Urinary Albumin Excretion and Insulin Metabolism in Clinically Healthy 58-Year-Old Men

Stefan Agewall and Fagerberg Björn

The aim of the present cross-sectional study was to investigate the relationship between urinary albumin excretion and insulin sensitivity, intact insulin and insulin propeptides in 104 clinically healthy 58-year-old men recruited from the general population. Insulin sensitivity (hyperinsulinemic euglycemic clamp) adjusted for lean body mass, fasting plasma insulin, proinsulin, split-proinsulin, C-peptide, and urinary albumin excretion and were determined. Urinary albumin excretion was significantly associated with body mass index (BMI), systolic and diastolic blood pressure, plasma insulin, and C-peptide ($P < .05$). Insulin sensitivity tended to be associated with urinary albumin excretion ($P = .07$). In a multiple regression analysis, urinary albumin excretion was independently and significantly associated with systolic blood pressure ($P < .05$). In conclusion, urinary albumin excretion was associated with insulin and C-peptide in clinically healthy 58-year-old men; however, this relationship became insignificant when blood pressure was taken into account in the regression analysis.

POOR METABOLIC control of diabetes patients is associated with microalbuminuria and it has been shown that improved diabetes care significantly diminishes the development of albuminuria and diabetes complications.[1,2] Several studies have demonstrated an association between urinary albumin excretion and impaired glucose tolerance[3,4] and insulin resistance[4,5] in different populations. In addition, it has been shown that acute hyperinsulinemia is followed by an increased urinary albumin excretion.[6] These findings may suggest that insulin resistance might be a pathogenetic factor in the disease process related to microalbuminuria.

Insulin resistance is in general accompanied by hyperinsulinemia[7] and it has been proposed that circulating insulin may contribute to the atherogenetic process. Several observations support this hypothesis. A meta-analysis of a number of prospective studies indicate that hyperinsulinemia is a risk factor for cardiovascular disease[8] and it has been demonstrated that insulin has trophic effects on tissue components in the atherosclerotic lesion.[9-11] There are also studies demonstrating a significant correlation between intima-media thickness of the carotid artery and insulin resistance or plasma insulin.[12-15] Circulating insulin propeptide levels are elevated among subjects with insulin resistance and stressed pancreatic β cells and it has been suggested that proinsulin may be more atherogenic than intact insulin.[16] Most previous studies have measured insulin by using a radioimunoassay (RIA) technique that is cross-reacting with the proinsulins. Hence, a careful examination of the association between different insulin peptides and atherosclerotic disease should preferably include measurements of both specific insulin and insulin propeptides.

Previous studies that have examined the relation between microalbuminuria and insulin action have either used surrogate variables as unspecific insulin, frequently sampled intravenous glucose tolerance test, or examined groups of subjects with hypertension or diabetes mellitus.[17-20]

Accordingly, the aim of the present study was to examine the relations between microalbuminuria and insulin sensitivity measured by the clamp technique, specific insulin, and different insulin propeptides in a sample of clinically healthy 58-year-old men selected from the general population.

MATERIALS AND METHODS

Subjects

The inclusion criteria were age 58 years, male sex, and Swedish ancestry. Exclusion criteria were cardiovascular or other clinically overt disease, treatment with cardiovascular drugs that might disturb the measurements performed in the study, or unwillingness to participate. The subjects were randomly selected among men in the County Council register and were invited to a screening examination.

The present report is a substudy to a trial that aimed to examine the relationship between insulin sensitivity and carotid artery intima-media thickness[14] and a larger study that examined the association between intact insulin, insulin propeptides, and ultrasound-assessed atherosclerosis.[21] The present study encompasses the subjects in the first-mentioned study.[14] An approach of stratified recruitment was applied to obtain men with different degrees of obesity and insulin sensitivity.

Thus, in connection with the screening examination the subjects were divided into quintiles of a body mass index (BMI)/blood glucose score, which allowed immediate stratification and selection for further studies. The following equation was used: BMI/blood glucose score = 46.22 − 1.27(BMI) − 0.84(whole-body glucose). This algorithm was based on a previous study of clinically healthy men of similar age who had undergone a euglycemic hyperinsulinemic clamp examination.[13] In the present population sample, this score correlated significantly with insulin sensitivity measured with the euglycemic hyperinsulinemic clamp method, when expressed as insulin-mediated glucose uptake adjusted both for body weight and for fat-free mass ($r = 0.69$, $P < .001$, and $r = 0.59$, $P < .001$, respectively, n = 104). A euglycemic hyperinsulinemic clamp examination was performed in a randomly

From the Department of Cardiology, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden; and the Department of Medicine, Sahlgrenska University Hospital, Göteborg University, Göteborg, Sweden.

Submitted August 10, 2001; accepted December 31, 2001.

Supported by grants from the Swedish Heart-Lung Foundation, the Swedish Medical Research Council (12270, 10880), King Gustav V and Queen Viktoria Foundation, and Astra Zeneca Mölndal, Sweden.

Address reprint requests to Stefan Agewall, MD, PhD, Department of Cardiology, Karolinska Institute, Huddinge University Hospital, Karolinska Institute, S-141 86 Stockholm, Sweden.

0026-0495/02/5106-0021$35.00/0

doi:10.1053/meta.2002.32805

selected subgroup (n = 104) of all subjects included in the larger study (n = 391) (see Bokemark et al for details[14,21]).

All men received both written and oral information before consenting to participate in the study. The ethical committee at Sahlgrenska University Hospital approved the study.

Screening Measurements

Resting blood pressure was measured phonographically (Korotkoff sounds recorded on electrocardiographic paper) in the right arm after supine rest according to a previous description.[22] Blood pressure was calculated to the nearest 1 mm Hg and the mean of 2 recordings was used. Venous blood was drawn after an overnight fast and after 5 minutes of supine rest for determination of blood glucose, serum levels of triglycerides, and total and HDL- cholesterol.[23] Patients were asked to bring 2 overnight (12-hour) urine samples for determination of urinary albumin excretion using an immunochemistry nephelometry method. The mean of the 2 measurements was used in the statistical analysis. Microalbuminuria was defined as 20 to 200 μg/min.

Measurement

An euglycemic hyperinsulinemic clamp examination was performed with a method that was a slight mofication of the method according to DeFronzo et al.[24,25]

After the clamp examination, fat-free mass was measured using the dual-energy x-ray absorptiometry body composition model.[26]

Insulin sensitivity was calculated as the glucose infusion rate per minute adjusted for fat-free mass (GIR_{FFM}) during the final 60 minutes of the clamp examination. Whole blood glucose was measured with the glucose oxidase technique. Specific plasma insulin was assayed on the Access Immunoassay System (Sanofi Pasteur Diagnostics, Chaska, USA) using a 1-step chemiluminescent immunoenzymatic assay. Cross-reactivity with intact proinsulin is less than 0.2%, 32,33 proinsulin less than 1%, both at 400 pmol/L. Between-run coefficient of variations are 6.6% at 28.6 pmol/L (n = 99), 4.8% at 153.1 pmol/L (n = 102), and 6.0% at 436.7 pmol/L (n = 99). Intact proinsulin and 32,33 split proinsulin were assayed in duplicate using a time-resolved fluorometric assay (DELFIA, Perkin Elmer, USA). The solid-phase antibody, bound to a microtiter plate, was the same in each case. The labeled antibody used in the 32,33 split proinsulin assay (CPT-3F11) was produced by Dako Diagnostics, England. The intact proinsulin assay shows less than 1% cross-reaction with insulin and 32,33 split proinsulin at concentrations of 2,500 pmol/L and 400 pmol/L, respectively. Between-batch coefficients of variation are 8.5% at 20 pmol/L. Plasma C-peptide was assessed by RIA (Guildhay, Guildford, UK).

Statistical Methods

Results are presented as means ±SD. The Mann-Whitney test was used to compare continuous variables.

Before performing the regression analyses, 10-logarithmic transformation was done if a variable was not normally distributed (urinary albumin excretion). Pearson's correlation coefficient was calculated and multiple regression analyses were performed. A 2-sided P value less than .05 was considered as statistically significant.

RESULTS

The group with microalbuminuria had significantly higher C-peptide levels, and systolic and diastolic blood pressure than the normoalbuminuric patients ($P < .05$) (Table 1).

Urinary albumin excretion was significantly and positively associated with BMI, systolic blood pressure, diastolic blood pressure, insulin, and C-peptide (Table 2).

Insulin sensitivity tended to be associated with urinary albumin excretion ($r = 0.18$, $P = .07$) . A stepwise multiple regression analysis with urinary albumin excretion as the dependent variable and the significant covariates in Table 2 as independent variables showed that systolic blood pressure was independently and significantly associated with urinary albumin excretion ($P < .05$).

C-peptide was significantly associated with systolic and diastolic blood pressure, triglycerides, high-density lipoprotein (HDL) cholesterol (negatively), insulin, proinsulin, split proinsulin, insulin sensitivity (negatively), and BMI (Table 3; $P < .05$).

DISCUSSION

This study showed that in a group of clinically healthy men, chosen from the general population, intact insulin and C-peptide levels were significantly associated with urinary albumin excretion; however, these relationship became insignificant when blood pressure was taken into account in the regression analysis. Insulin sensitivity measured by the euglycemic hyperinsulinemic clamp technique showed a weak association with microalbuminuria, that only reached borderline significance.

Table 1. Characteristics of Subjects With and Without Microalbuminuria

	Microalbuminuria (n = 10)	Normoalbuminuria (n = 94)
BMI (kg/m^2)	28.3 ± 4.8	26.1 ± 4.3
Systolic blood pressure (mm Hg)	157 ± 26	135 ± 19*
Diastolic blood pressure (mm Hg)	94 ± 18	82 ± 10*
Cholesterol (mmol/L)	6.0 ± 1.2	6.0 ± 1.1
HDL-cholesterol (mmol/L)	1.1 ± 0.2	1.3 ± 0.4
Triglycerides (mmol/L)	1.7 ± 0.8	1.6 ± 1.1
Blood glucose (mmol/L)	4.5 ± 0.4	4.7 ± 0.6
Insulin (pmol/L)	47 ± 29	36 ± 25
Proinsulin	7.6 ± 4.6	7.2 ± 5.6
Split proinsulin	11.5 ± 11.8	9.0 ± 8.2
C-peptide	760 ± 391	573 ± 270*
Hyperinsulinemic euglycemic clamp: glucose disposal (mg/kg lean body mass/min)	7.1 ± 3.0	8.4 ± 3.3
Current smoker (%)	2 (20)	22 (23)

NOTE. Values are means ± SD.
*$P < .05$.

Table 2. Univariate Correlation Coefficients Between the Logarithm of Urinary Albumin Excretion and Variables in Focus (n = 104)

	Urinary Albumin Excretion (*r*)
BMI	0.21*
Systolic blood pressure	0.35‡
Diastolic blood pressure	0.30†
Cholesterol	−0.09
HDL-cholesterol	−0.10
Triglycerides	0.15
Glucose	0.01
Insulin (pmol/L)	0.20*
Proinsulin	0.13
Split proinsulin	0.17
C-peptide	0.23*
Hyperinsulinemic euglycemic clamp: glucose disposal (mg/kg lean body mass/min)	0.18

*$P < .05$.
†$P < .01$.
‡$P < .001$.

We have previously reported a relationship between urinary albumin excretion and circulating insulin before and during an oral glucose tolerance test in hypertensive men.[4,27] In the present study of healthy men, we found a relationship between different stages in the insulin metabolism and urinary albumin excretion. The strongest relationship was observed between C-peptide and urinary albumin excretion. However, this relationship was not significant when blood pressure level was included into the regression analysis. There are several studies demonstrating an association between urinary albumin excretion and insulin resistance[4,5,27] in different populations. However, these studies included patients with hypertension or diabetes mellitus. One study of a population sample did not observe any association between insulin sensitivity as assessed by the homeostasis model assessment (HOMA) technique and microalbuminuria.[18] It has been shown that acute hyperinsulinemia is followed by an increased urinary albumin excretion.[6] However, the latter observation has been questioned by Catalano et al,[28] who reported that insulin increased the urinary excretion of albumin in non–insulin-dependent diabetes mellitus patients, but not in healthy subjects. Other studies have indicated an association between the glucose metabolism and microalbuminuria. In nondiabetic men, fasting blood glucose[29] or plasma glucose sum after a glucose load[4] were found to be related to microalbuminuria. In the elderly, occult hyperglycemia was associated with microalbuminuria.[3] However, in one study of a fairly large population sample, no positive relationship between microalbuminuria and blood glucose or plasma insulin was observed.[30]

The different variables in the insulin metabolism are closely related to each other. In this study, the correlation coefficients between C-peptide levels and other variables related to degradation of proinsulin were approximately 0.75. During the insulin synthesis, C-peptide is cleaved from proinsulin, stored in secretory granules, and eventually released into the bloodstream in amounts equimolar with those of insulin.[31] For a long time C-peptide was regarded as an inactive substance. However, administration of C-peptide to type 1 diabetes patients results in increased blood flow in the kidneys.[32] Patients with type 1 diabetes frequently develop glomerular hyperfiltration early in the course of their disorder.[33] Adequate insulin therapy does not correct this phenomenon.[34] In a group of patients with type 1 diabetes, C-peptide treatment resulted in a significant reduction in the level of urinary albumin excretion.[35] The same group has examined a group of normotensive subjects with microalbuminuria and type 1 diabetes. The patients received C-peptide plus insulin for 3 months and insulin only for 3 months.[36] During the C-peptide study period, urinary albumin excretion decreased progressively to significantly lower values than those found during the control period. The albumin excretion had decreased by approximately 40% at the end of the 3-month period, whereas no significant change occurred during the control period. The patients remained normotensive throughout the study, and glycemic control improved slightly but to the same extent in the 2 treatment groups. The mechanism underlying the beneficial effect of C-peptide on renal function in diabetes is not known.

It may be hypothesized that C-peptide can influence glomerular membrane permeability and transport, as well as regional blood flow of the kidney, possibly leading to improvements in renal function in the diabetic state. Thus, the positive association between C-peptide and urinary albumin excretion in this study of healthy men is not in line with previous reports from subjects with diabetes. The underlying pathophysiological mechanism behind this relationship is most likely different in subjects with type 1 diabetes and in healthy subjects.

Our population sample was representative of the general population of 58-year-old healthy men in Gothenburg with varying degrees of obesity and insulin sensitivity. Excluding subjects with cardiovascular disease prevented the confounding effects of different cardiovascular drugs on urinary albumin excretion.[37] Still, this study population represented subjects with a wide range of blood pressures and several men were also hypertensive, although untreated at the time-point for the examination. A limitation of our study is that the results cannot be inferred to women or subjects at different ages. It is also important to interpret the results carefully since several comparisons were made between the 2 groups and therefore a multisignificance problem is possible.

Table 3. Univariate Correlation Coefficients Between C-Peptide and Variables in Focus (n=104)

	C-Peptide (*r*)
BMI	0.62‡
Systolic blood pressure	0.25*
Diastolic blood pressure	0.33†
Cholesterol	0.08
HDL-cholesterol	−0.40‡
Triglycerides	0.51‡
Glucose	0.01
Insulin (pmol/L)	0.86‡
Proinsulin	0.71‡
Split proinsulin	0.77‡
Hyperinsulinemic euglycemic clamp: glucose disposal (mg/kg lean body mass/min)	−0.63‡

*$P < .05$.
†$P < .01$.
‡$P < .001$.

We conclude that urinary albumin excretion was associated with insulin and C-peptide in clinically healthy 58-year-old men; however, this relationship became insignificant when blood pressure was taken into account in the regression analysis.

ACKNOWLEDGMENT

We thank Dr Lena Bokemark for help with patient recruitment, and laboratory technologists Eva-Lena Alenhag, Anna Frödén, and Caroline Schmidt for excellent research assistance.

REFERENCES

1. The Diabetes Control and Complications Trial Research Group: The effect of intensive treament of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. N Engl J Med 329:977-986, 1993

2. Reichard P, Nilsson B-Y, Rosenquist U: The effect of long-term intensified insulin treatment on the development of microvascular complications of diabetes mellitus. N Engl J Med 329:304-309, 1993

3. Damsgaard EM, Mogensen CE: Microalbuminuria in elderly hyperglycaemic patients and controls. Diabetic Med 3:430-435, 1986

4. Agewall S, Fagerberg B, Attvall S, et al: Microalbuminuria, insulin sensitivity and haemostatic factors in non-diabetic treated hypertensive men. J Intern Med 237:195-203, 1995

5. Groop L, Ekstrand A, Forsblom C, et al: Insulin resistance, hypertension and microalbuminuria in patients with type 2 (non-insulin-dependent) diabetes mellitus. Diabetologia 36:642-647, 1993

6. Nestler JE, Barlascini CO, Tetrault GA, et al: Increased transcapillary escape rate of albumin in nondiabetic men in response to hyperinsulinemia. Diabetes 39:1212-1217, 1990

7. DeFronzo RA, Ferrannini E: Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. Diabetes Care 14:173-194, 1991

8. Ruige JB, Asselndelft WJJ, Dekker JM, et al: Insulin and risk of cardiovascular disease. A meta-analysis. Circulation 97:996-1001, 1998

9. Pfeifle B, Ditschuneit H: Effect of insulin on growth of cultured human arterial smooth muscle cells. Diabetologia 20:155-158, 1991

10. Stout RW: The effect of insulin and glucose on sterol synthesis in cultured rat arterial smooth muscle cells. Atherosclerosis 27:271-278, 1977

11. Froesch ER, Schmid C, Schwander J, et al: Actions of insulin-like growth factors. Annu Rev Physiol 47:443-467, 1985

12. Folsom AR, Eckfeldt JH, Weitzman S, et al: Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Stroke 25:66-73, 1994

13. Agewall S, Fagerberg B, Attvall S, et al: Carotid artery wall intima-media thickness is associated with insulin-mediated glucose disposal in men at high and low coronary risk. Stroke 26:956-960, 1995

14. Bokemark L, Wikstrand J, Attvall S, et al: Insulin resistance and intima-media thickness in the carotid and femoral arteries in clinically healthy 58-year-old men. The Atherosclerosis and Insulin Resistance Study (AIR). J Intern Med 249:59-67, 2001

15. Howard G, O'Leary D, Zaccaro D, et al: Insulin sensitivity and atherosclerosis. Circulation 93:1809-1817, 1996

16. Galloway JA, Hooper SA, Spradlin CT, et al: Biosynthetic human proinsulin. Review of chemistry, in vitro and in vivo receptor binding, animal and human pharmacology studies and clinical trial experiences. Diabetes Care 15:666-692, 1992

17. Mykkänen L, Zaccaro DJ, Wagenknecht LE, et al: Microalbuminuria is associated with insulin resistance in nondiabetic subjects. The insulin resistance atherosclerosis study. Diabetes 47:793-800, 1998

18. Jager A, Kostense PJ, Nijpels G, et al: Microalbuminuria is strongly associated with NIDDM and hypertension, but not with the insulin resistance syndrome: The Hoorn Study. Diabetologia 41:694-700, 1998

19. Cirillo M, Senigalliesi L, Laurenzi M, et al: Microalbuminuria in nondiabetic adults: Relation of blood pressure, body mass index, plasma cholesterol levels, and smoking: The Gubbio Population Study. Arch Intern Med 158:1933-1939, 1998

20. Tomura S, Kawada K, Saito Ket al: Prevalence of microalbuminuria and relationship to the risk of cardiovascular disease in the Japanese population. Am J Nephrol 19:13-20, 1999

21. Bokemark L, Wikstrand J, Fagerberg B: Intact insulin, insulin propeptides, and intima-media thickness in the femoralartery in 58-year-old clinical healthy men—The Atherosclerosis and Insulin Resistance Study. Angiology 52:237-245, 2001

22. Beckman-Suurküla M, Wikstrand J, Berglund G, et al: Body weight is more important than family history of hypertension for left ventricular function. Hypertension 17:661-668, 1991

23. Agewall S, Samuelsson O, Andersson OK, et al: The efficacy of multiple risk factor intervention in treated hypertensive men, during long term follow-up. J Int Med 236:651-659, 1994

24. DeFronzo R, Tobin JD, Andres R: Glucose clamp technique: A method for quantifying insulin secretion and resistance. Am J Physiol 237: E214-E223, 1979

25. Bokemark L, Frödén A, Attvall S, et al: The euglycemic hyperinsulinemic clamp examination: variability and reproducibility. Scand J Clin Lab Invest 60:27-36, 2000

26. Pietrobelli A, Formica C, Wang Z, et al: Dual-energy x-ray absorptiometry body composition model: Review of physical concepts. Am J Physiol 271:E941-E951, 1991

27. Agewall S, Persson B, Samuelsson O, et al: Microalbuminuria in treated hypertensive men at high risk of coronary disease. J Hypertens 11:461-469, 1993

28. Catalano C, Muscelli E, Quinones Galvan A, et al: Effect of insulin on systemic and renal handling of albumin in nondiabetic and NIDDM subjects. Diabetes 46:868-875, 1997

29. Woo J, Cockram CS, Swaminathan Ret al: Microalbuminuria and other cardiovascular risk factors in nondiabetic subjects. Int J Card 37:345-350, 1992

30. Haffner SM, Stern MP, Kozlowski Gruber M, et al: Microalbuminuria. Potential marker for increased cardiovascular risk factors in nondiabetic subjects? Arteriosclerosis 10:727-731, 1990

31. Steiner D, Cunningham D, Spigelman L, et al: Insulin biosynthesis: Evidence for a precursor. Science 157:697-700, 1967

32. Johansson B-L, Sjöberg S, Wahren J: The influence of human C-peptide on renal function and glucose utilization in type I (insulin-dependent) diabetic patients. Diabetologia 35:121-128, 1992

33. Mogensen, C, Andersen M: Increased kidney size and glomerular filtration rate in untreated juvenile diabetes. Normalisation by insulin treatment. Diabetologia 11:221-224, 1975

34. Sandahl-Christiansen J, Frandsen M, Parving H: The effect of intravenous insulin infusion on kidney function in insulin-dependent diabetes mellitus. Diabetologia 20:199-204, 1981

35. Johansson B-L, Kernell A, Sjöberg S, et al: Influence of combined C-peptide and insulin administration on renal function and metabolic control in diabetes type 1. J Clin Endocrinol Metab 77:976-981, 1993

36. Johansson B-L, Borg K, Fernqvist-Forbes E, et al: Beneficial effects of C-peptide on incipient nephropathy and neuropathy in patients with type I diabetes- a three-month study. Diabetic Med 17:181-189, 2000

37. Bianchi S, Bigazzi R, Baldari G, et al: Microalbuminuria in patients with essential hypertension: Effects of several antihypertensive drugs. Am J Med 93:525-528, 1992