Insulin Sensitivity and Albuminuria: The RISC Study

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OBJECTIVE

Accumulating evidence suggests an association between insulin sensitivity and albuminuria, which, even in the normal range, is a risk factor for cardiovascular diseases. We evaluated whether insulin sensitivity is associated with albuminuria in healthy subjects.

RESEARCH DESIGN AND METHODS

We investigated 1,415 healthy, nondiabetic participants (mean age 43.9 ± 8.3 years; 54.3% women) from the RISC (Relationship between Insulin Sensitivity and Cardiovascular Disease) study, of whom 852 participated in a follow-up examination after 3 years. At baseline, insulin sensitivity was assessed by hyperinsulinemic–euglycemic clamps, expressed as the M/I value. Oral glucose tolerance test–based insulin sensitivity (OGIS), homeostasis model assessment of insulin resistance (HOMA-IR), and urinary albumin-to-creatinine ratio (UACR) were determined at baseline and follow-up.

RESULTS

Microalbuminuria (UACR ≥30 mg/g) was present in fewer than 2% at either study visit. After multivariate adjustments, there was no cross-sectional association between UACR and any measure of insulin sensitivity. Neither OGIS nor HOMA-IR was significantly associated with follow-up UACR, but in a multivariate regression analysis, baseline M/I emerged as an independent predictor of UACR at follow-up (β-coefficient −0.14; *P* = 0.001).

CONCLUSIONS

In healthy middle-aged adults, reduced insulin sensitivity, assessed by hyperinsulinemic–euglycemic clamp, is continuously associated with a greater risk of increasing albuminuria. This finding suggests that reduced insulin sensitivity either is simply related to or might causally contribute to the initial pathogenesis of albuminuria.

Higher levels of albuminuria are associated with an increased risk of mortality and cardiovascular events in patients with diabetes mellitus or arterial hypertension and the general population (1–3). Insights into the pathophysiology of albuminuria are of high clinical relevance since therapeutic interventions to reduce albuminuria are often accompanied by improvements in cardiovascular outcomes (3). Microalbuminuria is also regarded as a marker of a certain form of endothelial dysfunction, but there exists still no clear picture on the direction of the cause and effect relationship (4–6). Alternatively, microalbuminuria and vascular diseases/endothelial dysfunction may also be caused by a common pathophysiologic process (7).

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**A complete list of RISC Study Investigators and participating centers can be found in the APPENDIX.*

While experimental data suggest reciprocal influences of albuminuria and insulin resistance, several clinical studies support the concept that insulin resistance might precede or contribute to the development of microalbuminuria (7–13). In fact, insulin signaling in podocytes seems to be important for their function to maintain the integrity of the glomerular filtration barrier (10–13). This is in line with experimental data suggesting that insulin resistance and hyperglycemia contribute to malfunction and loss of podocytes, which are considered initial steps in the development of diabetic nephropathy and albuminuria (10–13). Clinical studies addressing this issue were mainly performed in diabetic patients and have largely, but not consistently, confirmed an association between insulin resistance and microalbuminuria in this setting (10–18). Data in nondiabetic populations are sparse and show conflicting results (19–22). Among these, large studies in general populations are restricted to cross-sectional analyses and reported either a positive association of indirect measures of insulin resistance and microalbuminuria or no significant association (19,20). A prospective study on the relationship between insulin sensitivity and albuminuria in healthy individuals with insulin sensitivity assessed according to the gold standard hyperinsulinemic–euglycemic clamp is, however, still missing. Such data are of interest because large meta-analyses indicate that even high normal albuminuria is associated with fatal events (2). We therefore aimed to evaluate the cross-sectional and prospective association of insulin sensitivity and albuminuria in the European Relationship between Insulin Sensitivity and Cardiovascular Disease (RISC) study (23).

RESEARCH DESIGN AND METHODS

The rationale and design of the RISC study have been described previously (23). In brief, clinically healthy study participants aged 30 to 60 years were recruited from 19 study centers in 14 European countries. Exclusion criteria included prevalent cardiovascular disease and treatment for obesity, arterial hypertension, lipid disorders, or diabetes. Further exclusion criteria were systolic/diastolic blood pressure ≥140/90 mmHg, fasting plasma glucose ≥7.0 mmol/L, 2-h plasma glucose ≥11.1 mmol/L, total cholesterol ≥7.8 mmol/L, or triglycerides ≥4.6 mmol/L. Using a standardized protocol, the baseline assessments included anthropometric and blood pressure measurement, resting electrocardiogram, collection of spot urine samples, fasting blood sampling, oral glucose tolerance test (OGTT), hyperinsulinemic–euglycemic clamp, ultrasound measurement of carotid intima-media thickness (IMT), lifestyle and medical history questionnaires, and assessment of physical activity by the use of an accelerometer. Except for the hyperinsulinemic–euglycemic clamp and the accelerometer monitoring, these examinations as well as most baseline laboratory measurements were repeated after a 3-year follow-up period. In total, more than 1,500 study participants were examined at baseline. Data on urinary albumin-to-creatinine ratio (UACR) were available in 1,415 of these study participants who were thus included in the present work. Follow-up analyses were performed in a subgroup of 852 subjects with available UACR data at the 3-year follow-up examination.

The RISC study complies with the Declaration of Helsinki, and ethical approval was obtained by the local ethics committees. All participants gave written informed consent prior to study inclusion.

Laboratory Measurements

Laboratory measurements have been previously described in detail and were performed centrally (23–26). Early morning spot urine samples were obtained for measurements of the UACR. UACR was determined on two separate days, and the mean was recorded. Urine albumin was analyzed by using microalbumin antiserum for Beckman Array systems on a Beckman Array 360 protein analyzer. When albuminuria was under the detection limit of the assay (2 mg/L), the value was set to zero. The interassay coefficient of variation was 5.0%. Urine creatinine was measured by using the Jaffé creatinine reagent (Roche) on a modular P system (Roche) with an interassay coefficient of variation <2.0%. Albuminuria was classified according to UACR (in milligrams per gram) into optimal (≤5), intermediate normal (>5.0 to 9.9), high normal (10.0 to 29.9), microalbuminuria (30 to 300), and macroalbuminuria (>300; to convert milligrams per gram to milligrams per millimole, multiply by 0.113). This classification is based on previous surveys, including large meta-analyses showing that UACR, even within the normal range, is gradually associated with increased mortality and cardiovascular disease risk (2,27).

Estimates of Insulin Sensitivity

A 2-h hyperinsulinemic–euglycemic clamp was performed to directly assess insulin sensitivity. The basic concept of this technique is that, in the hyperinsulinemic state, hepatic glucose production is suppressed so that the glucose infusion rate should be equal to the glucose disposal rate (i.e., the M value) (28,29). Under a continuous insulin infusion rate of 240 pmol/min/m^2, a variable dextrose infusion was adjusted every 5 to 10 min to maintain a target plasma glucose concentration between 4.5 and 5.5 mmol/L. Stable isotope glucose tracer was infused to allow estimation of basal and end of clamp hepatic glucose production. The M value was averaged over the last 40 min of the clamp and normalized by the fat-free mass (28,29). Insulin sensitivity was assessed as the ratio of this M value to the mean plasma insulin concentration during the final 40 min of the clamp (M/I) (28,29).

OGTT-based insulin sensitivity (OGIS) was calculated as described by Mari et al. (30). This is an indirect measurement of insulin sensitivity, and it is an estimate of the glucose clearance during the hyperinsulinemic–euglycemic clamp (30). The homeostasis model assessment of insulin resistance (HOMA-IR) was calculated as fasting glucose (millimoles per liter) × fasting insulin (milliunits per liter) divided by 22.5. This surrogate index of insulin resistance reflects primarily hepatic insulin sensitivity/resistance, while the M/I value is a direct measure of whole body glucose disposal under conditions of a hyperinsulinemic state, with subsequently suppressed hepatic glucose production (28,29).

Statistical Analysis

Clinical and biochemical characteristics at baseline and at follow-up are presented according to the following UACR categories: ≤5, >5 to 9.9, 10 to 29.9, and ≥30 mg/g. Depending on the distribution of continuous variables, data are either presented as means with SD (normally distributed variables)

or as medians with interquartile ranges (skewed variables). Categorical data are presented as percentages. Variables following a non-normal distribution were log(e) transformed before use in parametric statistical analyses. Group differences were analyzed by either ANOVA (with *P* for trend) or χ^2 test (with *P* for linear by linear trend), and Fisher exact test was used for cases when the number of observations within one cell was below five. These analyses were performed at both study visits to evaluate whether associations between UACR and cardiovascular risk factors are modified due to changing cardiovascular risk patterns from baseline to the 3-year follow-up examination.

Univariate and multiple linear regression analyses were performed to evaluate associations of baseline measures of insulin sensitivity (explanatory variable) with UACR at the 3-year follow-up examination. These analyses were cumulatively adjusted for baseline variables as indicated, including potential confounders or mediating variables. Similar linear regression analyses were performed to assess cross-sectional associations of estimates of insulin sensitivity with UACR at baseline and at follow-up. Statistical analyses were performed by SPSS (version 20.0). A *P* value below 0.05 was considered statistically significant.

RESULTS

UACR measurements were available in 1,415 participants at baseline (mean age 43.9 ± 8.3 years; 54.3% women) and in 852 participants at the 3-year follow-up examination (mean age 45.2 ± 8.2 years; 55.6% women; 60% of the initial study cohort). There were no significant differences in clinical and laboratory characteristics between study participants with and without follow-up examinations (data not shown).

Study participant characteristics according to UACR categories are shown in Table 1 for the baseline examination. Among measures of insulin sensitivity/resistance there was a significant association of OGIS with UACR categories, but there was no significant association with M/I or HOMA-IR (see Table 1). In multivariate linear regression analyses adjusted for age, sex, study center, active smokers, high-sensitivity C-reactive protein (hs-CRP), systolic blood pressure, triglycerides, LDL and HDL cholesterol, waist circumference, adiponectin, and carotid IMT, there were no significant associations of any measure of insulin sensitivity

Table 1—Baseline characteristics according to UACR categories

Characteristic	UACR category: Optimal ≤5.0	Intermediate normal >5.0 to 9.9	High normal 10.0 to 29.9	Microalbuminuria ≥30.0	P_{trend} value
UACR (mg/g)	1.4 (0.00–2.8)	6.3 (5.7–7.7)	13.9 (11.5–18.9)	61.6 (40.7–107.5)	
Number	1,186	139	66	24	
Women (%)	52.7	66.9	68.8	52.2	0.004
Age (years)	43.8 ± 8.2	44.3 ± 9.3	55.0 ± 8.4	44.0 ± 9.1	0.305
Active smoker (%)	26.2	29.9	30.0	26.1	0.440
BMI (kg/m^2)	25.2 (22.9–28.0)	24.2 (21.7–27.4)	24.0 (22.0–26.4)	26.7 (23.4–28.3)	0.062
Waist circumference (cm)	87.4 ± 12.6	84.2 ± 13.2	83.4 ± 13.9	88.8 ± 14.8	0.022
Fat mass (kg)	54.5 ± 11.6	50.9 ± 10.9	50.0 ± 9.8	55.0 ± 12.9	0.001
Average daily physical activity (counts/min)	341 (262–432)	329 (248–434)	390 (309–499)	300 (228–371)	0.659
LDL cholesterol (mmol/L)	2.9 ± 0.8	2.9 ± 0.8	2.8 ± 0.8	3.0 ± 0.8	0.585
HDL cholesterol (mmol/L)	1.4 ± 0.4	1.4 ± 0.4	1.5 ± 0.4	1.3 ± 0.4	0.677
Triglycerides (mmol/L)	0.94 (0.68–1.32)	0.86 (0.63–1.14)	0.92 (0.63–1.25)	1.20 (0.91–1.45)	0.825
Systolic blood pressure (mmHg)	118 ± 13	117 ± 14	118 ± 15	116 ± 14	0.259
Diastolic blood pressure (mmHg)	75 ± 8	73 ± 8	75 ± 9	75 ± 8	0.646
Blood pressure ≥130/85 (%)	8.2	5.8	4.7	8.7	0.312
Heart rate (bpm)	68 ± 10	68 ± 10	70 ± 11	69 ± 11	0.372
M/I (μmol/min/kg$_{ffm}$/nM)	128 (92–175)	139 (98–188)	127 (93–180)	98 (69–177)	0.977
OGIS (mL/min/m^2)	436 (396–476)	455 (414–511)	453 (417–489)	423 (364–450)	0.014
HOMA-IR	1.00 (0.66–1.53)	0.97 (0.64–1.31)	0.86 (0.60–1.38)	1.23 (0.72–2.10)	0.852
Fasting glucose (mmol/L)	5.1 (4.7–5.4)	4.9 (4.6–5.3)	5.0 (4.6–5.3)	5.5 (4.9–5.7)	0.233
2-h glucose (mmol/L)	5.9 ± 1.7	5.6 ± 1.6	5.9 ± 2.0	6.5 ± 1.6	0.554
Fasting insulin (pmol/L)	31 (21–46)	31 (21–43)	27 (21–37)	35 (24–56)	0.951
2-h insulin (pmol/L)	153 (90–256)	140 (75–241)	145 (76–261)	177 (105–369)	0.818
Carotid IMT (mm)	0.60 ± 0.09	0.61 ± 0.10	0.62 ± 0.08	0.61 ± 1.00	0.160
hs-CRP (mg/L)	0.45 (0.20–0.99)	0.45 (0.19–1.26)	0.49 (0.31–1.03)	1.17 (0.51–2.22)	0.010
Adiponectin (pmol/L)	7.69 (5.45–10.2)	8.12 (6.24–11.7)	9.23 (5.68–11.7)	6.13 (4.75–8.69)	0.513
Alanine amino transferase (IU/L)	18 (13–25)	17 (13–22)	16 (12–20)	17 (12–27)	0.003
Aspartate amino transferase (IU/L)	20 (17–25)	19 (16–24)	19 (16–25)	22 (19–28)	0.468
γ-Glutamyl transferase (IU/L)	21 (16–30)	20 (15–28)	21 (15–31)	28 (18–33)	0.849

Continuous data are presented as mean ± SD or as median (interquartile range). Categorical data are presented as percentages. Data are analyzed by ANOVA and χ^2 tests with *P* for trend or Fisher exact test.

with UACR, with β-coefficients of 0.03 (P = 0.346) for M/I, 0.06 (P = 0.086) for OGIS, and 0.05 (P = 0.158) for HOMA-IR. Using log(e) transformed values, the Pearson correlation coefficients were −0.48 ($P < 0.001$) for M/I and HOMA-IR and 0.41 ($P < 0.001$) for M/I and OGIS. Among 24 participants (1.7%) with UACR ≥30 mg/g, there was only one with macroalbuminuria (UACR >300 mg/g). Restricting this baseline analysis to study participants with available follow-up values for UACR (n = 852) also revealed, in multivariate analyses, no significant association of any measure of insulin sensitivity with UACR (data not shown).

Data for the 3-year follow-up examination were also stratified according to UACR categories and are shown in Table 2. There were only nine participants (1.1%) with UACR ≥30 mg/g, including one with macroalbuminuria. Blood pressure and carotid IMT were significantly increased at higher UACR categories, whereas heart rate was slightly decreased (see Table 2). Use of antihypertensive drugs at follow-up was reported in only 4% of the population, with the highest prevalence in those with microalbuminuria. There was, however, no significant association of UACR categories with HOMA-IR or OGIS (see Table 2). In linear regression analyses adjusted for age, sex, study center, active smokers, systolic blood pressure, triglycerides, LDL and HDL cholesterol, waist circumference, and carotid IMT, the β-coefficients for an association with UACR (dependent variable) were 0.04 (P = 0.428) for OGIS and −0.02 (P = 0.631) for HOMA-IR.

Linear regression analyses on the association of baseline measures of insulin sensitivity and follow-up UACR are shown in Table 3. In these analyses, there was no significant association with OGIS or HOMA-IR, but the M/I value emerged as independently associated with follow-up UACR (see Table 3). In a multivariate model including baseline values of M/I, UACR, age, sex, study center, active smokers, hs-CRP, systolic blood pressure, triglycerides, LDL and HDL cholesterol, waist circumference, adiponectin, and carotid IMT as explanatory variables, the significant predictors of follow-up UACR and their β-coefficients were as follows: baseline UACR (β 0.30; $P < 0.001$), M/I (β −0.14; P = 0.001), systolic blood pressure (β 0.12; P = 0.004), and triglycerides (β −0.09; P = 0.047). Adjusting for or excluding patients on antihypertensive or lipid-lowering drugs at follow-up did not meaningfully change any of our results (data not shown). We included the one patient with macroalbuminuria into our analyses, but sensitivity analyses excluding this patient did not change our findings. When recalculating our cross-sectional and prospective statistical analyses by using the M value instead of the M/I value, we obtained similar results (data not shown), and we observed a highly significant correlation between the M value and the M/I value (Pearson correlation coefficient 0.83; $P > 0.001$). All of our statistical analyses were materially unchanged

Table 2—Characteristics according to UACR categories at the 3-year follow-up visit

Characteristic	UACR category: Optimal ≤5.0	Intermediate normal >5.0 to 9.9	High normal 10.0 to 29.9	Microalbuminuria ≥30.0	P_{trend} value
UACR (mg/g)	0.0 (0.0–2.7)	6.5 (5.7–7.6)	13.7 (12.0–16.6)	56.4 (31.0–111.9)	
Number	694	95	54	9	
Women (%)	54.5	61.1	61.1	55.6	0.221
Age (years)	48.4 ± 8.3	48.4 ± 8.1	51.0 ± 7.5	48.6 ± 5.5	0.092
Active smoker (%)	18.6	20.9	25.5	44.4	0.147
BMI (kg/m^2)	25.2 (22.8–27.8)	25.3 (22.6–28.5)	25.0 (23.0–28.2)	24.5 (19.9–27.7)	0.710
Waist circumference (cm)	88.0 ± 12.4	86.3 ± 11.1	88.2 ± 12.5	80.4 ± 12.7	0.247
Fat mass (kg)	53.6 ± 11.5	52.7 ± 12.3	52.3 ± 11.5	49.3 ± 10.0	0.193
LDL cholesterol (mmol/L)	3.0 ± 0.9	3.0 ± 0.8	3.1 ± 0.9	3.3 ± 0.8	0.734
HDL cholesterol (mmol/L)	1.5 ± 0.4	1.5 ± 0.4	1.4 ± 0.4	1.4 ± 0.4	0.270
Triglycerides (mmol/L)	0.95 (0.71–1.33)	0.93 (0.64–1.32)	1.01 (0.71–1.64)	1.04 (0.74–1.45)	0.446
Lipid-lowering drugs (%)	2.2	4.3	1.9	0.0	0.467
Systolic BP (mmHg)	120 ± 15	123 ± 15	125 ± 17	120 ± 23	0.003
Diastolic BP (mmHg)	75 ± 9	78 ± 10	77 ± 9	76 ± 13	0.013
BP ≥130/85 (%)	11.1	19.4	18.9	25.0	0.008
Antihypertensive drugs (%)	3.6	4.3	3.8	37.5	0.007
Heart rate (bpm)	66 ± 9	65 ± 11	63 ± 9	63 ± 9	0.037
OGIS (mL/min/m^2)	423 (385–468)	414 (384–475)	431 (378–467)	412 (371–525)	0.702
HOMA-IR	1.04 (0.69–1.53)	1.05 (0.68–1.37)	0.95 (0.67–1.80)	1.13 (0.73–1.80)	0.692
Fasting glucose (mmol/L)	5.2 (4.9–5.7)	5.2 (4.8–5.7)	5.3 (5.0–5.6)	5.0 (4.7–5.6)	0.954
2-h glucose (mmol/L)	5.8 (4.8–6.9)	5.6 (5.0–6.7)	6.0 (5.1–7.4)	5.7 (4.3–6.5)	0.463
Fasting insulin (pmol/L)	31 (22–44)	31 (23–40)	32 (21–48)	32 (24–51)	0.645
2-h insulin (pmol/L)	154 (94–253)	150 (96–233)	178 (87–285)	141 (93–378)	0.480
Carotid IMT (mm)	0.62 ± 0.09	0.63 ± 0.09	0.67 ± 0.08	0.65 ± 1.00	<0.001

Continuous data are presented as mean ± SD or as median (interquartile range). Categorical data are presented as percentages. Data are analyzed by ANOVA and χ^2 tests with P for trend or Fisher exact test. BP, blood pressure.

Table 3—Linear regression analyses of UACR at follow-up with baseline measures of insulin sensitivity/resistance and cumulative adjustments for baseline characteristics

	β-Coefficients (*P* value)		
Adjustments	M/I	OGIS	HOMA-IR
None	−0.11 (0.002)	0.02 (0.589)	0.02 (0.517)
Baseline UACR	−0.13 (<0.001)	0.00 (0.919)	0.03 (0.410)
Model 1	−0.13 (<0.001)	0.00 (0.914)	0.02 (0.487)
Model 2	−0.12 (<0.001)	0.01 (0.853)	0.02 (0.495)
Model 3	−0.12 (<0.001)	0.02 (0.562)	0.01 (0.868)
Model 4	−0.13 (<0.001)	0.02 (0.655)	0.01 (0.825)
Model 5	−0.14 (<0.001)	0.03 (0.680)	0.01 (0.851)
Model 6	−0.14 (0.001)	0.02 (0.659)	0.00 (0.965)

Model 1 additionally adjusted for age and sex. Model 2 additionally adjusted for study center. Model 3 additionally adjusted for active smokers, hs-CRP, and systolic blood pressure. Model 4 additionally adjusted for triglycerides and LDL and HDL cholesterol. Model 5 additionally adjusted for waist circumference. Model 6 additionally adjusted for adiponectin and carotid IMT.

when men and women were analyzed separately.

CONCLUSIONS

In the RISC study among healthy middle-aged adults, we observed that reduced insulin sensitivity, as determined by a hyperinsulinemic–euglycemic clamp, was associated with a higher risk of albuminuria after 3 years of follow-up. Other measures of insulin sensitivity or insulin resistance (i.e., OGIS and HOMA-IR) were not significantly related to UACR at follow-up.

Our work is, to the best of our knowledge, the first study to investigate the relationship between insulin sensitivity, determined by the gold standard clamp method, with prospective changes in albuminuria in a large study cohort. Previous studies on insulin sensitivity and UACR have mainly categorized their study participants according to the presence of microalbuminuria, which is usually defined as an UACR of 30 to 300 mg/g (14–22). Large meta-analyses and prospective studies have, however, questioned the use of these cutoffs and suggest rather a linear relationship of UACR and clinical end points (2,27,31,32). In this context, it was documented that cardiovascular and mortality risk start to significantly increase at UACR levels as low as ~5 mg/g (27,32). It can therefore be concluded that albuminuria, even in the normal range, is independent of known risk factors and in a linear relationship associated with an increased risk of cardiovascular disease and mortality. This was the rationale for our study to explore associations of insulin sensitivity with UACR concentrations that were mainly below the classic cutoff levels for microalbuminuria. While several studies have, by the majority, reported on a significant association of insulin resistance and albuminuria in diabetic patients, this issue has only been rarely addressed in large nondiabetic or population-based cohorts (9–21). Among these studies, cross-sectional analyses of the Insulin Resistance Atherosclerosis Study in 982 nondiabetic individuals aged 40 to 69 years showed that insulin resistance was significantly associated with microalbuminuria (20). By contrast, in the Hoorn study, microalbuminuria was not statistically significantly associated with insulin resistance in 622 participants of this general older population (19). These inconsistent data on indirect measures of insulin resistance and albuminuria in general populations underline the need for our current investigation. In this context, it should also be stressed that we observed a continuous relationship between albuminuria and insulin sensitivity, suggesting that previous investigations might have been limited by strictly using certain cutoffs for albuminuria.

In our cross-sectional analyses at both the baseline and the follow-up examination, we did not, after adjustment for various covariates, observe any significant association of M/I, OGIS, or HOMA-IR with UACR, but baseline levels of M/I emerged as a strong and independent predictor of follow-up UACR. These data must be interpreted in light of the fact that we investigated a healthy population, which was carefully selected by excluding all individuals with significant cardiovascular risk factors. This, in turn, led to a very low cardiovascular risk pattern, which might have limited the power to detect significant associations of UACR with measures of insulin sensitivity. Furthermore, some expected associations of UACR with cardiovascular risk factors (e.g., blood pressure) at baseline were either missing or showed a U-shaped relationship. This might be a consequence of selection bias, e.g., by excluding hypertensive patients that would have been more prevalent in groups with higher UACR. On the other hand, the nature of our study cohort is also a strength of the current work since we were able to evaluate very early stages of albuminuria in a cohort with a low risk of confounding comorbidities. In this context, the significant association of blood pressure with follow-up UACR in our study is an important confirmation of existing knowledge. The fact that this association of blood pressure and UACR was not apparent at baseline but became significant in our prospective and follow-up analyses further underlines that our study population is indeed reliable to investigate the initial pathogenesis of albuminuria.

Our finding that low insulin sensitivity, determined by the M/I value, predicts progression of UACR levels may reflect key processes related to the initial pathogenesis of albuminuria. From a pathophysiological point of view, this makes sense because podocytes of the glomerular filtration barrier are insulin-sensitive cells (10–13). With reference to this, studies in mice suggest that insulin resistance of podocytes increases their susceptibility to cell death and may thus contribute to early diabetic nephropathy (11,33). Various other mechanisms may hypothetically mediate the association of insulin resistance and albuminuria and include, for example, decreased expression of nephrin, which is important for the barrier function of podocytes, increased salt sensitivity, or hyperinsulinemia, which may cause glomerular hyperfiltration and compromised nitric oxide production with endothelial dysfunction (5–13). In line with this, it has been shown that thiazolidinediones, which improve insulin sensitivity, also decrease albuminuria (34). Our findings support this notion that improving

insulin sensitivity might also reduce or prevent albuminuria, in particular, at very early stages. This is of clinical interest when considering the role of albuminuria as a cardiovascular risk factor that is closely related to endothelial dysfunction and predicts cardiovascular outcome and mortality in diabetic as well as nondiabetic populations (2,3,6,8,31,35). In this context, our work also supports the link between insulin resistance and endothelial dysfunction because previous data suggest that albuminuria may indicate a specific form of endothelial dysfunction (6–9). In general, there exists a reciprocal relationship between insulin resistance and endothelial dysfunction, with our data supporting the notion that insulin resistance precedes and may probably contribute to endothelial dysfunction (9). On the other hand, we cannot exclude the possibility that both conditions occur simply in parallel as a consequence of similar vascular and metabolic deteriorations or that endothelial dysfunction may itself cause microalbuminuria (6–9).

Apart from this, we must note that in contrast to the association of M/I with the progression of albuminuria, there were no significant relationships between baseline OGIS or HOMA-IR and prospective changes in UACR. These inconsistent associations may reflect differences or advantages of the gold standard method for assessment of insulin sensitivity, the hyperinsulinemic–euglycemic clamp technique, in comparison with indirect measures such as OGIS or HOMA-IR (28,29). In particular HOMA-IR is considered a poor measure of peripheral insulin sensitivity as shown by Pisprasert et al. (36).

Our results are limited by the fact that the range of UACR in this initially healthy population was limited and mainly below the range for microalbuminuria. Although there were no meaningful differences between people who participated in the follow-up visit and those who were lost to follow-up (data not shown), we cannot rule out selection bias for our follow-up data. Considering that we studied a healthy cohort, our findings may not be fully generalizable to other populations, including patients with diabetes. Furthermore, our observational study design does not allow drawing definite conclusions regarding causality. Another limitation of our work is the short follow-up of 3 years in a relatively young population, because a stronger relationship between albuminuria and insulin sensitivity might have emerged after a longer follow-up time. Finally, we cannot rule out residual confounding as well as overadjustments or underadjustments of our multivariate statistical analyses, but we have to note that the association between M/I and follow-up UACR remained materially unchanged throughout all statistical models (see Table 3).

In summary, reduced insulin sensitivity, measured by a hyperinsulinemic–euglycemic clamp, predicts follow-up albuminuria in a healthy cohort with UACR mainly below the cutoff for microalbuminuria. These data may suggest that reduced insulin sensitivity is related to the initial pathogenesis of albuminuria. It remains, however, to be clarified whether insulin resistance and albuminuria emerge in parallel as a consequence of a common pathogenic pathway (e.g., endothelial dysfunction) or whether insulin resistance is a causal factor for the pathogenesis of albuminuria. Further studies are therefore needed to confirm our findings and to evaluate whether improving insulin sensitivity impacts on the initiation and progression of albuminuria.

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Author Contributions. S.P. wrote the manuscript and performed statistical analyses. F.R. contributed to the discussion and reviewed and edited the manuscript. G.N., C.D.A.S., K.H., J.J.N., B.B., and J.M.D. researched data, contributed to the discussion, and reviewed and edited the manuscript. S.P. is the guarantor of this work and, as such, had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis.

Appendix

RISC Study Recruiting Centers. Amsterdam, the Netherlands: R.J. Heine, J.M. Dekker, S. de Rooij, G. Nijpels, and W. Boorsma; Athens, Greece: A. Mitrakou, S. Tournis, K. Kyriakopoulou, and P. Thomakos; Belgrade, Serbia and Montenegro: N. Lalic, K. Lalic, A. Jotic, L. Lukic, and M. Civcic; Dublin, Ireland: J.J. Nolan, T.P. Yeow, M. Murphy, C. DeLong, G. Neary, M.P. Colgan, and M. Hatunic; Frankfurt, Germany: T. Konrad, H. Böhles, S. Fuellert, F. Baer, and H. Zuchhold; Geneva, Switzerland: A. Golay, E. Harsch Bobbioni, V. Barthassat, V. Makoundou, T.N.O. Lehmann, and T. Merminod; Glasgow, Scotland: J.R. Petrie, C. Perry, F. Neary, C. MacDougall, K. Shields, and L. Malcolm; Kuopio, Finland: M. Laakso, U. Salmenniemi, A. Aura, R. Raisanen, U. Ruotsalainen, T. Sistonen, M. Laitinen, and H. Saloranta; London, England: S.W. Coppack, N. McIntosh, J. Ross, L. Pettersson, and P. Khadobaksh; Lyon, France: M. Laville, F. Bonnet, A. Brac de la Perriere, C. Louche-Pelissier, C. Maitrepierre, J. Peyrat, S. Beltran, and A. Serusclat; Madrid, Spain: R. Gabriel, E.M. Sanchez, R. Carraro, A. Friera, and B. Novella; Malmö, Sweden: *1*) P. Nilsson, M. Persson, and G. Östling and *2*) O. Melander and P. Burri; Milan, Italy: P.M. Piatti, L.D. Monti, E. Setola, E. Galluccio, F. Minicucci, and A. Colleluori; Newcastle-upon-Tyne, England: M. Walker, I.M. Ibrahim, M. Jayapaul, D. Carman, C. Ryan, K. Short, Y. McGrady, and D. Richardson; Odense, Denmark: H. Beck-Nielsen, P. Staehr, K. Hojlund, V. Vestergaard, C. Olsen, and L. Hansen; Perugia, Italy: G.B. Bolli, F. Porcellati, C. Fanelli, P. Lucidi, F. Calcinaro, and A. Saturni; Pisa, Italy: E. Ferrannini, A. Natali, E. Muscelli, S. Pinnola, and M. Kozakova; Rome, Italy: G. Mingrone, C. Guidone, A. Favuzzi, and P. Di Rocco; Vienna, Austria: C. Anderwald, M. Bischof, M. Promintzer, M. Krebs, M. Mandl, A. Hofer, A. Luger, W. Waldhäusl, and M. Roden.

Project Management Board. B. Balkau (Villejuif, France), S.W. Coppack (London, England), J.M. Dekker (Amsterdam, the Netherlands), E. Ferrannini (Pisa, Italy), A. Mari (Padova, Italy), A. Natali (Pisa, Italy), M. Walker (Newcastle, England).

Core Laboratories and Reading Centers. Lipids, Dublin, Ireland: P. Gaffney, J.J. Nolan, and G. Boran; hormones, Odense, Denmark: C. Olsen, L. Hansen, and H. Beck-Nielsen; albumin:creatinine, Amsterdam, the Netherlands: A. Kok and J. Dekker; genetics, Newcastle-upon-Tyne, England: S. Patel and M. Walker; stable isotope laboratory, Pisa, Italy: A. Gastaldelli and D. Ciociaro; ultrasound reading center, Pisa, Italy: M. Kozakova; electrocardiogram reading, Villejuif, France: M.T. Guillanneuf; data management, Villejuif, France: B.B. and L. Mhamdi; data management, Padova, Italy: A. Mari; data management, Pisa, Italy: L. Mota; mathematical modeling and website management, Padova, Italy: A. Mari, G. Pacini, and C. Cavaggion; coordinating office, Pisa, Italy: S.A. Hills, L. Landucci, and L. Mota.

Further information on the RISC study and participating centers can be found on www.egir.org.

References

1. Basi S, Fesler P, Mimran A, Lewis JB. Microalbuminuria in type 2 diabetes and hypertension: a marker, treatment target, or innocent bystander? Diabetes Care 2008;31(Suppl. 2): S194–S201

2. Matsushita K, van der Velde M, Astor BC, et al.; Chronic Kidney Disease Prognosis Consortium. Association of estimated glomerular filtration rate and albuminuria with all-cause and

cardiovascular mortality in general population cohorts: a collaborative meta-analysis. Lancet 2010;375:2073–2081
3. Redon J, Martinez F. Microalbuminuria as surrogate endpoint in therapeutic trials. Curr Hypertens Rep 2012;14:345–349
4. Malik AR, Sultan S, Turner ST, Kullo IJ. Urinary albumin excretion is associated with impaired flow- and nitroglycerin-mediated brachial artery dilatation in hypertensive adults. J Hum Hypertens 2007;21:231–238
5. Weir MR. Microalbuminuria and cardiovascular disease. Clin J Am Soc Nephrol 2007;2: 581–590
6. Stehouwer CD, Yudkin JS, Fioretto P, Nosadini R. How heterogeneous is microalbuminuria in diabetes mellitus? The case for 'benign' and 'malignant' microalbuminuria. Nephrol Dial Transplant 1998;13:2751–2754
7. Stehouwer CD, Smulders YM. Microalbuminuria and risk for cardiovascular disease: Analysis of potential mechanisms. J Am Soc Nephrol 2006;17:2106–2111
8. Bornfeldt KE, Tabas I. Insulin resistance, hyperglycemia, and atherosclerosis. Cell Metab 2011;14:575–585
9. Kim JA, Montagnani M, Koh KK, Quon MJ. Reciprocal relationships between insulin resistance and endothelial dysfunction: molecular and pathophysiological mechanisms. Circulation 2006;113:1888–1904
10. El-Atat FA, Stas SN, McFarlane SI, Sowers JR. The relationship between hyperinsulinemia, hypertension and progressive renal disease. J Am Soc Nephrol 2004;15:2816–2827
11. Welsh GI, Coward RJ. Podocytes, glucose and insulin. Curr Opin Nephrol Hypertens 2010;19:379–384
12. De Cosmo S, Menzaghi C, Prudente S, Trischitta V. Role of insulin resistance in kidney dysfunction: insights into the mechanism and epidemiological evidence. Nephrol Dial Transplant 2013;28:29–36
13. Jauregui A, Mintz DH, Mundel P, Fornoni A. Role of altered insulin signaling pathways in the pathogenesis of podocyte malfunction and microalbuminuria. Curr Opin Nephrol Hypertens 2009;18:539–545
14. Hsu CC, Chang HY, Huang MC, et al. Association between insulin resistance and development of microalbuminuria in type 2 diabetes: a prospective cohort study. Diabetes Care 2011;34:982–987
15. Parvanova AI, Trevisan R, Iliev IP, et al. Insulin resistance and microalbuminuria: a cross-sectional, case-control study of 158 patients with type 2 diabetes and different degrees of urinary albumin excretion. Diabetes 2006;55: 1456–1462
16. De Cosmo S, Minenna A, Ludovico O, et al. Increased urinary albumin excretion, insulin resistance, and related cardiovascular risk factors in patients with type 2 diabetes: evidence of a sex-specific association. Diabetes Care 2005;28: 910–915
17. Nielsen S, Schmitz O, Orskov H, Mogensen CE. Similar insulin sensitivity in NIDDM patients with normo- and microalbuminuria. Diabetes Care 1995;18:834–842
18. Mottl AK, Lauer A, Dabelea D, et al.; SEARCH for Diabetes in Youth Study Group. Albuminuria according to status of autoimmunity and insulin sensitivity among youth with type 1 and type 2 diabetes. Diabetes Care 2013;36:3633–3638
19. Jager A, Kostense PJ, Nijpels G, Heine RJ, Bouter LM, Stehouwer CD. Microalbuminuria is strongly associated with NIDDM and hypertension, but not with the insulin resistance syndrome: the Hoorn Study. Diabetologia 1998;41:694–700
20. Mykkänen L, Zaccaro DJ, Wagenknecht LE, Robbins DC, Gabriel M, Haffner SM. Microalbuminuria is associated with insulin resistance in nondiabetic subjects: the insulin resistance atherosclerosis study. Diabetes 1998;47:793–800
21. Forsblom CM, Eriksson JG, Ekstrand AV, Teppo AM, Taskinen MR, Groop LC. Insulin resistance and abnormal albumin excretion in non-diabetic first-degree relatives of patients with NIDDM. Diabetologia 1995;38:363–369
22. Toft I, Bønaa KH, Eikrem J, Bendiksen AL, Iversen H, Jenssen T. Microalbuminuria in hypertension is not a determinant of insulin resistance. Kidney Int 2002;61:1445–1452
23. Hills SA, Balkau B, Coppack SW, et al.; EGIR-RISC Study Group. The EGIR-RISC STUDY (The European group for the study of insulin resistance: relationship between insulin sensitivity and cardiovascular disease risk): I. Methodology and objectives. Diabetologia 2004;47:566–570
24. Mari A, Tura A, Natali A, et al.; RISC Investigators. Influence of hyperinsulinemia and insulin resistance on in vivo β-cell function: their role in human β-cell dysfunction. Diabetes 2011;60:3141–3147
25. de Rooij SR, Dekker JM, Kozakova M, et al.; RISC Group Investigators. Fasting insulin has a stronger association with an adverse cardiometabolic risk profile than insulin resistance: the RISC study. Eur J Endocrinol 2009;161:223–230
26. Kozakova M, Palombo C, Eng MP, et al.; RISC Investigators. Fatty liver index, gamma-glutamyltransferase, and early carotid plaques. Hepatology 2012;55:1406–1415
27. Blecker S, Matsushita K, Köttgen A, et al. High-normal albuminuria and risk of heart failure in the community. Am J Kidney Dis 2011;58: 47–55
28. Muniyappa R, Lee S, Chen H, Quon MJ. Current approaches for assessing insulin sensitivity and resistance in vivo: advantages, limitations, and appropriate usage. Am J Physiol Endocrinol Metab 2008;294:E15–E26
29. Ferrannini E, Mari A. How to measure insulin sensitivity. J Hypertens 1998;16:895–906
30. Mari A, Pacini G, Murphy E, Ludvik B, Nolan JJ. A model-based method for assessing insulin sensitivity from the oral glucose tolerance test. Diabetes Care 2001;24:539–548
31. Fox CS, Matsushita K, Woodward M, et al.; Chronic Kidney Disease Prognosis Consortium. Associations of kidney disease measures with mortality and end-stage renal disease in individuals with and without diabetes: a meta-analysis. Lancet 2012;380:1662–1673
32. Xu J, Knowler WC, Devereux RB, et al. Albuminuria within the "normal" range and risk of cardiovascular disease and death in American Indians: the Strong Heart Study. Am J Kidney Dis 2007;49:208–216
33. Tejada T, Catanuto P, Ijaz A, et al. Failure to phosphorylate AKT in podocytes from mice with early diabetic nephropathy promotes cell death. Kidney Int 2008;73:1385–1393
34. Sarafidis PA, Stafylas PC, Georgianos PI, Saratzis AN, Lasaridis AN. Effect of thiazolidinediones on albuminuria and proteinuria in diabetes: a meta-analysis. Am J Kidney Dis 2010;55:835–847
35. Groop PH, Thomas MC, Moran JL, et al.; FinnDiane Study Group. The presence and severity of chronic kidney disease predicts all-cause mortality in type 1 diabetes. Diabetes 2009;58:1651–1658
36. Pisprasert V, Ingram KH, Lopez-Davila MF, Munoz AJ, Garvey WT. Limitations in the use of indices using glucose and insulin levels to predict insulin sensitivity: impact of race and gender and superiority of the indices derived from oral glucose tolerance test in African Americans. Diabetes Care 2013;36:845–853