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Changes in insulin sensitivity, renal function, and markers of endothelial dysfunction in hypertension—the impact of microalbuminuria: a 13-year follow-up study

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Abstract

Microalbuminuria (MA) clusters with the metabolic syndrome and insulin resistance and may reflect endothelial dysfunction. Microalbuminuria may also represent renal dysfunction. The aim of the present follow-up study was to assess changes over 13 years in insulin sensitivity, markers of endothelial dysfunction, and renal function in hypertensive subjects with and without MA in 1992-1993, matched for age, sex, and body mass index. Fourteen subjects with and 17 without MA at baseline (1992-1993) participated. At follow-up (2005-2006), MA status was unchanged in 75% of the subjects. The groups had comparable age, blood pressure, body mass index, markers of endothelial dysfunction, and metabolic traits, assessed by oral glucose tolerance test and hyperglycemic clamp. Estimated glomerular filtration rate decreased significantly in the MA group ($P = .049$) and tended to be lower in the MA than the non-MA group in 2005-2006 (79.9 ± 24.5 vs 90.8 ± 13.3 mL min^{-1} (1.73 m^2)$^{-1}$, $P = .2$). Urinary albumin excretion in 1992-1993 predicted estimated glomerular filtration rate in 2005-2006 in adjusted analysis ($\beta = -0.47$, $P = .006$). Estimated glomerular filtration rate less than 60 mL min^{-1} (1.73 m^2)$^{-1}$ was more frequent in the MA than non-MA group at follow-up ($P = .03$). In conclusion, long-standing MA was not associated with progression of metabolic disturbances or markers of endothelial dysfunction in hypertensive individuals. A decline in renal function predicted by urinary albumin excretion was suggested. Microalbuminuria may not be a metabolic trait, but a marker mainly of renal endothelial dysfunction.

1. Introduction

Microalbuminuria (MA) is an independent risk factor for cardiovascular disease (CVD), mortality, and nephropathy in diabetic populations [1,2], nondiabetic hypertensives [3,4], as well as in general and nondiabetic, nonhypertensive populations [5-7].

Microalbuminuria is associated with metabolic traits and the metabolic syndrome (MetS) [8-10]. However, whether MA is causally linked to the progression of the MetS is not clear. Insulin resistance (IR) and compensatory hyperinsulinemia are considered the milestones of the MetS, hence the term *insulin resistance syndrome*; and MA could be a consequence of IR. However, some investigators have found no association between MA and IR [11,12]; and others have shown that MA precedes the development of type 2 diabetes mellitus (DM) [13,14].

It has been suggested that MA reflects a state of generalized vascular leak caused by endothelial dysfunction [15,16], preceding change in insulin sensitivity (IS). Furthermore, a fall in renal function may ensue [17]; and eventually, CVD may develop. However, the association between generalized transcapillary leak and MA has been contradicted [18]; and studies on this subject often have been cross-sectional, done in persons with DM.

In a case-control study of nondiabetic, hypertensive subjects with and without MA, Toft et al [12] reported that MA was not associated with IR when the study groups were matched for age, sex, and body mass index (BMI). Neither was there any association between MA and markers of endothelial dysfunction or renal function. In the present

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study, we report 13-year follow-up data of a subset of these persons. The aim of the study was to evaluate if MA would predict changes in IS, markers of endothelial dysfunction and inflammation, or estimated glomerular filtration rate (eGFR) during long-term follow-up.

2. Methods

2.1. Participants

The Tromsø Study is a population-based, prospective study with repeated health surveys of inhabitants of the municipality of Tromsø, Northern Norway. The third Tromsø survey was run by the University of Tromsø in cooperation with The National Health Screening Service during 1986-1987 [19]. Eighty-four subjects with untreated hypertension (diastolic blood pressure [DBP] ≥90 mm Hg), randomly selected from the third Tromsø survey, participated in a substudy in 1992-1993 [12]. Oral glucose tolerance test (OGTT), hyperglycemic clamp, blood tests, and urine collection were completed for all participants. Urinary albumin excretion (UAE) of 20 to 200 μg/min was detected in 26 subjects (MA group). These subjects were matched (by sex, age, and BMI) with participants who had UAE of less than 20 μg/min (non-MA group, n = 32) [12]. In 2005, all participants still alive and residing in Tromsø from both the MA group (23 subjects) and the non-MA group (24 of the original 32 subjects plus 3 persons without MA from the 1992-1993 substudy) were invited to a follow-up. Finally, 14 subjects from the MA group and 17 from the non-MA group agreed to participate in the present follow-up study. All participants gave their written consent. The invited subjects who did not attend consented to the use of information from their hospital records about development of CVD, DM, and chronic kidney disease. Because the University Hospital of North Norway is distanced from the nearest hospital by 260 km, these hospital records give complete information about hospital-treated disease and laboratory measurements [20]. The Regional Committee for Medical Research Ethics and The Data Inspectorate approved the study.

2.2. Clinical and laboratory measurements

Baseline data (1992-1993) were obtained from previous records, and the methods were described in detail by Toft et al [12]. To minimize the influence of antihypertensive drugs on the measurements at follow-up (2005-2006), the participants were asked to withdraw their drugs 2 weeks in advance and gradually start the medication after the survey. If blood pressure exceeded 180 mm Hg systolic (SBP) and/or 110 mm Hg diastolic, moxonidine was given. β-Blockers used for cardiac disease were continued as prescribed. Antidiabetic medication was withdrawn for up to 1 week before the investigation, and participants were excluded from the OGTT and hyperglycemic clamp if fasting plasma glucose was greater than 10 mmol/L and/or postprandial glucose was greater than 15 mmol/L.

All measurements were done by trained nurses and completed during a 5-day period. On 3 separate days, 3 measurements of blood pressure were made at 2-minute intervals, using an automatic device (Propaq 102 EL monitor; Protocol Systems, Beaverton, OR), after the participant had been seated for 10 minutes. The mean of the second and the third measurements of all 3 days was calculated. Twenty-four–hour ambulatory blood pressure was measured with a Spacelabs 90207 ABP Monitor (Spacelabs, Redmond, WA). The mean SBP and DBP during the whole recording period, as well as the mean SBP and DBP at daytime (7 AM to midnight) and nighttime (midnight to 7 AM), were registered. Height and body weight were measured once, and BMI was calculated (kilograms per square meter). The waist circumference (WC) was measured at the level midway between the inferior border of the rib cage and the superior border of the iliac crest, and hip circumference was measured at the maximal circumference of the buttocks. The mean value of 2 measurements was recorded for each. Two 24-hour urine collections were delivered and analyzed immediately for albumin by rate nephelometry (Beckman Coulter, Brea, CA). Venous blood samples were drawn on 2 occasions after an overnight fast (8-10 hours). The mean value of each variable was used. Plasma creatinine, cholesterol, high-density lipoprotein, and low-density lipoprotein cholesterol were analyzed using an enzymatic colorimetric method; plasma triglycerides were analyzed with a colorimetric method; and an immunoturbidimetric method was used for plasma high-sensitive C-reactive protein (hsCRP). Modular P (Roche, Basel, Switzerland) was applied for all these plasma analyses. Glycosylated hemoglobin (HbA_{1c}) was analyzed with a high-performance liquid chromatography method by VARIANT II (Bio-Rad, Hercules, CA). Monocyte chemoattractant protein 1 (MCP-1) was assessed from serum and morning spot urine samples using an enzyme immunoassay technique ((R&D Systems, Minneapolis, MN). Fibrinogen and D-dimer were analyzed by clotting method and turbidimetric method, respectively (STA-R Bergman Diagnostika, Lillestrøm, Norway). Immunoassays from Hyphen BioMed, Neuville-Sur-Oise, France (Zymutest), were used to assess plasminogen activator inhibitor type 1 activity and tissue plasminogen activator (tPA) antigen.

Oral glucose tolerance test with 1 g of dextrose per kilogram body weight or a maximum of 75 g of glucose was carried out. Postload glucose response was calculated as incremental area units over the 2-hour sampling time (area under the glucose curve [AUC glucose]). On a separate day, a standard hyperglycemic clamp was performed to assess insulin secretion as well as glucose disposal and suppression of free fatty acids (FFA) under physiologic insulin stimulation [21,22]. Glucose was infused into an antecubital vein and kept stable at 10 mmol/L for 180 minutes. Blood samples were drawn from an antecubital vein of the contralateral arm, which was kept in a heating device to

arterialize the blood [23]. Plasma glucose was checked bedside every 5 minutes using a Yellow Spring Instruments glucose analyzer (2300 STAT PLUS; YSI, Yellow Springs, OH). First-phase insulin release was calculated as the area under the insulin curve (AUC insulin) over the initial 10 minutes; and second-phase insulin release, as the AUC insulin from 120 to 180 minutes of the clamp period. Insulin sensitivity was assessed as the mean glucose infusion rate (micromoles per kilogram per minute) during the third clamp hour and as the insulin sensitivity index (ISI), that is, the mean glucose infusion rate (micromoles per kilogram per minute) divided by the mean of the insulin concentrations (picomoles per liter) at 120, 140, 160, and 180 minutes of the clamp.

Plasma insulin was measured with an enzyme-linked immunosorbent assay method (DakoCytomation, Ely, United Kingdom). Because plasma insulin was analyzed with a radioimmunoassay method at baseline (1992-1993), plasma samples from the baseline hyperglycemic clamp (−30, 0, 120, and 180 minutes) were thawed and reanalyzed using the enzyme-linked immunosorbent assay method. Pearson correlation coefficients with the corresponding insulin values assessed with radioimmunoassay technique in 1992-1993 were 0.67 ($P < .001$) for the mean of the fasting values (−30 and 0 minutes) and 0.94 ($P < .001$) for the mean of the values at 120 and 180 minutes. FFAs were measured with the Wako nonesterified fatty acid test kit (Wako Chemicals, Neuss, Germany) by an ABX Pentra 400 autoanalyzer (Horiba ABX, Northampton, United Kingdom); and suppression of FFA during the hyperglycemic clamp was calculated as $[(FFA_{-30\text{-}0} - FFA_{120\text{-}180})/FFA_{-30\text{-}0}] \times 100$, where $FFA_{-30\text{-}0}$ was the mean FFA concentration at −30 and 0 minutes and $FFA_{120\text{-}180}$ was the mean concentration at 120, 160, and 180 minutes of the clamp. The FFA_{IS} index was calculated as $FFA_{120\text{-}180}$ divided by the mean insulin concentration at 120 to 180 minutes and then multiplied by 1000.

eGFR was calculated using the 4-variable Modification of Diet in Renal Disease study equation. Because creatinine was measured by the Jaffe method in 1992-1993, the original 4-variable version (eGFR = 186 × [s-creatinine {micromoles per liter}/88.4]$^{-1.154}$ × age$^{-0.203}$ × 0.742 if female) [24] was applied at baseline, whereas the recalibrated version (eGFR = 175 × [s-creatinine {micromoles per liter}/88.4]$^{-1.154}$ × age$^{-0.203}$ × 0.742 if female) [25] was used at follow-up.

2.3. Statistical analyses

Before initiation of the study, statistical power to detect significant ($P < .005$) changes in ISI of 0.07 μmol·kg^{-1}·min^{-1}/pmol·L^{-1} and in HbA_{1c} of 1% by inclusion of 40 individuals was calculated. Standard deviations (SDs) derived from repeated hyperglycemic clamps and blood tests in 1992-1993 were applied. The power was 97% and 93%, respectively, and decreased to 91% and 86% by inclusion of 30 individuals. The SD for between-group differences was somewhat larger for ISI, reducing the power for detection of these differences.

Frequency distribution was checked for all variables. Data are given as mean ± SD if normally distributed and median (interquartile range) if distributions were skewed. The participants were allocated into the MA group and the non-MA group according to MA status in 1992-1993. Independent-samples *t* tests were used to compare normally distributed continuous variables; otherwise, Mann-Whitney test was applied. Within-group comparison between baseline and follow-up was assessed using a paired *t* test or Wilcoxon signed rank sum test as appropriate. Fisher exact test was used for between-group comparison and McNemar test for within-group comparison of categorical variables. Multiple linear regression models were run, by which the association of UAE in 1992-1993 to ISI, glucose infusion rate, first-phase insulin release during the clamp, HbA_{1c}, eGFR, and markers of inflammation and endothelial dysfunction was tested. Sex and age were entered as covariates.

P values less than .05 were considered statistically significant. The statistical analyses were run using SPSS software version 15.0 (SPSS, Chicago, IL) for Windows.

3. Results

Two persons from the MA group and 6 from the non-MA group died between baseline (1992-1993) and follow-up (2005-2006). Among the 31 participants at follow-up (n = 14 in the MA group and n = 17 in the non-MA group), 26 completed all tests. Five persons in the MA and 3 in the non-MA group reported having DM in 2005-2006, and 5 of these (4 in the MA and 1 in the non-MA group) had fasting blood glucose of at least 10 mmol/L and/or postprandial glucose of at least 15 mmol/L and hence were excluded from the OGTT and hyperglycemic clamp. The subjects with DM at follow-up had significantly higher BMI and WC at baseline (29 ± 3 vs 27 ± 3 kg/m^2, $P = .05$ and 105 ± 11 vs 95 ± 10 cm, $P = .003$ for DM vs non-DM) and lower glucose infusion rate during the baseline hyperglycemic clamp (16 ± 5 vs 35 ± 14 μmol/kg min^{-1}, $P < .001$ for DM vs non-DM). Furthermore, they increased more in BMI ($P = .02$) during follow-up and borderline significantly more in WC ($P = .07$, data not shown). Fourteen subjects (9 in the MA group, 5 in the non-MA group) continued antihypertensive treatment throughout the study.

3.1. Participant characteristics

In the MA group, 9 of the 14 subjects still had UAE of at least 20 μg/min in 2005-2006. Four had UAE values less than 10 μg/min, whereas one had a value of 18 μg/min. Fourteen subjects in the non-MA group maintained UAE of less than 20 μg/min at follow-up, and 3 had developed MA. From the non-MA group, none progressed to manifest proteinuria (UAE ≥200 μg/min). Three subjects in the MA group were proteinuric in 2005-2006.

Characteristics of the MA and non-MA groups at baseline (1992-1993) and follow-up (2005-2006) are

given in Table 1. The 2 groups had similar age and sex distribution, BMI and WC, lipid values, fibrinogen, pattern of smoking, and physical activity. Furthermore, the change in these variables from baseline to follow-up did not differ between the groups. Office SBP increased more in the non-MA group than in the MA group from 1992-1993 to 2005-2006, whereas there was a greater fall in DBP in the MA group.

HbA_{1c} was significantly higher in the MA group than in the non-MA group at follow-up, but the difference was no longer significant when the subjects with DM were excluded (MA group, 6.0% ± 0.4% vs non-MA group, 5.8% ± 0.3%; P = .1). Kidney function, measured as eGFR, decreased significantly from 1992-1993 to 2005-2006 only in the MA group.

Within-group comparison of traditional and novel markers of inflammation and endothelial dysfunction was limited by the fact that different methods were applied at baseline and follow-up, and some of the analyses were not available in 1992-1993. D-dimer, tPA antigen, plasminogen activator inhibitor type 1 activity, hsCRP, and MCP-1 at follow-up were similar in both groups (MA group vs non-MA group), as follows: D-dimer—0.50 (0.40-0.64) vs 0.40 (0.40-0.60) μg fibrinogen equivalent units per milliliter, P = .5; tPA antigen—12.4 ± 4.9 vs 12.1 ± 4.7 ng/mL, P = .8; PAI activity—2.7 (1.6-25.3) vs 4.0 (2.8-5.8) ng/mL, P = .9; hsCRP—2.6 (1.1-5.9) vs 1.8 (1.2-3.7) mg/L, P = .5; serum MCP-1—419 ± 101 vs 403 ± 135 pg/mL, P = .7; and urinary MCP-1 to creatinine ratio—19.1 (14.8-26.5) vs 19.2 (10.0-25.8) ng/mmol, P = .5.

Ambulatory blood pressure was only measured during follow-up. No differences in blood pressure values or dipping patterns were noted among the groups (data not shown).

At follow-up, most of the participants were on regular antihypertensive treatment; but more subjects in the MA than in the non-MA group used blockers of the renin-angiotensin system (RAS blockers) (71% vs 18%, P = .004). Regular intake of cod liver oil or omega-3 fatty acids was reported more frequently in the non-MA than in the MA group (29% vs 71%, P = .03 for the MA group vs the non-MA group), whereas the trend was opposite for statins (57% vs 24%, P = .08).

3.2. Glucose and lipid metabolism

Results from the OGTT and the hyperglycemic clamp are shown in Table 2. Mean fasting glucose was higher than normal in both groups at baseline and follow-up, even when the participants with DM were excluded at follow-up (MA group, 6.3 ± 1.0 mmol/L vs non-MA group, 5.7 ± 0.6 mmol/L; P = .2). Fasting insulin was within the reference range and did not differ between the groups, and the same was also true when the diabetic subjects were excluded from the 2005-2006 measurements (MA group, 65.4 ± 33.0 pmol/L vs non-MA group, 61.0 ± 34.2 pmol/L; P = .8). As in 1992-1993, mean glucose value at 120 minutes of the OGTT was in the glucose intolerance range in both groups at follow-up. In contrast to the MA group, the non-MA group showed a significant increase in mean glucose value at 120 minutes and AUC glucose during the OGTT; but the groups were not significantly different at any instance. All metabolic variables measured during the hyperglycemic clamp remained similar between groups at both surveys.

3.3. Multivariable analyses

Linear regression analyses were run, and the results are shown in Table 3. Urinary albumin excretion measured in

Table 1
Characteristics at baseline and follow-up of the participants with MA (UAE ≥20 μg/min) (MA group) and without MA (non-MA group) at the survey in 1992-1993

	Baseline (1992-1993)			Follow-up (2005-2006)			Baseline vs follow-up	
	MA group	Non-MA group	P value	MA group	Non-MA group	P value	MA group, P value	Non-MA group, P value
n	14	17		14	17			
Sex, male/female, n	11/3	13/4	1.0	11/3	13/4	1.0		
Age, y	56 ± 7	52 ± 9	.2	69 ± 7	66 ± 10	.4		
SBP (office), mm Hg	154 ± 10	148 ± 15	.3	158 ± 19	161 ± 22	.6	.4	.02
DBP (office), mm Hg	99 ± 5	98 ± 6	.4	90 ± 9	92 ± 9	.5	.002	.06
WC, cm	100 ± 12	96 ± 10	.4	105 ± 12	103 ± 10	.6	.03	<.001
BMI, kg/m^2	28 ± 3	27 ± 2	.7	29 ± 4	29 ± 4	1.0	.2	.006
UAE, μg/min	40 (29-132)	7 (7-11)	<.001	60 (8-235)	5 (4-9)	.001	.07	.3
eGFR, mL min^{-1} $(1.73\ m^2)^{-1}$	89 ± 19	95 ± 15	.4	80 ± 25	91 ± 13	.2	.049	.3
HbA_{1c}, %	5.9 ± 0.5	5.8 ± 0.6	.6	6.7 ± 1.2	5.9 ± 0.6	.04	.048	.2
Cholesterol, mmol/L	6.1 ± 0.6	6.3 ± 1.1	.6	4.7 ± 0.8	5.2 ± 1.2	.2	<.001	.006
Triglycerides, mmol/L	1.3 (0.9-1.4)	1.2 (0.9-1.6)	1.0	1.2 (0.9-1.6)	1.2 (1.0-1.5)	.8	.4	.7
HDL cholesterol, mmol/L	1.4 ± 0.6	1.4 ± 0.3	.8	1.3 ± 0.3	1.4 ± 0.3	.9	.7	1.0
LDL cholesterol, mmol/L	4.0 ± 0.7	4.3 ± 1.0	.3	3.2 ± 0.8	3.7 ± 0.9	.1	.006	.03
Fibrinogen, g/L	2.2 ± 0.8	2.3 ± 0.8	1.0	3.5 ± 0.6	3.7 ± 1.0	.5	<.001	<.001
Current use of antihypertensive agents, n (%)	1 (7)	3 (18)	.6	13 (93)	13 (77)	.3	<.001	.002
Current smoking, n (%)	5 (36)	3 (18)	.4	3 (21)	2 (12)	.6	.7	1.0
Physical activity ≥1 h/wk previous year, n (%)	5 (36)	6 (35)	1.0	5 (36)	9 (56)	.3	1.0	.5

The data are given as mean ± SD, median (interquartile range), or number (percentage). HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

Table 2
Glucose and lipid metabolism at baseline and follow-up according to the presence or absence of MA in 1992-1993 (UAE ≥20 μg/min)

	Baseline (1992-1993)			Follow-up (2005-2006)			Baseline vs follow-up	
	MA group (n = 14)	Non-MA group (n = 17)	*P* value	MA group (n = 14) (n = 10)[a]	Non-MA group (n = 17) (n = 16)[a]	*P* value	MA group, *P* value	Non-MA group, *P* value
Fasting glucose, mmol/L	6.1 ± 0.9	5.6 ± 0.7	0.2	7.4 ± 2.5	6.2 ± 1.4	.1	.02	.02
Glucose 120 min after dextrose ingestion, mmol/L[a]	9.2 ± 3	8.2 ± 2	0.3	8.8 ± 3.8	8.9 ± 2.6	.9	.4	.007
AUC glucose, pmol/L[a]	14.7 ± 6.9	14.7 ± 5	1.0	15.1 ± 9.5	16.9 ± 6.4	.6	.2	.07
Fasting insulin, pmol/L	68 ± 58	59 ± 29	0.6	74 ± 41	58 ± 33	.2	.7	.9
1st-phase insulin release, pmol/L[a]	664 ± 537	711 ± 484	0.8	600 ± 588	540 ± 421	.8	.09	.02
2nd-phase insulin release, pmol/L[a]	892 ± 524	854 ± 599	0.9	1190 ± 1479	889 ± 572	.6	.7	.9
Mean insulin 2nd phase, pmol/L[a]	291 ± 140	272 ± 166	0.8	363 ± 378	283 ± 164	.5	.6	.9
Mean glucose infusion rate 2nd phase, $\mu mol \cdot kg^{-1} \cdot min^{-1}$[a]	29 ± 18	31 ± 12	0.8	30 ± 20	29 ± 15	.9	.1	.4
ISI, $\mu mol \cdot kg^{-1} \cdot min^{-1}/pmol \cdot L^{-1}$[a]	0.13 ± 0.09	0.18 ± 0.14	0.2	0.12 ± 0.10	0.13 ± 0.09	.8	.3	.3
FFA, fasting, mmol/L	0.47 ± 0.16	0.48 ± 0.11	0.8	0.43 ± 0.15	0.40 ± 0.14	.5	.5	.002
FFA during HG clamp, mmol/L[a]	0.07 ± 0.03	0.06 ± 0.04	0.9	0.08 ± 0.09	0.04 ± 0.04	.2	.3	.04
Suppression of FFA during clamp, %[a]	86 ± 6	87 ± 7	0.7	83 ± 17	91 ± 9	.2	.4	.3
FFA_{IS} index[a,b]	0.3 (0.2-0.3)	0.3 (0.1-0.5)	0.1	0.09 (0.03-0.2)	0.2 (0.02-1.5)	.8	.4	.04
Lowest quartile of glucose infusion rate, n (%)[a,c]	4 (29)	4 (24)	1.0	4 (40)	2 (13)	.2	.5	1.0
Lowest quartile of IS index[a,d]	4 (29)	3 (18)	0.7	4 (40)	2 (13)	.2	1.0	1.0
Highest quartile of HbA_{1c}[e]	3 (21)	4 (24)	1.0	5 (36)	3 (18)	.4	.6	1.0

[a] At follow-up (2005-2006), 4 persons in the MA group and 1 person in the non-MA group were excluded from the OGTT and the hyperglycemic clamp because of fasting blood glucose greater than 10 mmol/L and/or non-fasting blood glucose greater than 15 mmol/L.

[b] The *FFA_{IS} index* is defined as the mean FFA during the clamp (120-180 minutes) divided by the mean plasma insulin during the same period and then multiplied by 1000.

[c] Lowest quartile of glucose infusion rate: less than 19.8 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at baseline (1992-1993); less than 15 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at follow-up (2005-2006).

[d] Lowest quartile of ISI: less than 0.07 $\mu mol \cdot kg^{-1} \cdot min^{-1}/pmol \cdot L^{-1}$ at baseline; less than 0.05 $\mu mol \cdot kg^{-1} \cdot min^{-1}/pmol \cdot L^{-1}$ at follow-up.

[e] Highest quartile of HbA_{1c}: greater than or equal to 6.3% at baseline; greater than or equal to 6.4% at follow-up.

1992-1993, adjusted for age and sex, was a significant predictor of eGFR in 2005-2006. Additional adjustment for BMI did not change this relationship. Metabolic traits or markers of endothelial dysfunction in 2005-2006 were not predicted by UAE.

3.4. Development of disease

Development of atherosclerotic CVD, DM, and eGFR less than 60 mL min^{-1} $(1.73\ m^2)^{-1}$ between the surveys was registered for all 31 participants, for the 8 who had died, and for the 19 who did not attend the follow-up survey for other reasons. The results are presented in Table 4. There was a significant difference between the groups with respect to the development of eGFR less than 60 mL min^{-1} $(1.73\ m^2)^{-1}$, whereas statistical significance was not reached for the other end points. The individuals who died or did not attend the 2005-2006 survey had similar age compared with the rest of the participants (70.1 ± 7.4 vs 67.5 ± 8.5 years, $P = .2$).

4. Discussion

In this observational study of well-characterized individuals with long-standing hypertension, initially recruited from the general population, we demonstrated that MA

Table 3
Multiple linear regression

	B	95% confidence interval	Standardized β	*P* value
UAE 1992-1993 predicting ISI 2005-2006	0.000	−0.001 to 0.000	−0.28	.2
UAE 1992-1993 predicting mean glucose infusion rate[a] in 2005-2006	0.05	−0.07 to 0.16	0.17	.4
UAE 1992-1993 predicting 1st-phase insulin release 2005-2006	2.8	−0.44 to 6.1	0.37	.09
UAE 1992-1993 predicting HbA_{1c} 2005-2006	−0.001	−0.007 to 0.006	−0.04	.9
UAE 1992-1993 predicting eGFR 2005-2006	−0.16	−0.27 to −0.52	−0.47	.006
UAE 1992-1993 predicting log hsCRP 2005-2006	−0.002	−0.009 to 0.006	−0.08	.7
UAE 1992-1993 predicting serum MCP-1 2005-2006	0.005	−0.79 to 0.80	0.002	1.0
UAE 1992-1993 predicting urinary MCP-1 to creatinine ratio 2005-2006	−0.04	−0.17 to 0.08	−0.13	.5

All models are adjusted for age and sex.

[a] Mean glucose infusion rate during 120 to 180 minutes of the hyperglycemic clamp.

Table 4
Development of disease after the baseline survey[a] in 1992-1993, according to the presence or absence of MA (UAE ≥20 μg/min)

	MA group (n = 25)	Non-MA group (n = 33)	*P* value
Diabetes mellitus, n (%)	6 (24)	4 (12)	.30
Atherosclerotic vascular disease, n (%)	12 (48)	11 (33)	.29
Chronic kidney disease (eGFR <60 mL min^{-1} (1.73 m^2)$^{-1}$), n (%)	7 (28)	2 (6)	.03

Diseases were registered in 2006.
[a] Including subjects who died between baseline and follow-up (n = 8) or did not participate at follow-up for other reasons.

tended to persist for many years. Conversely, hypertensive individuals without MA had a tendency to remain non-albuminuric for 13 years. Microalbuminuria status 13 years earlier did not predict increased IR, impaired insulin secretion, or increased levels of traditional and novel markers of inflammation and endothelial dysfunction, but seemed to be associated with the rate of renal function decline.

Microalbuminuria in nondiabetic individuals has frequently been found to cluster with components of the MetS such as IR [19,26], obesity [27,28], lipid disturbances [29,30], and hypertension [26,31]. Most studies investigating the association between MA and other risk factors have been cross-sectional, and differences in BMI and WC often have been insufficiently controlled for. In the present follow-up study, stimulated insulin secretion and IS were assessed with a clamp method, which is considered the gold standard [21,22]. Furthermore, the follow-up time was long enough to expect clinically interesting changes in IS, possibly linked with MA, to occur during the observation time. The fact that the study groups were similar in BMI and WC at baseline and follow-up prevented the IS data from being biased by differences in body fat. Because no difference in insulin secretion or IS was observed, we conclude that MA in hypertensive individuals who have not developed diabetes does not seem to be associated with an increase in IR, even during long-term follow-up of already glucose-intolerant, overweight, and elderly individuals.

In recent literature, MA has been linked to generalized endothelial dysfunction [15,16] and to a decline in eGFR in hypertensive persons [32]. Our finding that MA affects the rate of eGFR decrease is supported both by epidemiologic data [17,33] and by reports considering MA as mainly a marker of localized renal, and not necessarily generalized, endothelial dysfunction [34]. Microalbuminuria may also develop in a subgroup of hypertensives with abnormalities in structures of the filtration barrier other than the endothelium [18]. Mildly reduced GFR has been recognized as a strong independent predictor of CVD [35,36], and some of the increased cardiovascular risk associated with MA may be transmitted through an effect of MA on GFR. The low-grade inflammation and signs of endothelial dysfunction observed in both groups may have been caused by decades of hypertension, in consort with obesity, cigarette smoking, or low-grade renal dysfunction.

End point data in the present study are limited and should be interpreted with caution. However, the finding of a higher rate of eGFR less than 60 mL min^{-1} (1.73 m^2)$^{-1}$ in the MA group than in the non-MA group supports the other results of the present study.

Our study has several important limitations. Although the follow-up time was long and clinically relevant differences would be expected to appear after 13 years, the number of subjects was low and the risk of making a type II error was considerable. Moreover, we did not measure the complete metabolic profile in the diabetic persons with high glucose levels. Hence, we cannot exclude that smaller differences in IS, insulin secretion, and markers of endothelial dysfunction exist between hypertensive individuals with and without MA. However, the power of the present study to detect clinically interesting changes in ISI of 0.07 μmol·kg^{-1}·min^{-1}/pmol·L^{-1} or HbA_{1c} of 1% was high. Smaller changes may have been undetected.

The study groups were well characterized and known to have similar patterns of metabolic and inflammatory variables at baseline and follow-up. This advantage may contribute to elucidate the link between MA per se and the development of IR.

At baseline, creatinine was measured with a noncalibrated Jaffe method. Calibration errors may bias estimations of GFR, especially in the reference range [37]. However, calibrated creatinine values were used at follow-up; and because the groups had similar distribution of age, sex, and BMI, creatinine calibration problems are unlikely to be an important source of bias in the present study.

Antihypertensive agents, including blockers of the RAS, had been used by most participants for a variable length of time. Although medication was stopped 2 weeks before the measurements, it probably has affected the rate of change in the metabolic variables. Blockers of the RAS, which were used by more participants in the MA than the non-MA group, are thought to improve endothelial function [38] and prevent the development of type 2 DM and IR [39]. The use of modern treatment may have contributed to the stabilization of the metabolic profile observed in the present study, even in persons with long-standing hypertension and MA, abnormal postload glucose levels, and inflammatory markers in the upper range. Thus, the use of medication may have blunted an eventual difference between the groups that otherwise would have appeared.

Finally, although originally recruited from the general population, the group of hypertensive participants had been followed for 20 years; and survival bias may have been introduced. Therefore, the results may not be generally valid in all hypertensive persons.

In conclusion, MA tended to persist for many years in this small cohort of well-characterized hypertensive subjects. The presence of MA seemed to be associated with a decline in renal function, but not with a change in insulin secretion,

IR, other metabolic variables, or markers of endothelial dysfunction. Some of the risk of CVD associated with UAE elevation may be mediated through renal factors, not only through metabolic factors or the presence of generalized endothelial dysfunction.

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