic patients.

RESEARCH DESIGN AND METHODS

This study is part of the ongoing Finnish Diabetic Nephropathy (FinnDiane) study, a comprehensive, multicenter, nationwide project with the aim to characterize 25% ($n = 8,000$) of all adult patients with type 1 diabetes in Finland. In 1999, there were 1,700 patients in the FinnDiane database representing ~5% of all type 1 diabetic patients in Finland. All patients with an ascertained renal status ($n = 1,006$) were included in the present study. These patients had been recruited from 20 referral centers. The patients were required to be C-peptide negative (<0.3 nmol/l) and to have permanent insulin treatment initiated before the age of 35 years and within 1 year of the diabetes diagnosis. Six patients with residual C-peptide secretion and four with evidence of nondiabetic kidney disease were excluded. Renal status was based on the albumin excretion rate (AER) in at least two of three consecutive overnight or 24-h urine collections or the presence of end-stage renal disease (ESRD). A total of 321 patients had normal AER (<20 $\mu\text{g}/\text{min}$ or <30 mg/24 h), 166 patients had microalbuminuria (AER 20–200 $\mu\text{g}/\text{min}$ or 30–300 mg/24 h), and 325 patients overt proteinuria (AER >200 $\mu\text{g}/\text{min}$ or >300 mg/24 h). A total of 184 patients were classified as having ESRD and were either on dialysis ($n = 44$) or had had a kidney transplant ($n = 140$). Patients with normal AER were required to have had diabetes for >15 years to qualify for the study. Plasma NPY was measured in all normoalbuminuric and proteinuric patients ($n = 76$) with the Pro7/– genotype and in 164 age- and BMI-matched normoalbuminuric and proteinuric patients with the wild-type genotype. Information about antihypertensive treatment, lipid medication, and cardiovascular disease, including CHD, acute myocardial infarction, and stroke, was also obtained from medical records. The patient had CHD when there was a verified acute myocardial infarction, previous coronary artery bypass surgery or angioplasty, or permanent nitroglycerine

treatment. Informed consent was obtained from all subjects participating in the study. The protocol followed the principles expressed in the Declaration of Helsinki and was approved by the local ethics committees of each center.

Assays

Serum and urine creatinine levels for calculation of creatinine clearance were measured at a central laboratory by a modified Jaffé reaction (Boehringer Mannheim, Mannheim, Germany). Urinary albumin concentration was measured with radioimmunoassay (RIA) (Pharmacia, Uppsala, Sweden) and serum C-peptide concentrations with a Human C-peptide RIA kit (Linco Research, St. Charles, MO). HbA_{1c}, serum cholesterol, and serum triglycerides were locally measured using standardized assays in each referral center. The normal range for HbA_{1c} was 4.0–6.0% in 75% of the local laboratories and the upper normal value was $<7.0\%$ in all centers, whereas the normal range for triglycerides was 0.4–1.7 mmol/l in 55% of the centers, the upper normal range was <2.0 mmol/l in 90% of local laboratories and <3.0 mmol/l in all centers. Serum cholesterol and lipoproteins were analyzed using routine standardized laboratory methods at each center. All local laboratories participated in a national quality assessment program. The estimated glucose disposal rate, which measures insulin sensitivity, was calculated according to a formula as suggested by Williams et al. (11). Plasma NPY was measured using a commercial RIA kit (EURIA-NPY; Euro-Diagnostica, Malmö, Sweden).

Genotyping

Prepro-NPY genotypes were determined by an investigator unaware of the phenotypes using the previously described method (7). Due to failure of the PCR, one patient remained ungenotyped.

Statistical analysis

Data are means \pm SE or median (range) value unless otherwise indicated. Categorical variables were compared using the χ^2 test or Fisher's exact test. Normally distributed continuous variables were tested with Student's *t* test, and non-normally distributed variables were logarithmically transformed or assessed with the Kruskal-Wallis or Mann-Whitney tests. Data were adjusted for HbA_{1c} and BMI when appro-

prate. Pearson correlation coefficient was used to evaluate the correlation between the plasma NPY level and a continuous variable. To evaluate the independent association between categorical or continuous variables, a multiple logistic or linear regression model was used with a means substitution of absent data. Analyses were carried out using a Statistica 4.1 statistical package (Tulsa, OK). A *P* value <0.05 was considered statistically significant.

RESULTS— The clinical characteristics of the study population are shown in Table 1. A more detailed description has been previously reported (12).

Patients homo- and heterozygous for the Leu7Pro genotype were pooled in all further analyses because there were only four patients homozygous for the Pro7 allele. The frequency of the Pro7 substitution was 10.9% in the patients with normal AER, 10.2% with microalbuminuria, 16.2% with proteinuria, and 13.0% with ESRD ($\chi^2 = 5.48$ and $df = 3$, $P = 0.14$). The Pro7 substitution was thus more common in patients with overt nephropathy compared with the patients with normal AER ($\chi^2 = 4.00$ and $df = 1$, $P < 0.05$). The genotype frequencies in all four groups were in Hardy-Weinberg equilibrium.

Patients with the Pro7 substitution had higher values of HbA_{1c}, triglycerides, and BMI and a higher prevalence of CHD (Table 2). Since a majority of patients with ESRD had a renal transplant and were normoalbuminuric at the time of investigation, and since there may be a survival bias in this group, analyses were also performed without ESRD patients. HbA_{1c}, triglycerides, BMI, and the prevalence of CHD were also significantly higher when ESRD patients were excluded (Table 3). In addition, total cholesterol was also higher in these patients, whereas the estimated glucose disposal rate was lower and insulin dose higher in females with the Pro7 substitution.

The plasma NPY was 111.5 ± 3.0 in those with the wild-type genotype and 110.3 ± 4.4 in those with the Pro7 substitution ($P = \text{NS}$). No differences were detected when males and females were analyzed separately. There were no differences when those with proteinuria and normal AER were analyzed separately or when the patients were divided into quintiles according to their plasma NPY (data not shown). The plasma NPY was $96.7 \pm$

Table 1—Clinical characteristics of 996 type 1 diabetic patients included in the study

	NORMO	MICRO	PROT	ESRD
n	321	166	325	184
M/F (%)	41/59	64/36	59/41	59/41
Age (years)	40.4 ± 0.5	38.1 ± 0.9	40.3 ± 0.5	44.0 ± 0.6
Duration of diabetes (years)	26.7 ± 0.4	25.1 ± 0.7	28.0 ± 0.4	32.0 ± 0.6
BMI (kg/m ²)	24.9 ± 0.2	25.7 ± 0.3	25.9 ± 0.2	23.8 ± 0.3
Waist-to-hip ratio				
Male	0.90 ± 0.01	0.91 ± 0.01	0.94 ± 0.01	0.95 ± 0.01
Female	0.81 ± 0.01	0.82 ± 0.01	0.83 ± 0.01	0.87 ± 0.01
Antihypertensive therapy	47 (15)	99 (62)	304 (96)	154 (91)
Cardiovascular disease	10 (3)	6 (4)	42 (13)	48 (27)
Acute myocardial infarction	5 (2)	4 (2)	21 (7)	23 (13)
CHD	9 (3)	6 (4)	33 (11)	37 (21)
Stroke	2 (1)	0 (0)	17 (5)	18 (10)
Retinal laser treatment	92 (29)	84 (51)	260 (81)	179 (99)
Systolic blood pressure (mmHg)	132 ± 1	138 ± 1	144 ± 1	154 ± 2
Diastolic blood pressure (mmHg)	79 ± 1	82 ± 1	83 ± 1	87 ± 1
HbA _{1c} (%)	8.0 ± 0.1	8.7 ± 0.1	8.9 ± 0.1	8.4 ± 0.1
AER				
(μg/min)*	5 (1–17)	38 (3–198)	—	—
(mg/24 h)*	—	—	1,063 (10–16,600)	—
Creatinine clearance (ml · s ⁻¹ · 1.73 m ⁻²)	1.62 ± 0.02	1.51 ± 0.04	1.02 ± 0.04	0.81 ± 0.07

Data are means ± SE, median (range), or n (%). *Some patients with previously abnormal AER had responded to antihypertensive treatment and showed a regression of AER at the time of investigation. NORMO, normal AER; MICRO, microalbuminuria; PROT, proteinuria; ESRD, end-stage renal disease.

2.7 in those with normal AER compared with 121.3 ± 3.5 in those with proteinuria ($P < 0.001$). There were correlations between plasma NPY and HbA_{1c} ($r = 0.24$, $P < 0.001$), total cholesterol ($r = 0.40$, $P < 0.001$), LDL cholesterol ($r = 0.23$, $P < 0.001$), and serum creatinine ($r = 0.51$, $P < 0.001$). However, the correlation between plasma NPY and these variables were significant only when proteinuric patients were included in the analysis, but nonsignificant when only patients with normal AER were analyzed (data not shown).

In a multiple backward stepwise linear regression analysis, the Leu7Pro polymorphism of the prepro-NPY was independently associated with HbA_{1c} (Table 4). Similarly, the polymorphism was independently associated with proteinuria and CHD in a multiple backward stepwise logistic regression analysis (Table 5).

CONCLUSIONS— In this study, the Pro7 substitution of Leu7Pro polymorphism of the prepro-NPY was associated with HbA_{1c}, triglycerides, BMI, and total cholesterol. Furthermore, the Pro7 substitution was also independently associated with proteinuria and CHD in

multiple regression analyses. We cannot exclude the possibility that an unknown polymorphism in close linkage disequilibrium with the Leu7Pro could be responsible for the results. However, experimental studies indicate that the Leu7Pro polymorphism influences the heart rate level, growth hormone, free fatty acid, insulin concentration, and plasma NPY (13,14), indicating that the polymorphism is potentially functional. There is a considerable variance in NPY levels during normal activities, and therefore differences in plasma NPY between genotypes have been observed only during standardized conditions, i.e., during cycle-ergometer exercise test, in healthy young subjects (13,15). This may explain why we could not detect any differences in the plasma NPY levels. The correlation between plasma NPY, serum lipids, and proteinuria is most likely explained by higher plasma NPY levels in patients with impaired renal function (16), since triglyceride-rich lipoproteins are a key feature of diabetic nephropathy (17). It is of note that the half-life of plasma NPY is rather short, only 4 min (18), which can also explain the lack of association between the polymorphism and the plasma levels. Furthermore, since NPY is present in the

central and peripheral nervous system, kidney, and endothelium, it is possible that the effect is not mediated via plasma NPY.

Our patients with the Pro7 substitution had higher serum triglycerides, a phenomenon that was previously seen in preschool-aged boys (8). However, in our study the association was observed within the entire cohort, predominantly in females. A suggested effect of the Pro7 substitution on lipolysis has also been studied in healthy nondiabetic control subjects, where subjects with the Pro7 substitution had a lower free fatty acid level (19). This finding does not, however, contradict our results because free fatty acid levels do not appear to be an important determinant of triglyceride levels (20). The differences in triglycerides between the genotypes were observed when analyzed both with and without the patients with ESRD and after adjusting for HbA_{1c}. At this point, how the polymorphism causes a potentially altered NPY effect on serum lipids is not known, although an altered triglyceride metabolism via specific stimulation of lipoprotein lipase has been suggested (21). The Leu7Pro polymorphism has also been suggested to alter the composition of the lipoprotein particles affecting the postprandial lipid metabolism (22).

The observed association between the prepro-NPY Leu7Pro polymorphism and HbA_{1c} is a novel finding and was detected in both sexes, although the association was stronger in women. HbA_{1c} was higher in those with the Pro7 substitution both when patients with and without ESRD were analyzed and after adjusting for BMI. It was also independently associated with HbA_{1c} in a multiple linear regression analysis. The mechanism is unknown but could be due to differences in insulin sensitivity, although a recent study (19) did not show an association with Leu7Pro and insulin sensitivity, insulin secretion, or glucose metabolism. That study was, however, performed in healthy control subjects, whereas our study comprised type 1 diabetic patients with various stages of late complications. The association between HbA_{1c} and the Leu7Pro genotype and its possible connection with insulin sensitivity is also supported by the fact that the estimated glucose disposal rate was lower and insulin dose higher in female patients with the Pro7 substitution. Interestingly, the association between the Leu7Pro genotype

Table 2—Clinical characteristics according to the Leu7Pro polymorphism of the NPY gene in all patients

	All			Male			Female		
	Leu7Leu	Pro7/—	P	Leu7Leu	Pro7/—	P	Leu7Leu	Pro7/—	P
	n (%)	n (%)		n (%)	n (%)		n (%)	n (%)	
BMI (kg/m ²)	25.1 ± 0.1	25.8 ± 0.4	0.037	25.3 ± 0.3	25.7 ± 0.2	0.413	24.8 ± 0.2	25.9 ± 0.2	0.029
Waist-to-hip ratio	0.880 ± 0.003	0.887 ± 0.008	NS	0.927 ± 0.004	0.930 ± 0.008	NS	0.824 ± 0.004	0.837 ± 0.010	NS
Coronary heart disease	68 (8)	17 (14)	0.043	47 (11)	9 (14)	0.447	21 (5)	8 (14)	0.017
Acute myocardial infarction	44 (5)	9 (7)	NS	31 (7)	7 (10)	NS	13 (3)	2 (3)	NS*
Stroke	33 (4)	4 (3)	1.000*	29 (6)	1 (1)	0.157*	4 (1)	3 (5)	0.050*
Antihypertensive medication	525 (63)	78 (62)	NS	322 (71)	44 (66)	NS	203 (52)	34 (58)	NS
HbA _{1c} (%)†	8.5 ± 0.0	8.8 ± 0.1	0.004	8.6 ± 0.3	8.9 ± 0.1	0.068	8.3 ± 0.1	8.8 ± 0.2	0.024
Total cholesterol (mmol/l)	5.20 ± 0.01	5.39 ± 0.11	NS	5.17 ± 0.05	5.38 ± 0.15	NS	5.22 ± 0.06	5.40 ± 0.16	NS
HDL cholesterol (mmol/l)	1.51 ± 0.02	1.52 ± 0.05	NS	1.37 ± 0.02	1.34 ± 0.05	NS	1.69 ± 0.03	1.74 ± 0.09	NS
LDL cholesterol (mmol/l)	3.10 ± 0.03	3.07 ± 0.09	NS	3.15 ± 0.05	3.15 ± 0.11	NS	3.03 ± 0.05	2.98 ± 0.15	NS
Triglycerides (mmol/l)‡	1.35 ± 0.03	1.65 ± 0.11	0.003	1.49 ± 0.05	1.77 ± 0.19	0.135	1.19 ± 0.03	1.50 ± 0.11	0.003

Data are means ± SE or n (%). * Fisher's exact two-tailed test. †P = 0.005, P = 0.047, and P = 0.048 in all, male, and female, respectively, when adjusted for BMI. ‡P = 0.029, P = 0.275, and P = 0.028 in all, male, and female, respectively, when adjusted for HbA_{1c}.

and insulin dose disappeared when it was adjusted for HbA_{1c}, suggesting a possible link between Leu7Pro, insulin sensitivity, and HbA_{1c}. Our results may indicate a role for NPY in the glucose metabolism, although the mechanisms need to be further investigated.

The lipid profile, BMI, and HbA_{1c} may partly explain why there was more CHD and proteinuria in patients with the Pro7 substitution than in those with the wild-type genotype. This is supported by the outcome in a forward logistic regression model with proteinuria as dependent variable. When the variables most strongly associated with the Leu7Pro in a univariate analysis (HbA_{1c} and serum triglycerides) were added to this model, the association between proteinuria and Leu7Pro was no longer significant (data not shown). However, the Leu7Pro polymorphism was independently associated with proteinuria and CHD in a backward stepwise logistic regression analysis with known cardiovascular risk factors as covariates. In this backwards model, Leu7Pro was independently associated with proteinuria although serum triglycerides and HbA_{1c} were included in the model, thus suggesting that there could also be a direct effect of the Leu7Pro on the renal function. This is further supported by experimental studies showing that higher growth hormone levels increase the AER (23), which is interesting because the Leu7Pro polymorphism has been shown to increase growth hormone levels (14). Furthermore, in vitro studies have shown a possible link between NPY and atherosclerosis because NPY has been shown to stimulate vascular growth (24). NPY is also a potent vasoconstrictor of renal arteries (25) and decreases renal blood flow and glomerular filtration (26), which could indicate a direct influence on the kidneys contributing to the pathogenesis of diabetic nephropathy.

The association between the Leu7Pro and diabetic nephropathy was only seen between those with normoalbuminuria and proteinuria. The fact that there was no association between the Leu7Pro and microalbuminuric patients may be explained by the fact that these patients had a rather long duration of diabetes and, according to recent epidemiological data, there is frequently a regression toward normoalbuminuria in this phenotype (27). Consequently, these patients cannot necessarily be considered to have true di-

Table 4—Multiple backward stepwise linear regression analysis with HbA_{1c} as dependent variable when excluding the patients with ESRD

	Coefficient	SE	P
Age	-0.023	0.005	<0.001
Triglycerides	1.205	0.260	<0.001
LDL cholesterol	0.290	0.060	<0.001
Waist-to-hip ratio	1.492	0.617	0.016
NPY Leu7Pro	0.555	0.156	<0.001

Complete data were available from 679 patients. Variables included in the model: sex, age, serum creatinine, triglycerides, LDL cholesterol, waist-to-hip ratio, systolic blood pressure, lipid lowering medication, and NPY Leu7Leu vs. Pro7/— polymorphism. Adjusted R² = 0.14.

abetic nephropathy. The lack of association between the Leu7Pro polymorphism and those with ESRD could be due to a survival bias, since those with ESRD have a high mortality rate. This is further supported by the fact that the Leu7Pro was also associated with CHD and several cardiovascular risk factors in this study. Furthermore, any population substratification with respect to Finnish ancestral history is unlikely in this study because the prevalence of the Pro7 substitution and the main results did not differ when the southwest and “isolate” eastern regions were analyzed separately (data not shown), as suggested by Perola et al. (28). In conclusion, the Leu7Pro polymorphism of the prepro-NPY may contribute to the genetic susceptibility of diabetic nephropathy and CHD, possibly by influencing the glycemic control and lipid metabolism, in type 1 diabetic patients.

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Table 3—Clinical characteristics according to the Leu7Pro polymorphism of the NPY gene excluding ESRD patients

	All		P	Male		P	Female		P
	Leu7Leu	Pro7/—		Leu7Leu	Pro7/—		Leu7Leu	Pro7/—	
n (%)	706 (87)	105 (13)	—	372 (87)	57 (13)	—	334 (87)	48 (13)	—
Age (years)	40.0 ± 0.4	40.0 ± 1.2	NS	41.5 ± 0.5	41.8 ± 1.3	NS	39.7 ± 0.5	38.7 ± 1.5	NS
Diabetes duration (years)	26.9 ± 0.3	27.0 ± 0.9	NS	26.7 ± 0.4	28.0 ± 1.3	NS	27.0 ± 0.4	25.8 ± 1.2	NS
BMI (kg/m ²)	25.3 ± 0.1	26.1 ± 0.4	0.042	25.6 ± 0.2	25.9 ± 0.6	0.528	25.0 ± 0.2	26.3 ± 0.6	0.021
Waist-to-hip ratio	0.872 ± 0.004	0.882 ± 0.009	NS	0.921 ± 0.004	0.927 ± 0.009	NS	0.817 ± 0.004	0.826 ± 0.011	NS
Cardiovascular disease	46 (7)	12 (12)	0.061	31 (8)	6 (11)	0.567	15 (4)	6 (13)	0.020
CHD	37 (5)	11 (11)	0.033	22 (6)	6 (11)	0.200	15 (5)	5 (11)	0.086*
Acute myocardial infarction	24 (3)	6 (6)	NS	16 (4)	5 (9)	NS*	8 (2)	1 (2)	NS*
Stroke	16 (2)	3 (3)	NS*	13 (4)	1 (2)	NS*	3 (1)	2 (4)	NS*
Antihypertensive medication	393 (57)	56 (55)	NS	236 (65)	33 (60)	NS	157 (47)	13 (49)	NS
Lipid lowering medication	67 (10)	12 (12)	NS	45 (12)	7 (13)	NS	22 (7)	5 (11)	NS*
Systolic blood pressure (mmHg)	138 ± 1	136 ± 2	NS	141 ± 1	138 ± 2	NS	135 ± 1	134 ± 3	NS
Diastolic blood pressure (mmHg)	81 ± 1	80 ± 1	NS	83 ± 1	81 ± 1	NS	79 ± 1	79 ± 2	NS
HbA _{1c} (%) [†]	8.5 ± 0.1	9.0 ± 0.1	0.0006	8.6 ± 0.1	9.0 ± 0.2	0.0613	8.3 ± 0.1	9.0 ± 0.2	0.0027
eGDR (mg · kg ⁻¹ · min ⁻¹)	6.13 ± 0.10	5.61 ± 0.26	0.067	5.11 ± 0.13	4.92 ± 0.31	0.60	7.27 ± 0.14	6.43 ± 0.40	0.034
Insulin dose (IU/kg) [‡]	0.70 ± 0.01	0.73 ± 0.02	0.170	0.72 ± 0.01	0.70 ± 0.03	0.659	0.68 ± 0.01	0.77 ± 0.03	0.010
Total cholesterol (mmol/l)	5.10 ± 0.04	5.38 ± 0.13	0.023	5.10 ± 0.04	5.44 ± 0.17	0.036	5.12 ± 0.06	5.29 ± 0.15	0.315
HDL cholesterol (mmol/l)	1.52 ± 0.02	1.50 ± 0.06	NS	1.37 ± 0.02	1.33 ± 0.05	NS	1.71 ± 0.03	1.70 ± 0.10	NS
LDL cholesterol (mmol/l)	3.04 ± 0.04	3.03 ± 0.10	NS	3.12 ± 0.05	3.18 ± 0.12	NS	2.95 ± 0.05	2.86 ± 0.16	NS
Triglycerides (mmol/l) [§]	1.27 ± 0.03	1.72 ± 0.13	<0.0001	1.42 ± 0.05	1.82 ± 0.21	0.0521	1.10 ± 0.03	1.60 ± 0.13	<0.0001

Data are means ± SE or n (%). *Fisher's exact two-tailed test. †P = 0.001, P = 0.061, and P = 0.006 in all, male, and female, respectively, when adjusted for BMI. ‡P = 0.630, P = 0.375 and P = 0.090 in all, male, and female, respectively, when adjusted for HbA_{1c}. §P = 0.001, P = 0.117 and, P = 0.001 in all, male, and female, respectively, when adjusted for HbA_{1c}. eGDR, estimated glucose disposal rate.

Table 5—Multiple backward stepwise logistic regression analyses

	Coefficient	SE	P
Proteinuria versus normal AER as dependent variable*			
Duration of diabetes	-0.031	0.015	0.036
HbA _{1c}	0.207	0.090	0.022
Triglycerides	2.942	0.613	<0.001
LDL cholesterol	0.543	0.168	0.001
Antihypertensive medication	5.287	0.598	<0.001
NPY Leu7Pro	1.250	0.457	0.006
Coronary heart disease as dependent variable in all patients†			
Age	0.092	0.010	<0.001
HbA _{1c}	0.160	0.055	<0.005
Antihypertensive medication	1.645	0.365	<0.001
NPY Leu7Pro	0.634	0.229	0.006

*Complete data were available from 679 patients. Variables included in the model were sex, duration of diabetes, BMI, HbA_{1c}, triglycerides, LDL cholesterol, current smoking, antihypertensive medication, and NPY Leu7/Leu vs. Pro7/- polymorphism. R² = 0.69. †Complete data were available from 957 patients. Variables included in the model were sex, age, HbA_{1c}, LDL cholesterol, waist-to-hip ratio, current smoking, antihypertensive medication, and NPY Leu7/Leu vs. Pro7/- polymorphism. R² = 0.14.

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References

1. Quinn M, Angelico MC, Warram JH, Krolewski AS: Familial factors determine the development of diabetic nephropathy in patients with IDDM. *Diabetologia* 39: 940-945, 1996
2. Bischoff A, Michel MC: Renal effects of neuropeptide Y. *Pflugers Arch* 435:443-453, 1998
3. Playford RJ, Mehta S, Upton P, Rentch R,

- Moss S, Calam J, Bloom S, Payne N, Ghatei M, Edwards R: Effect of peptide YY on human renal function. *Am J Physiol* 268:F754-F759, 1995
4. Lewis EJ, Hunsicker LG, Bain RP, Rohde RD, The Collaborative Study Group: The effect of angiotensin-converting-enzyme inhibition on diabetic nephropathy. *N Engl J Med* 329:1456-1462, 1993
5. von Heijne G: The signal peptide. *J Membr Biol* 115:195-201, 1990
6. Karvonen MK, Valkonen VP, Lakka TA, Salonen R, Koulu M, Pesonen U, Tuomainen TP, Kauhanen J, Nyyssonen K, Lakka HM, Uusitupa MI, Salonen JT: Leucine7 to proline7 polymorphism in the preproneuropeptide Y is associated with the progression of carotid atherosclerosis, blood pressure and serum lipids in Finnish men. *Atherosclerosis* 159:145-151, 2001
7. Karvonen MK, Pesonen U, Koulu M, Niskanen L, Laakso M, Rissanen A, Dekker JM, Hart LM, Valve R, Uusitupa MI: Association of a leucine(7)-to-proline(7) polymorphism in the signal peptide of neuropeptide Y with high serum cholesterol and LDL cholesterol levels. *Nat Med* 4:1434-1437, 1998
8. Karvonen MK, Koulu M, Pesonen U, Uusitupa MI, Tammi A, Viikari J, Simell O, Ronnema T: Leucine 7 to proline 7 polymorphism in the preproneuropeptide Y is associated with birth weight and serum triglyceride concentration in preschool aged children. *J Clin Endocrinol Metab* 85:1455-1460, 2000
9. Niskanen L, Karvonen MK, Valve R, Uusitupa MI, Tammi A, Viikari J, Simell O, Ronnema T: Leucine 7 to proline 7 polymorphism in the neuropeptide Y gene is associated with enhanced carotid athero-

- sclerosis in elderly patients with type 2 diabetes and control subjects. *J Clin Endocrinol Metab* 85:2266-2269, 2000
10. Niskanen L, Voutilainen-Kaunisto R, Tersavirta M, Karvonen MK, Valve R, Pesonen U, Laakso M, Uusitupa MI, Koulu M: Leucine 7 to proline 7 polymorphism in the neuropeptide y gene is associated with retinopathy in type 2 diabetes. *Exp Clin Endocrinol Diabetes* 108:235-236, 2000
11. Williams KV, Erbey JR, Becker D, Arslanian S, Orchard TJ: Can clinical factors estimate insulin resistance in type 1 diabetes? *Diabetes* 49:626-632, 2000
12. Pettersson-Fernholm K, Forsblom C, Perola M, Groop PH: Polymorphisms in the nephrin gene and diabetic nephropathy in type 1 diabetic patients. *Kidney Int* 63:1205-1210, 2003
13. Kallio J, Pesonen U, Kaipio K, Karvonen MK, Jaakkola U, Heinonen OJ, Uusitupa MI, Koulu M: Altered intracellular processing and release of neuropeptide Y due to leucine 7 to proline 7 polymorphism in the signal peptide of preproneuropeptide Y in humans. *FASEB J* 15:1242-1244, 2001
14. Kallio J, Pesonen U, Karvonen MK, Kojima M, Hosoda H, Kangawa K, Koulu M: Enhanced exercise-induced GH secretion in subjects with Pro7 substitution in the prepro-NPY. *J Clin Endocrinol Metab* 86:5348-5352, 2001
15. Kallio J, Pesonen U, Jaakkola U, Karvonen MK, Helenius H, Koulu M: Changes in diurnal sympatho-adrenal balance and pituitary hormone secretion in subjects with Leu7Pro polymorphism in the prepro-NPY. *J Clin Endocrinol Metab* 88: 3278-3283, 2003
16. Satoh C, Satoh F, Takahashi K, Murakami O, Sone M, Totsune K, Yabe T, Ohneda M, Fukuda M, Sugimura K, Ogawa S, Nagakubo I, Sato T, Mouri T: Elevated plasma immunoreactive neuropeptide Y concentrations and its increased urinary excretion in patients with advanced diabetic nephropathy. *Endocr J* 46:139-146, 1999
17. Groop PH, Elliott T, Ekstrand A, Franssila-Kallunki A, Friedman R, Viberti GC, Taskinen MR: Multiple lipoprotein abnormalities in type I diabetic patients with renal disease. *Diabetes* 45:974-979, 1996
18. Pernow J, Lundberg JM, Kaijser L: Vasoconstrictor effects in vivo and plasma disappearance rate of neuropeptide Y in man. *Life Sci* 40:47-54, 1987
19. Pihlajamäki J, Karhapää P, Vauhkonen I, Kekalainen P, Kareinen A, Viitanen L, Pesonen U, Kallio J, Uusitupa M, Laakso M: The Leu7Pro polymorphism of the neuropeptide Y gene regulates free fatty acid metabolism. *Metabolism* 52:643-646, 2003

20. Carpentier A, Patterson BW, Leung N, Lewis GF: Sensitivity to acute insulin-mediated suppression of plasma free fatty acid is not a determinant of fasting VLDL triglyceride secretion in healthy humans. *Diabetes* 51:1867–1875, 2002
21. Billington CJ, Briggs JE, Harker S, Grace M, Levine AS: Neuropeptide Y in hypothalamic paraventricular nucleus: a center coordinating energy metabolism. *Am J Physiol* 266:R1765–R1770, 1994
22. Schwab US, Agren JJ, Valve R, Hallikainen MA, Sarkkinen ES, Jauhiainen M, Karvonen MK, Pesonen U, Koulu M, Uusitupa MI, Savolainen MJ: The impact of the leucine 7 to proline 7 polymorphism of the neuropeptide Y gene on postprandial lipemia and on the response of serum total and lipoprotein lipids to a reduced fat diet. *Eur J Clin Nutr* 56:149–156, 2002
23. Landau D, Israel E, Rivkis I, Kachko L, Schrijvers BF, Flyvbjerg A, Phillip M, Segev Y: The effect of growth hormone on the development of diabetic kidney disease in rats. *Nephrol Dial Transplant* 18:694–702, 2003
24. Zukowska-Grojec Z, Karwowska-Prokopczuk E, Fisher TA, Ji H: Mechanisms of vascular growth-promoting effects of neuropeptide Y: role of its inducible receptors. *Regul Pept* 75–76:231–238, 1998
25. Michel MC, Gaida W, Beck-Sickinger AG: Further characterization of neuropeptide Y receptor subtypes using centrally truncated analogs of neuropeptide Y: evidence for subtype-differentiating effects on affinity and intrinsic efficacy. *Mol Pharmacol* 42:642–648, 1992
26. Echtenkamp SF, Dandridge PF: Influence of renal sympathetic nerve stimulation on renal function in the primate. *Am J Physiol* 257:F204–F209, 1989
27. Perkins BA, Ficociello LH, Silva KH, Finkelstein DM, Warram JH, Krolewski A: Regression of microalbuminuria in type 1 diabetes. *N Engl J Med* 348:2285–2293, 2003
28. Perola M, Sajantila A, Sarti C, Stengard J, Tamminen M, Puska P, Huttunen J, Tuomilehto J, Peltonen L: Angiotensin-converting enzyme genotypes in the high- and low-risk area for coronary heart disease in Finland. *Genet Epidemiol* 12:391–399, 1995