Insulin sensitivity measured with euglycemic clamp is independently associated with glomerular filtration rate in a community-based cohort

Elisabet Nerpin, MMed, Ulf Risérus, MMed, PhD, Erik Ingelsson, MD, PhD, Johan Sundström, MD, PhD, Magnus Jobs, MSc, PhD, Anders Larsson, MD, PhD, Samar Basu, MSc PhD, and Johan Årnlöv, MD, PhD

From the Department of Public Health and Caring Sciences/Geriatrics (E.I., J.Ä.) and Section of Clinical Nutrition (U.R, S.B.), Department of Medical Sciences (J.S.,A.L.) Uppsala University, Uppsala, Sweden and the Department of Health and Social Sciences, Högskolan Dalarna, Falun, Sweden (E.N., M.J., J.Ä.)

Correspondence to: Johan Årnlöv, Department of Public Health and Caring Sciences/Geriatrics, Uppsala Science Park, SE-75185 Uppsala, Sweden E-mail: johan.arnlov@pubcare.uu.se

Running title: Insulin sensitivity and renal function

Received 20 February 2008 and accepted 15 May 2008.
**Objective:** To investigate the association between insulin sensitivity and glomerular filtration rate (GFR) in the community, with pre-specified subgroup analyses in normoglycemic individuals with normal GFR.

**Research Design and Methods:** We investigated the cross-sectional association between insulin sensitivity (M/I, assessed using euglycemic clamp) and cystatin C-based GFR in a community-based cohort of elderly men (Uppsala Longitudinal Study of Adult Men, ULSAM; n=1070). We also investigated whether insulin sensitivity predicted the incidence of renal dysfunction at a follow-up examination after 7 years.

**Results:** Insulin sensitivity was directly related to GFR (multivariable-adjusted regression coefficient for 1-unit higher M/I 1.19, 95% CI 0.69-1.68, p<0.001) after adjusting for age, glucometabolic variables (fasting plasma glucose, fasting plasma insulin, 2-hour glucose after an oral glucose tolerance test), cardiovascular risk factors (hypertension, dyslipidemia, smoking), and lifestyle factors (BMI, physical activity, consumption of tea, coffee and alcohol). The positive multivariable-adjusted association between insulin sensitivity and GFR remained statistically significant also in participants with normal fasting plasma glucose, normal glucose tolerance and normal GFR (n=443, p<0.02). In longitudinal analyses, higher insulin sensitivity at baseline was associated with lower risk of impaired renal function (GFR<50ml/min/1.73 m$^2$) during follow-up independently of glucometabolic variables (multivariable-adjusted odds ratio for 1-unit higher of M/I 0.58, 95% CI 0.40-0.84, p<0.004).

**Conclusion:** Our data suggest that impaired insulin sensitivity may be involved in the development of renal dysfunction at an early stage, prior to the onset of diabetes or pre-diabetic glucose elevations. Further studies are needed in order to establish causality.
Insulin sensitivity and renal function

Reduced insulin sensitivity is a key component in the pathogenesis of diabetes, and diabetic nephropathy is a leading cause of end-stage renal disease (ESRD)(1). However, lower insulin sensitivity has also been suggested to be associated with impaired renal function in individuals without overt diabetes (2). For instance, insulin resistance has been shown to predict ESRD in patients with mild renal impairment due to IgA-nephritis (3). Furthermore, the opposite chain of events has also been observed, patients with ESRD without diabetes have been shown to develop insulin resistance in the later stage of the disease (3; 4). Based on previous data, we hypothesized that reduced insulin sensitivity could be involved in the development of renal dysfunction via pathways that are not primarily mediated via increased glucose levels.

We are aware of a few previous community-based studies that have reported the association of reduced insulin sensitivity to diminished renal function(2; 5; 6). These studies have however been limited by the use of surrogate markers of insulin sensitivity or by the use of creatinine-based glomerular filtration rate (GFR). Furthermore, all previous studies have included patients with impaired fasting glucose and impaired glucose tolerance, making it difficult to fully evaluate whether the association between insulin sensitivity and GFR is independent of elevated fasting and post-load glucose levels. Moreover, most previous studies (2; 5) have included patients with impaired fasting glucose and impaired glucose tolerance, making it difficult to fully evaluate whether the association between insulin sensitivity and GFR is independent of elevated fasting and post-load glucose levels.

Thus, we investigated the association between insulin sensitivity, evaluated by euglycemic clamp, and cystatin C-based GFR in a community-based cohort of elderly men with pre-specified sub-group analyses in individuals with normal fasting glucose, normal glucose tolerance and normal GFR. We also investigated the longitudinal association between insulin sensitivity and renal dysfunction during follow-up and evaluated whether this association was independent of other glucometabolic factors.

RESEARCH DESIGN AND METHODS

**Study sample:** The design and selection criteria of the Uppsala Longitudinal Study of Adult Men (ULSAM) have been described previously (7) and further details can be found on the Internet (http://www.pubcare.uu.se/ULSAM/). At the third examination cycle (1991-1995), 1221 men (mean age 71 years) were investigated. We excluded 151 men for the following reasons: unavailable clamp data (n=61), unavailable cystatin C data (n=26); hospitalization for kidney failure prior to the baseline (n=4) and use of diabetes medicine (n=60). Thus, the present study sample comprised 1070 individuals. We also performed analyses in participants with normal fasting glucose and glucose tolerance (n=517) and participants with normal fasting glucose and glucose tolerance, and normal GFR (>50 ml/min/1.73m^2, n=433). Follow-up data at the fourth examination cycle (1998-2002, mean age 77) was available in 694 participants. All participants gave written informed consent and the Ethics Committee of Uppsala University approved the study protocol.

**Clinical and biochemical evaluation at baseline:** Serum cystatin C was measured by latex enhanced reagent (N Latex Cystatin C, Siemens) using a BN ProSpec analyzer (Siemens). GFR was calculated from serum cystatin C results in mg/L by the formula $y = 77.24x^{-1.2623}$ which have been shown to be closely correlated with iohexol clearance(8).

The euglycemic hyperinsulinemic clamp technique according to DeFronzo (9) was used, with a slight modification to suppress hepatic glucose production (10), for
estimation of in vivo sensitivity to insulin. Insulin (Actrapid Human®, Novo, Copenhagen, Denmark) was infused in a primary dose for the first 10 min and then as a continuous infusion (56 mU/min per body surface area (m²), where DeFronzo(9) used 40 mU/min per body surface area (m²)) for two hours to maintain steady state hyperinsulinemia. The target plasma glucose level was 5.1 mmol/L, maintained by measuring plasma glucose every five minutes. The glucose infusion rate during the last hour was used as a measure of insulin sensitivity (M-value). The insulin sensitivity index (M/I ratio) was calculated by dividing M by the mean insulin concentration during the same period of the clamp. M/I thus represents the amount of glucose metabolized per unit of plasma insulin.

An oral glucose tolerance test (OGTT) was performed where subjects ingested 75 g glucose dissolved in 300 ml of water, and blood samples for plasma glucose and insulin were drawn immediately before, and 30, 60, 90, and 120 min after ingestion of glucose. Plasma glucose was measured by the glucose dehydrogenase method (Gluc-DH; Merck, Darmstadt, Germany). Plasma insulin was assayed using an enzymatic-immunological assay (Enzymmun, Boehringer Mannheim, Germany) performed in an ES300 automatic analyzer (Boehringer Mannheim).

Urinary albumin excretion rate was calculated on the amount of albumin in the urine collected during the night. The subjects were instructed to void immediately before going to bed and to record the time. All samples during the night and the first sample of urine after rising were collected and used for the analysis (Albumin RIA 100, Pharmacia, Uppsala, Sweden).

Coffee, tea and alcohol consumption were recorded using a 7-day pre-coded food diary after instructions of a dietician. Daily intakes were calculated using a computer program and the Swedish National Food Administration database (SLV Database, 1990). Participants reported leisure-time physical activity on a standardized questionnaire (7). Smoking status was based on interview reports performed by a nurse.

Diabetes mellitus was diagnosed as fasting plasma glucose ≥7.0 mmol/l (≥126 mg/dl), 2 h post-load glucose level >11.1 mmol/l (>200 mg/dl), or by the use of oral hypoglycaemic agents or insulin. Impaired glucose tolerance was defined as a 2 h post-load value of 7.8-11 mmol/l (140-199 mg/dl). Impaired fasting glucose was defined as fasting plasma glucose of 5.6-6.9 mmol/l (100-125 mg/dl). Body mass index (BMI) was calculated as body weight/(body height)² (kg/m²). Hypertension was defined as use of antihypertensive medication or a systolic blood pressure ≥140 mm Hg or diastolic blood pressure ≥90 mm Hg. Dyslipidemia was defined as total cholesterol ≥5 mmol/l (>200 mg/dl) or high-density lipoprotein cholesterol ≤1.0 mmol/l (<40 mg/dl) or the use of lipid-lowering medication.

Follow-up and outcome: Renal impairment during follow-up was defined as having a GFR <50ml/min/1.73m² at the fourth examination cycle (after approximately 7 years) or being hospitalized for renal failure during follow-up. We defined renal dysfunction as GFR <50ml/min/1.73m² according to the current definition used in clinical practice in Sweden for individuals older than 50 years. Subjects who were hospitalized for renal failure were identified by the Swedish hospital discharge register using the following international classification of disease (ICD) codes: renal failure; 584-588 (ICD-9), N17-N19 (ICD-10).

Statistical analysis: If necessary, logarithmic transformation was performed to achieve normal distribution (fasting plasma glucose, 2-hour plasma-glucose and fasting plasma insulin and 2-hour plasma insulin).

Linear regression analyses were used to assess the cross-sectional associations
between insulin sensitivity index (M/I; independent variable) and cystatin C-based GFR (dependent variable). The following models were used:

- Model A: age-adjusted;
- Model B (Glucometabolic model): adjusted for age, fasting plasma glucose, fasting plasma insulin and 2-hour glucose;
- Model C (Cardiovascular risk factor model): adjusted for age, hypertension, dyslipidemia, and smoking;
- Model D (Lifestyle model): adjusted for age, BMI, physical activity and consumption of tea, coffee and alcohol;
- Model E (Combined model): adjusted for covariates in model A-D.

We also performed the above analyses in the following pre-specified subgroups: 1) normal fasting glucose and normal glucose tolerance (n=517); 2) normal fasting glucose and normal glucose tolerance, and normal GFR (>50 ml/min /1.73m², n=433). In secondary analyses, the separate addition of urinary albumin excretion rate (modeled as normoalbuminuria [<20 μg/min], microalbuminuria [20-200μg/min] or macroalbuminuria [>200 μg/min]), serum triglycerides and 2-hour plasma insulin at the OGTT to multivariable model E were also investigated. We also exchanged the hypertension- and dyslipidemia-variables in model C to systolic and diastolic blood pressure and use of alpha-blockers, beta blockers, ACE-inhibitors, diuretics, calcium antagonist and to total cholesterol, HDL cholesterol and use of lipid lowering medication, respectively. Moreover, we also investigated the association of insulin sensitivity to creatinine-based GFR (Modification of Diet in Renal Disease, MDRD)(11) Also, we investigated the association between the glucose disposal rate (M) and GFR and the association between 2-hour plasma insulin (as a marker for daylong hyperinsulinemia) and GFR. The potential non-linearity of the association between M/I and GFR was investigated using penalized regression splines.

Logistic regression was used to relate insulin sensitivity to renal dysfunction during follow-up. In these analyses, 108 participants with impaired GFR at baseline (<50ml/min/1.73m²) were excluded, which leaves 586 participants with longitudinal data. Given the moderate sample size and the low number of participants that developed renal dysfunction during follow-up (n=32), we limited the multivariable modeling to the above models A and B. We also performed these longitudinal analyses in participants with normal fasting glucose and normal glucose tolerance. In secondary analyses, we added baseline GFR to the longitudinal multivariable logistic regression models. A two-sided p-value <0.05 was regarded as significant in all analyses. The statistical software package STATA 10.0 (Stata Corp College Station, TX, USA) was used for all analyses.

RESULTS

Baseline characteristics: Baseline characteristics of the study population and the different sub-samples are presented in Table 1.

Cross-sectional association between insulin sensitivity and GFR

In the whole cohort, 1 unit higher of M/I was significantly associated with 0.85-1.19 ml/min/1.73m² higher GFR adjusting for age (model A), glucometabolic variables (model B), cardiovascular risk factors (model C), lifestyle factors (model D), and the combination of all covariates in models A-D (model E, Table 2). In participants with normal fasting glucose and normal glucose tolerance, the positive association between insulin sensitivity and GFR remained essentially the same in all models (models A-
After further exclusion of participants with impaired GFR (<50 ml/min/1.73 m$^2$) the association between insulin sensitivity and GFR remained statistically significant in all models but with lower regression coefficients (Table 2).

In secondary analyses, the association between insulin sensitivity and GFR was not substantially altered after further adjustment for urinary albumin excretion rate, serum triglycerides or 2-hour plasma insulin at an OGTT, or when exchanging the hypertension variable in model C for systolic and diastolic blood pressure, use of alpha-blockers, beta blockers, ACE-inhibitors, diuretics and calcium antagonist, or the dyslipidemia variable for total cholesterol, HDL-cholesterol and use of lipid lowering medication (data not shown). The association of insulin sensitivity to creatinine-based GFR was essentially similar as compared to the association of insulin sensitivity and cystatin C-based GFR (Model E, $\beta$-coefficient 1.05 95% CI (0.61-1.50), p<0.001). The association between M and GFR was similar as the association between M/I and GFR (Model E, $\beta$-coefficient 1.09 95% CI (0.44-1.75), p<0.001). No deviation from linearity was detected in the association of M/I and GFR as evaluated by regression splines. Moreover, 2-hour plasma insulin was inversely associated with GFR in all multivariable models (A-E, p<0.001 for all) as well as after adding M/I to multivariable model E ($\beta$-coefficient -2.07, 95% CI -3.95-(-0.19), p=0.03).

**CONCLUSIONS**

We identified a significant positive association between insulin sensitivity and GFR in a community-based sample of elderly men. This association was consistent also in participants with normal fasting glucose, normal glucose tolerance and normal renal function and after taking glucometabolic variables, cardiovascular risk factors and lifestyle factors into account in multivariable analyses. Moreover, in longitudinal analyses, insulin sensitivity at baseline predicted subsequent renal dysfunction independently of other glucometabolic variables. Our data suggest that the association between impaired insulin sensitivity and lower GFR is not primarily mediated via elevated glucose levels and that this association is evident already in individuals without any clinical signs of kidney dysfunction or glucose dysregulation.

**Comparison with the literature:** Our findings are in accordance with previous community based studies that have
investigated the cross-sectional association of insulin sensitivity and GFR (2; 5; 6). In these studies, decreased insulin sensitivity (assessed by serum insulin levels or HOMA insulin sensitivity) was associated with impaired renal function. However, both fasting insulin and HOMA insulin sensitivity are limited as indicators of insulin sensitivity because they are also highly influenced by the individual’s beta cell function, i.e. insulin secretion. The association between insulin sensitivity as evaluated by the gold standard euglycaemic clamp technique and GFR has not been reported previously. Furthermore, no previous studies have analyzed this association in individuals with normal glucose levels and normal GFR.

We are aware of one previous study that has evaluated the longitudinal association between insulin sensitivity and incidence of renal dysfunction. In contrast to the present study, Fox and co-workers reported that HOMA insulin resistance did not significantly predict renal dysfunction in participants with normal glucose levels (12). The discrepant results could perhaps be explained by differences between the studies in the assessment of insulin sensitivity or that our study sample consisted exclusively of elderly men. However, no firm conclusions should be drawn from the present study with regard to the longitudinal association between insulin sensitivity and incident renal impairment due to the moderate number of events during follow-up, particularly in the sub-sample with normal glucose levels. Further studies are needed in order to shed further light on this issue.

**Possible mechanisms for observed associations:** Impaired insulin sensitivity and compensatory hyperinsulinemia have been suggested to contribute to development of renal injury via a number of different pathophysiologic pathways: Insulin per se stimulates the expression and activation of insulin-like growth factor 1 (IGF-1), transforming growth factor beta (TGF-β), endothelin-1 and components of the renin-angiotensin-aldosterone system (13). These factors have been shown to promote mitogenic and fibrotic processes in the kidney, such as proliferation of mesangial cells and extracellular matrix expansion (13). Moreover, insulin resistance and hyperinsulinemia is closely associated with oxidative stress (14), which could promote renal injury via decreased production and availability of nitric oxide (15), accelerated formation of glycooxidation and lipid peroxidation products (16-18). Also, insulin resistance is linked to increased activity of pro-inflammatory cytokines and adipokines, factors that have been suggested to contribute to the progression of renal disease (19). There is also data suggesting that renal insufficiency suppresses renal clearance of insulin which leads to higher circulating levels of insulin and thus further stimulates the deleterious effect of insulin on the kidney, i.e. leading to a vicious circle (20). We are not able to fully evaluate the potential influence of reduced renal clearance of insulin in our study, but the strong positive association between the glucose disposal rate (M) and GFR as well as the similar results in individuals with normal renal function indicates that the potential impact of a reduced renal clearance of insulin on the present results is not likely to be major. The fact that the association between insulin sensitivity and GFR remained robust after adjustment for both fasting and 2-hour post load insulin would argue against hyperinsulinemia as the sole explanation of our findings. Also, as 2-hour post load insulin was associated with GFR independently of insulin sensitivity and all potential confounders in model E it is possible that daylong hyperinsulinemia per se independently contributes to a reduced GFR. Since these two conditions are so closely interrelated, it is not possible to fully disentangle the individual contribution of
Insulin sensitivity and renal function

hyperinsulinemia and insulin resistance to GFR in the present study.

There are also some other potential mechanisms that merit discussion: Reduced insulin sensitivity may lead to diabetes, which is one of the leading causes of renal failure. However, as insulin sensitivity was associated with GFR in individuals with otherwise normal glucose metabolism after adjustment for both fasting and post-load glucose levels, higher glucose levels is not a likely explanation for the results in the present study. Also, insulin sensitivity is associated with several glucometabolic factors, cardiovascular risk factors and lifestyle factors that have been shown to be associated with a reduced GFR and the development of chronic kidney disease (2; 12; 21-23). The fact that insulin sensitivity remained significantly associated with GFR in all multivariable models suggest that confounding by these factors does not explain our findings.

Clinical implementations: While the cross-sectional regression coefficients suggest that the magnitude of the association between insulin sensitivity and GFR could be modest, the odds ratios from the longitudinal logistic regression analyses imply that the potential impact of lower insulin sensitivity on the development of renal dysfunction over time could be substantial. Since no firm conclusions regarding causality and effect size should be drawn from observational data, intervention trials are needed to properly investigate these issues.

Strengths and limitations: The strengths of our investigation include the large, homogenous, community-based study sample with detailed characterization of glucometabolic variables, cardiovascular risk factors, and lifestyle factors. Moreover, the ULSAM-cohort is the largest cohort in the world examined with the euglycaemic clamp technique. Furthermore, serum levels of cystatin C was used to estimate GFR, which is considered to be a more reliable indicator of kidney function than creatinine-based GFR, particularly in the elderly (24).

Limitations include the unknown generalizability to women and other age and ethnic groups. Also, multiple statistical analyses in several different subgroups were performed. Yet, the consistency of results across all models and sub-sample makes it unlikely that the observed associations arose by chance due to multiple testing. Moreover, we have not used the gold standard method to measure GFR (isotope clearance measurements). However, isotope clearance measurements are seldom used in epidemiological research as it is a very time-consuming and costly procedure. Importantly, cystatin C-based GFR have been shown to be closely correlated with GFR assessed by isotope clearance measurements (8).

In summary, in a community-based sample of elderly men, lower insulin sensitivity was associated with lower renal function, even in individuals with normal glucose levels and normal GFR. Our data suggest that impaired insulin sensitivity may be involved in the development of renal dysfunction at an early stage, prior to the onset of diabetes or pre-diabetic glucose elevations. Further studies are needed to establish causality and to evaluate the clinical implication of our findings.

ACKNOWLEDGEMENTS

This study was supported by The Swedish Research Council (2006-6555), Swedish Heart-Lung foundation, Erik, Karin och Gösta Selanders foundation, Loo och Hans Ostermans foundation, Stiftelsen Sigurd och Elsa Goljes Minne, Högskolan Dalarna and Uppsala University. The funding sources did not play any role in the design and conduct of the study; collection, management, analysis, and interpretation of the data; and preparation, review, or approval of the
manuscript. There are no conflicts of interest for any of the authors.

REFERENCES
1. Muntner P, He J, Hamm L, Loria C, Whelton PK: Renal insufficiency and subsequent death resulting from cardiovascular disease in the United States. J Am Soc Nephrol 13:745-753, 2002
2. Chen J, Muntner P, Hamm LL, Fonseca V, Batuman V, Whelton PK, He J: Insulin resistance and risk of chronic kidney disease in nondiabetic US adults. J Am Soc Nephrol 14:469-477, 2003
3. Kaartinen K, Syrjanen J, Porsti I, Harmoinen A, Pasternack A, Huhtala H, Niemela O, Mustonen J: Insulin resistance and the progression of IgA glomerulonephritis. Nephrol Dial Transplant 22:778-783, 2007
4. DeFronzo RA, Alvestrand A, Smith D, Hendler R, Hendler E, Wahren J: Insulin resistance in uremia. J Clin Invest 67:563-568, 1981
5. Kubo M, Kiyohara Y, Kato I, Iwamoto H, Nakayama K, Hirakata H, Fujishima M: Effect of hyperinsulinemia on renal function in a general Japanese population: the Hisayama study. Kidney Int 55:2450-2456, 1999
6. Onat A, Hergenc G, Uyarel H, Ozhan H, Esen AM, Karabulut A, Albayrak S, Can G, Keles I: Association between mild renal dysfunction and insulin resistance or metabolic syndrome in a random nondiabetic population sample. Kidney Blood Press Res 30:88-96, 2007
7. Byberg L, Zethelius B, McKeigue PM, Lithell HO: Changes in physical activity are associated with changes in metabolic cardiovascular risk factors. Diabetologia 44:2134-2139, 2001
8. Larsson A, Malm J, Grubb A, Hansson LO: Calculation of glomerular filtration rate expressed in mL/min from plasma cystatin C values in mg/L. Scand J Clin Lab Invest 64:25-30, 2004
9. DeFronzo RA, Tobin JD, Andres R: Glucose clamp technique: a method for quantifying insulin secretion and resistance. Am J Physiol 237:E214-223, 1979
10. Pollare T, Vessby B, Lithell H: Lipoprotein lipase activity in skeletal muscle is related to insulin sensitivity. Arterioscler Thromb 11:1192-1203, 1991
11. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, Roth D: A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130:461-470, 1999
12. Fox CS, Larson MG, Leip EP, Meigs JB, Wilson PW, Levy D: Glycemic status and development of kidney disease: the Framingham Heart Study. Diabetes Care 28:2436-2440, 2005
13. Sarafidis PA, Ruilope LM: Insulin resistance, hyperinsulinemia, and renal injury: mechanisms and implications. Am J Nephrol 26:232-244, 2006
14. Riserus U, Basu S, Jovinge S, Fredrikson GN, Arnlov J, Vessby B: Supplementation with conjugated linoleic acid causes isomer-dependent oxidative stress and elevated C-reactive protein: a potential link to fatty acid-induced insulin resistance. Circulation 106:1925-1929, 2002
15. Prabhakar SS: Role of nitric oxide in diabetic nephropathy. Semin Nephrol 24:333-344, 2004
16. Horie K, Miyata T, Maeda K, Miyata S, Sugiyama S, Sakai H, van Ypersel de Strihou C, Monnier VM, Witztum JL, Kurokawa K: Immunohistochemical colocalization of glyoxidation products and lipid peroxidation products in diabetic renal glomerular lesions. Implication for glycoxidative stress in the pathogenesis of diabetic nephropathy. J Clin Invest 100:2995-3004, 1997
17. Suzuki D, Miyata T, Saotome N, Horie K, Inagi R, Yasuda Y, Uchida K, Izuhara Y, Yagame M, Sakai H, Kurokawa K: Immunohistochemical evidence for an increased oxidative stress and carbonyl modification of proteins in diabetic glomerular lesions. *J Am Soc Nephrol* 10:822-832, 1999
18. Miyata T, Sugiyama S, Suzuki D, Inagi R, Kurokawa K: Increased carbonyl modification by lipids and carbohydrates in diabetic nephropathy. *Kidney Int Suppl* 71:S54-56, 1999
19. Knight SF, Imig JD: Obesity, insulin resistance, and renal function. *Microcirculation* 14:349-362, 2007
20. Rabkin R, Ryan MP, Duckworth WC: The renal metabolism of insulin. *Diabetologia* 27:351-357, 1984
21. Chen J, Gu D, Chen CS, Wu X, Hamm LL, Muntner P, Batuman V, Lee CH, Whelton PK, He J: Association between the metabolic syndrome and chronic kidney disease in Chinese adults. *Nephrol Dial Transplant* 22:1100-1106, 2007
22. Laaksonen DE, Lindstrom J, Lakka TA, Eriksson JG, Niskanen L, Wikstrom K, Aunola S, Keinanen-Kiukaanniemi S, Laakso M, Valle TT, Ilanne-Parikka P, Louheranta A, Hamalainen H, Rastas M, Salminen V, Cepaitis Z, Hakumaki M, Kaikkonen H, Harkonen P, Sundvall J, Tuomilehto J, Uusitupa M: Physical activity in the prevention of type 2 diabetes: the Finnish diabetes prevention study. *Diabetes* 54:158-165, 2005
23. Perneger TV, Whelton PK, Puddey IB, Klag MJ: Risk of end-stage renal disease associated with alcohol consumption. *Am J Epidemiol* 150:1275-1281, 1999
24. Laterza OF, Price CP, Scott MG: Cystatin C: an improved estimator of glomerular filtration rate? *Clin Chem* 48:699-707, 2002
**Table 1** Baseline characteristics of the whole study population and different sub-samples

| Variable                                                                 | Total cohort (n=1070) | Normal fasting glucose and normal glucose tolerance (n=517) | Normal fasting glucose and glucose tolerance and GFR >50ml/min/1.73m² (n=433) |
|--------------------------------------------------------------------------|-----------------------|-------------------------------------------------------------|--------------------------------------------------------------------------------|
| Age (years)                                                              | 71.0±0.59             | 71.0±0.58                                                   | 71.0±0.56                                                                     |
| Glomerular filtration rate (ml/min/1.73m²)                               | 61.5±13.7             | 61.5±13.0                                                   | 65.3±10.4                                                                     |
| Urinary albumin excretion rate (µg/min)                                  | 21.9±84.0             | 19.0±86.6                                                   | 11.9±33.0                                                                     |
| Fasting plasma glucose (mmol/l)                                         | 5.5±0.9               | 5.1±0.3                                                     | 5.1±0.3                                                                        |
| Insulin sensitivity (M/I) (100*mg/kg body weight/min/mU/l)              | 5.2±2.5               | 6.2±2.4                                                     | 6.3±2.4                                                                        |
| Oral glucose tolerance test 2-hour glucose (mmol/l)                      | 7.7±3.0               | 5.8±1.2                                                     | 5.7±1.2                                                                        |
| Fasting plasma insulin (mu/l)                                           | 12.5±7.2              | 10.6±5.3                                                    | 10.3±4.9                                                                      |
| Systolic blood pressure (mmHg)                                          | 146.7±18.5            | 143.9±18.3                                                  | 143.3±17.3                                                                    |
| Diastolic blood pressure (mmHg)                                         | 83.9±9.4              | 82.7±9.3                                                    | 82.5±8.9                                                                      |
| Body mass index (kg/m²)                                                 | 26.2±3.4              | 25.2±3.0                                                    | 25.1±2.9                                                                      |
| Coffee (cups per day)                                                   | 3.4±1.6               | 3.4±1.7                                                     | 3.5±1.6                                                                        |
| Tea (cups per day)                                                      | 0.67±1.0              | 0.73±1.2                                                    | 0.73±1.18                                                                     |
| Alcohol (g/day)                                                         | 6.4±7.0               | 6.4±7.0                                                     | 6.6±7.1                                                                        |
| Diabetes mellitus                                                       | 61 (5.7)              | -                                                           | -                                                                              |
| Impaired fasting glucose                                                | 365 (34.0)            | -                                                           | -                                                                              |
| Impaired glucose tolerance                                              | 410 (38.3)            | -                                                           | -                                                                              |
| Smoking                                                                 | 221 (20.7)            | 125 (24.2)                                                  | 100 (23.1)                                                                     |
| Dyslipidemia                                                            | 938 (87.7)            | 446 (86.3)                                                  | 372 (85.9)                                                                     |
| Hypertension                                                            | 789 (73.7)            | 351 (67.9)                                                  | 289 (66.7)                                                                     |
| Physical activity level                                                 |                      |                                                             |                                                                                |
| Sedentary                                                               | 36 (3.7)              | 15 (3.2)                                                    | 12 (3.0)                                                                       |
| Moderate                                                                | 324 (33.4)            | 151 (31.8)                                                  | 124 (31.2)                                                                     |
| Regular                                                                 | 551 (56.8)            | 276 (58.2)                                                  | 229 (57.8)                                                                     |
| Athletic                                                                | 60 (6.2)              | 32 (6.8)                                                    | 31 (7.8)                                                                       |

*Date are mean±SD for continuous variables and n (%) for dichotomous variables*
### Table 2
The association of insulin sensitivity index (M/I) and glomerular filtration rate (GFR): Multivariable linear regression

| Model | Total cohort (n= 1070) | Normal fasting glucose and normal glucose tolerance (n=517) | Normal fasting glucose, normal glucose tolerance and GFR >50ml/min/1.73m² (n=433) |
|-------|------------------------|----------------------------------------------------------|----------------------------------------------------------|
|       | β-coefficient (95% CI) | p-value                                                  | β-coefficient (95% CI)                                    | p-value                                    |
| A     | 0.86 (0.53-1.19)       | <0.001                                                  | 1.03 (0.57-1.50)                                          | <0.001                                                   |
| B     | 1.10 (0.67-1.53)       | <0.001                                                  | 0.79 (0.25-1.33)                                          | 0.004                                                   |
| C     | 0.85 (0.52-1.19)       | <0.001                                                  | 1.03 (0.56-1.56)                                          | 0.001                                                   |
| D     | 0.88 (0.45-1.31)       | <0.001                                                  | 1.09 (0.51-1.67)                                          | <0.001                                                   |
| E     | 1.19 (0.69-1.68)       | <0.001                                                  | 0.86 (0.23-1.49)                                          | 0.007                                                   |

Data are regression coefficients for a 1-unit higher M/I. Model A is adjusted for age; model B is adjusted for age and glucometabolic factors (fasting plasma glucose, fasting plasma insulin and 2-hour plasma glucose at an oral glucose tolerance test); model C is adjusted for age and cardiovascular risk factors (hypertension, dyslipidemia and smoking), model D is adjusted for age and lifestyle factors (BMI, physical activity, consumption of tea, coffee and alcohol), model E is adjusted for all covariates in models A-D.

### Table 3
The association of insulin sensitivity index (M/I) and the incidence of renal dysfunction in participants with GFR >50ml/min/1.73m² at baseline: Multivariable logistic regression

| Model | Total Cohort (no of events/no at risk (32/586)) | Normal fasting glucose and normal glucose tolerance (no of events/no at risk (16/295)) |
|-------|------------------------------------------------|--------------------------------------------------------------------------------------|
|       | Odds ratio (95% CI) | p value       | Odds ratio (95% CI) | p value       |
| Model A | 0.85 (0.72-1.00) | 0.055 | 0.67 (0.51-0.89) | 0.006 |
| Model B | 0.82 (0.65-1.02) | 0.071 | 0.58 (0.40-0.84) | 0.004 |

Data are odds ratios for a 1-unit higher M/I. Model A is adjusted for age; model B is adjusted for age, fasting plasma glucose, fasting plasma insulin and 2-hour glucose at an oral glucose tolerance test.