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Epidemiological and Clinical Studies on Insulin Resistance and Diabetes

Hans Lithell, Johan Sundström, Johan Ärnlöv, Kristina Björklund, Arvo Hänni, Anu Hedman, Björn Zethelius, Liisa Byberg, Lena Kilander, Richard Reneland

Department of Public Health and Caring Sciences/Geriatrics, Uppsala University, Uppsala, Sweden

ABSTRACT

In Uppsala, extensive epidemiological and clinical studies on insulin resistance and diabetes have been ongoing for the past 30 years. A prospective cohort study of men born 1920–24, living in Uppsala County, was initiated during 1969-74 (the Uppsala Longitudinal Study of Adult Men, ULSAM). Risk factors for cardiovascular disease were examined in 2,322 men, and re-examinations have been performed every 10 years. At the first follow-up, when the men were 60 years old, insulin resistance was found to be a risk factor for development of hypertension and diabetes. In addition, treatment with antihypertensive medication was an independent risk factor for development of diabetes. These findings resulted in a series of clinical studies on metabolic effects of antihypertensive agents. At the second follow-up, when the men were 70 years old, the development of hypertension and diabetes was once again in focus, but at this time, cross-sectional and prospective studies of other cardiovascular determinants, such as circadian blood pressure pattern, left ventricular geometry and function, muscle morphology, ion status, fibrinolysis and cognitive function, were also performed. The cohort has furthermore been linked to the Swedish census and hospital discharge and cause of death registries, it has been used for studies on relationships between birth weight and cardiovascular disease, and genetic analyses have been performed, taking advantage of the long observation time obtained in this cohort. The cohort is currently being re-examined for the third time, and will hopefully continue to provide valuable information on the epidemiology of diabetes and cardiovascular disease in the future.

INTRODUCTION

In the late 1960s, an interest in the pathogenesis for type 2 diabetes mellitus emerged. In Uppsala, a prospective cohort study was planned and executed during 1969–74 (27). It was based on a cohort of 50-year-old men living in Uppsala county, born 1920–24 (the Uppsala Longitudinal Study of Adult Men, ULSAM). Of the 2,841 men available, 2,322 (82%) accepted the invitation to participate in a health

survey. A large number of investigations were performed. A questionnaire regarding physical exercise habits, smoking habits and family history of cardiovascular disease was filled out by the subjects. Concentrations of fasting lipids, glucose and insulin were measured, and in 1,692 men an intravenous glucose tolerance test (IVGTT) was carried out. At that time there were still no official guidelines on how to investigate glucose intolerance and it was not until 1980 that the first recommendations to use oral glucose tolerance tests (OGTT) appeared. However, the use of IVGTT turned out to be fruitful in the epidemiological context. It was also decided that follow-up examinations would be performed every 10 years. This is a brief summary of past and ongoing research that has its basis in this cohort study or has been initiated due to findings in the cohort study.

FINDINGS AT AGE 60

Insulin resistance and hypertension

The first follow-up was carried out in 1980–84, when the men were 60 years old. A total of 1,860 men (87.5% of the eligible men from the original cohort) participated in the follow-up examination. This time the focus was on identifying those men who had developed diabetes or hypertension. For development of hypertension, the blood pressure itself was found to be the most important risk factor. In addition, fasting and late (at 60 minutes of the IVGTT) insulin concentrations, body mass index (BMI), abdominal skinfold thickness and heredity for hypertension were identified as contributing factors. In particular, late insulin concentration and increase in BMI were important risk factors for development of hypertension. We believe this was the first prospective study to demonstrate insulin resistance (as reflected in insulin concentrations) being a risk factor for hypertension (66).

Insulin resistance and type 2 diabetes mellitus

Amongst the 1,834 men who were normoglycemic at age 50 and re-investigated at age 60, 77 (4.4%) had developed diabetes at age 60. The most powerful risk factors for development of diabetes were a high fasting insulin concentration (an indicator of insulin resistance), a low insulin response to glucose injection at the IVGTT, BMI, systolic blood pressure and a high glucose value (within the normal range). In addition, treatment with anti-hypertensive medication was an independent risk factor (67).

FINDINGS AT AGE 70

Non-dipping and the insulin resistance syndrome

Non-dipping is a circadian blood pressure pattern that sometimes accompanies states of autonomic failure (41), diabetes mellitus (11) and hypertension (69), but also occurs in apparently healthy subjects. Whether non-dipping adds to an increased risk of end organ damage or not has been disputed. A blunted nocturnal

blood pressure reduction has been associated with left ventricular hypertrophy (78), renal dysfunction (6), silent cerebrovascular disease (65) and cognitive deterioration (31). In addition, non-dippers have an increased mortality risk compared with dippers (50). In contrast, some investigators have failed to find a relationship between an impaired nocturnal blood pressure fall and target organ damage in hypertensive individuals (9, 63). In the ULSAM cohort, 24-hour blood pressure was evaluated in 1,057 70-year-old men. Preliminary data from a cross-sectional investigation indicate that non-dippers (n=66), in addition to insulin resistance, diabetes and hypertriglyceridaemia, have an unfavourable lipid profile with elevated levels of free fatty acids (FFA) in serum and an increased proportion of palmitic, palmitoleic and oleic acid in serum cholesterol esters.

Ion status, hypertension and insulin resistance

A disturbed magnesium balance, with reduced intracellular ionised magnesium concentration, has been observed in patients with essential hypertension and in subjects with diabetes mellitus (62). Magnesium supplementation in humans has led to increased insulin sensitivity, lowered blood pressure and improved lipid status (55, 61, 80). Increased serum magnesium concentrations during antihypertensive captopril treatment were correlated to improved insulin sensitivity (18). During bendrofluazide treatment serum magnesium decreased significantly and there was an inverse correlation between the changes in serum magnesium and mean fasting glucose concentrations (14). An association between the magnesium and calcium balance in skeletal muscle and blood pressure response to antihypertensive treatment has been reported (17). The changes in serum magnesium concentrations, and in the ratio between calcium and magnesium concentrations, correlated to changes in insulin sensitivity and to serum triglyceride concentrations during antihypertensive treatment with different angiotensin converting enzyme (ACE) inhibitors (15, 19). It has been questioned whether the total magnesium concentration in blood reflects the biological activity of magnesium since only about 60 % of the magnesium in the blood is in a free unbound state. In fact, atherogenic lipid fractions and some glucometabolic variables were more closely correlated with circulating ionised magnesium than with total magnesium concentration (21). The circulating ionised magnesium concentration in patients with essential hypertension and in insulin resistant but otherwise healthy subjects was within the normal range (21). Euglycaemic hyperinsulinaemia increases the circulating ionised magnesium concentration while no significant change was observed of the total magnesium level (16, 20).

Relationships between muscle morphology and insulin resistance

Skeletal muscle accounts for the largest part of glucose uptake when stimulated by insulin (as much as 90%). The capacity of the muscle to utilise glucose depends largely on muscle structure. Muscle rich in type I (oxidative) fibres, as well as muscle rich in capillaries, apparently show much higher glucose uptake than muscle containing mainly type IIB (glycolytic) fibres. The percentage of type I fibres and

capillary density in skeletal muscle increases with increasing level of physical activity. In contrast, type IIB fibres are prevalent in obese people.

Muscle biopsies were taken in 515 men (43%) at age 70 in order to analyse muscle histology. Results of this analysis showed a clear association between insulin sensitivity and muscle structure. Insulin sensitivity showed a positive relationship with percentage of type I fibres and capillary density and a negative correlation with percentage of type IIB fibres (25). These associations were independent of the level of physical activity and obesity. Moreover, diabetic subjects showed a significantly higher percentage of type IIB muscle fibres compared to healthy controls.

A more intriguing and less studied aspect is the degree to which muscle structure, i.e. fibre composition and capillary density, is related to hypertension. For that purpose, a group of normotensive (n=113) and hypertensive (n=43) men were compared in order to test the hypothesis of a relationship between heart rate, development of hypertension and muscle morphology in a state of normal glucose tolerance. These groups did not differ significantly with regard to fibre type distribution, but the hypertensive subjects had a significantly lower capillary supply than the controls, when analysed as number of capillaries around different fibre types. Capillary rarefaction in the hypertensive subjects in the present study was closely associated with the increase in blood pressure over a 20-year period. Heart rate, a marker of the balance between sympathetic and parasympathetic tone, was inversely correlated to capillary supply, which supports the hypothesis of involvement of increased sympathetic tone in a reduction of the capillary net in skeletal muscle (26).

Insulin has also been found to increase the blood flow in skeletal muscle beds through vasodilation. This increase in blood flow is believed to contribute to glucose uptake by the muscle. The magnitude of the increase in blood flow during hyperinsulinaemia, which is endothelium-dependent, depends not only on the degree of vasodilation in large vessels, but also possibly on the number of capillaries present in the muscle bed. Preliminary data show that the capillary density is associated to insulin-induced changes in leg blood flow. This indicates that endothelial function is closely linked to the capillarisation in skeletal muscle.

Proinsulin in relation to development of type 2 diabetes and cardiovascular disease

In longitudinal studies, the fasting concentration of total proinsulin-like molecules predicts the development of type 2 diabetes over periods of follow-up ranging from 2 to 4 years (76). Fasting concentrations of specific insulin, intact and 32–33 split proinsulin have been measured in plasma samples, stored frozen since baseline in liquid nitrogen, by the specific two-site immunometric assay technique (68). Zethelius and co-workers have published preliminary results (83) indicating that proinsulin may be a long-term predictor for diabetes when compared to known baseline risk factors for type 2 diabetes in this cohort (glucose and immunoreactive insulin concentrations, fasting and at 60 min after an intravenous glucose load, respectively, and use of antihypertensive drug treatment).

Insulin has been shown to be a risk factor for cardiovascular disease (64). The extent to which proinsulin may contribute to the association between insulin and cardiovascular disease is largely unknown. The aim of one of our studies was to identify the longitudinal relationship between proinsulin and coronary heart disease in the ULSAM cohort. The diagnosis of incident disease was collected from the official in-patient and causes-of-death registers. In a preliminary report (82), Zethelius and co-workers have shown results indicating that proinsulin is a predictor for cardiovascular disease and may be independent of known confounders.

Heart failure and the insulin resistance syndrome

Heart failure is the only major cardiovascular disorder that is increasing in incidence and prevalence (44), with substantial morbidity and mortality and a huge economic impact on the public health systems of western society (28). In spite of this, the epidemiological data on heart failure are relatively scarce. Previous studies demonstrate an association between insulin resistance and heart failure (53, 74) and an impaired insulin-mediated glucose uptake has been shown to be a prognostic factor in heart failure patients (56). Preliminary results from Ärnlöv and co-workers (84) indicate that factors related to insulin resistance (10, 49, 52, 79), such as an increased heart rate, increased serum concentrations of proinsulin, a high proportion of dihomogammalinolenic acid in serum cholesterol esters and hypophosphataemia are predictors of left ventricular systolic dysfunction in elderly males after 20 years follow-up, independently of myocardial infarction, hypertension, diabetes and the use of cardiovascular medication.

Left ventricular hypertrophy and the insulin resistance syndrome

Left ventricular hypertrophy (LVH) is an important risk factor for cardiovascular and all-cause mortality and morbidity (32, 37), and an increased left ventricular relative wall thickness (RWT, wall thickness divided by ventricular diameter) also has an adverse prognosis (32, 77). The aetiology of LVH is largely unknown. Cross-sectional studies (23, 36, 42, 60, 70) have shown that male sex, age, hypertension, obesity, valvular disease, previous myocardial infarction, heredity and alcohol use are important in LVH. Recently, associations between LVH and the insulin resistance syndrome have been found (39, 43, 54), although some studies (51, 71) have found insulin resistance or impaired glucose tolerance to be more closely related to increased RWT than to LVH. In the ULSAM cohort, electrocardiographic and echocardiographic examinations were performed in 583 men at 70 years of age. Thus, this cohort is well suited for the much needed further study of the epidemiology of LVH, including detailed cross-sectional and prospective studies of the relationship between aspects of the insulin resistance syndrome and left ventricular geometry, as well as development of new diagnostic electrocardiographic criteria for LVH.

In a cross-sectional study of the cohort at age 70 (71), we found that several components of the insulin resistance syndrome (hyperinsulinaemic euglycaemic clamp insulin sensitivity index, fasting insulin, 32-33 split proinsulin, triglycerides, FFA, OGTT glucose and insulin levels, waist/hip ratio, BMI, 24-h blood pressure and

heart rate) were related to left ventricular RWT. Only 24-h systolic pressure and heart rate were related to left ventricular mass index. Several metabolic parameters were adversely altered in left ventricular concentric remodelling, but only 24-h blood pressure and heart rate in LVH.

Preliminary data from a small study using positron emission tomography to determine myocardial, skeletal muscle and whole-body insulin sensitivity indicate that myocardial insulin sensitivity may be related to RWT and not to left ventricular mass index (73). Preliminary studies of predictors for LVH over 20 years indicate that dyslipidaemia and indices of a low dietary intake of linoleic acid and high intake of saturated and monounsaturated fats, as well as hypertension and obesity, at age 50 are predictive of the prevalence of LVH 20 years later, suggesting that lipids may be important in the aetiology of LVH (72).

Insulin resistance and PAI-1

Elevated levels of plasminogen activator inhibitor-1 (PAI-1) are regarded as a component of the insulin resistance syndrome (1). PAI-1 is an important factor in the regulation of fibrinolysis and inhibits the degradation of solid fibrin by forming an inactive complex with tissue-type plasminogen activator (t-PA). Plasminogen is cleaved by t-PA to form active plasmin that degrades fibrin clots. It has been suggested that PAI-1 is one of the factors linking insulin resistance with cardiovascular disease (29).

PAI-1 activity was analysed in the ULSAM cohort at age 70 (8). BMI, waist/hip ratio, blood pressure, fasting glucose, insulin and serum triglyceride concentrations were all positively related with PAI-1 activity. Insulin sensitivity index, measured by clamp, and HDL cholesterol concentrations were inversely related with PAI-1 activity. In a multivariate regression analysis, BMI, waist/hip ratio, serum triglyceride concentrations, and insulin sensitivity were related to the PAI-1 activity, all independent of each other. PAI-1 activity was also higher in men with type 2 diabetes and impaired glucose tolerance than in men with normal glucose tolerance. This difference was not explained by variations in BMI, waist/hip ratio, serum triglycerides, insulin sensitivity, blood pressure medication or fasting glucose concentrations.

Insulin resistance and cognitive function

In recent years, it has been recognised that cerebrovascular disease is associated not only with stroke and vascular dementia, but also with late-onset Alzheimer's disease. Dementia research has now focused on risk factors associated with early stages of cognitive impairment, from which a further progress to dementia may be prevented. A subgroup of the 70-year-old men (999 out of 1,221) participated in cognitive function testing. The Mini Mental State Examination (12) and the Trail Making Tests (38) were used, tests which are sensitive for subcortico-frontal dysfunction. Results have been previously published in detail (30, 31). In brief, men with diabetes according to an OGTT (n=130) had significantly poorer results on the cognitive tests, even when stroke, hypertension and atrial fibrillation were taken into account. Similarly, clamp measurements of insulin sensitivity were related to

cognitive function: men in the highest tertile of insulin sensitivity performed significantly better than those in the lowest tertile.

STUDIES ON LOW BIRTH WEIGHT, INSULIN RESISTANCE AND DIABETES

Low birth weight predicts physiological disturbances in adult life, such as raised blood pressure (3, 33, 34), type 2 diabetes (22, 40, 45), impaired glucose tolerance (22, 59) and insulin resistance (40, 46, 58), all constituents of the insulin resistance syndrome. All of these conditions are associated with an increased risk of and mortality from cardiovascular disease, as is small size at birth (5, 35). Low birth weight has also been associated with the insulin resistance syndrome itself (4, 75).

Birth weight, in this cohort, is inversely associated with fasting concentrations of immunoreactive insulin and insulin resistance at age 50 and positively associated with BMI at age 50 (40) and insulin sensitivity at age 70 (46). Birth weight was also related to blood pressure at both ages (33, 34) but not with the concentrations of serum triglycerides or HDL cholesterol at age 50 (40). In a recent study we demonstrated that low birth weight is associated with physiological disturbances in adult age that are a subset of the disturbances clustered in the general population as the insulin resistance syndrome (7). Birth weight was inversely associated with the subscapular/triceps skinfold ratio investigated at age 50 and used as a measure of truncal fat. Low birth weight predicted a smaller hip but not a larger waist and thus the inverse relationship of birth weight with the waist/hip ratio at age 70 does not reflect an association of low birth weight with central adiposity. Birth weight was also inversely associated with the activity of PAI-1 and the concentrations of specific insulin and proinsulin at age 70. There were no associations of birth weight with the concentrations of serum triglycerides or HDL cholesterol at age 70.

In short, low birth weight predicts high blood pressure, insulin resistance, truncal obesity and high PAI-1 activity but not the abdominal obesity or dyslipidaemia present in the insulin resistance syndrome. Understanding why low birth weight predicts only some components of the insulin resistance syndrome and not others may depend on identifying a specific physiological pathway that links this subset of disturbances. One possible mechanism could be increased concentrations of or sensitivity to glucocorticoids. Supporting this hypothesis are the observations that long-term treatment of glucocorticoids cause accumulation of truncal fat, glucocorticoids increase the production of PAI-1 (48), and the sensitivity to glucocorticoids could be programmed in foetal life (57).

EFFECTS OF ANTIHYPERTENSIVE DRUGS ON INSULIN RESISTANCE

In eight different intervention studies, the actively treated comparison groups have been treated with a selective beta-blocker (atenolol). The average effect on insulin sensitivity was a reduction by 19% from the basal value after 4-6 weeks placebo

treatment. The average effect of two similar diuretics, given in moderate high doses, was a decrease by 19% in insulin sensitivity. In four studies of calcium-channel blockers, no significant effect on insulin sensitivity was found. In the class of ACE inhibitors, the results ranged from +16% with captopril to –10% with lisinopril. The average effect was +2%. With two different angiotensin$_1$-receptor blockers, similar non-significant (+2%) effects on insulin sensitivity were demonstrated. The average of three different studies with alpha-1 selective inhibitors was an improvement of insulin sensitivity by 23%. The effect of moxonidine treatment was an improvement of insulin sensitivity by 12%. However, the improvement was restricted to a subgroup of very insulin resistant patients, predefined in the protocol, who improved by 21%. Moxonidine belongs to a class of centrally acting inhibitors of the sympathetic nervous system. The agonistic effect these drugs exhibit on the imidazoline I_1 receptors causes an inhibition of the sympathetic nervous system.

CANDIDATE GENES FOR INSULIN RESISTANCE

For several years, the Geriatrics Section of the Department of Public Health and Caring Sciences has actively collaborated with Professor Oluf Pedersen's research group at Steno Diabetes Centre in Gentofte, Denmark. The Steno group investigates candidate genes for type 2 diabetes. Variants of candidate genes are identified either from the literature or in-house at Steno. Associations between these genetic variants and relevant phenotypes such as type 2 diabetes, insulin resistance, and insulin secretion are then sought in the ULSAM material. In ULSAM, DNA has been collected from >95% of participants and IVGTT, OGTT and hyperinsulinaemic, euglycaemic clamp data are available.

In the first study, hepatocyte nuclear factor 4alpha (HNF-4α), the MODY-1 gene, was investigated, and two single nucleotide polymorphisms that changed the amino acid sequence were found, one previously reported (Thr130Ile) and one new (Val255Met). In a Danish sample, the frequency of the Thr130Ile variant was significantly higher in 509 type 2 diabetes patients (4.7%) compared to control subjects (1.7%, p=0.008). This finding could not be replicated in the Swedish ULSAM population, however, in which frequencies of the variant were similar in subjects with type 2 diabetes (5.4%) and glucose tolerant control subjects (5.1%). The novel Val255Met variant was originally only found in type 2 diabetes patients (4/477) and not in controls (0/217). Interestingly, the Val255Met variant could not be found in any of the 894 ULSAM men investigated (47).

In a second study, the previously identified Gly1057Asp variant of the insulin receptor substrate-2 (IRS-2) gene was analysed in four different groups of glucose tolerant subjects, one of which was derived from ULSAM. No consistent effect on insulin sensitivity or secretion was found (2).

A third study investigated the influence of two common genetic variants in the PPP1R3 gene encoding the glycogen targeting subunit of type-1 protein phosphatase (24). A previous study in Pima Indians indicated an association between the Asp905Tyr, Arg883Ser, or PP1ARE polymorphisms, and insulin sensitivity (81).

The Arg883Ser variant was not included in the Danish/Swedish study since it could not be found in 82 Caucasian volunteers. In ULSAM there was no association between either the Asp905Tyr or the PP1ARE variants and diabetes incidence between 50 and 70 years of age. There was no association between the Asp905Tyr variant and insulin sensitivity, but there was a weak correlation between the PP1ARE variant and whole body insulin sensitivity. In view of the multiple tests involved in the study, however, this correlation was not considered significant by the authors (24).

DISCUSSION

The past and on-going research of our group reviewed in this paper demonstrates the usefulness of an epidemiological study as a basis for research. Not only can valuable information be obtained from the study itself, but other studies may develop from some of the, often unexpected, results. In this case, some of the findings resulted in a series of clinical studies on metabolic effects of antihypertensive agents. In addition, the cohort was useful for studies on relationships between birth weight and cardiovascular disease, and for genetic analyses.

In the first follow-up of the ULSAM cohort (at age 60), two major findings were made. Development of hypertension at age 60 had been preceded by high insulin concentrations at age 50. Insulin resistance as a predictor of hypertension has been found in some but not all other studies, and the question of whether insulin is causally related to hypertension is still debated. The other major finding was that high fasting insulin concentrations (as indicators of insulin resistance) and low insulin response to glucose injection at the IVGTT (as a measure of insulin secretion capacity) were strong predictors of future diabetes. The importance of insulin resistance for diabetes was further manifested in the 20-year follow-up. We used stored, frozen serum samples and analysed for specific insulin, proinsulin and 32–33 split proinsulin. Proinsulin (which reflects insulin resistance) was a powerful risk factor for development of diabetes, particularly during the first ten years.

At the second follow-up (at age 70), skeletal muscle biopsies were studied in about 500 of the participants. Investigations of muscle fibre composition and capillarisation indicated that hypertensive, glucose-tolerant 70-year-old men have a lower percentage of type I fibres and more type II fibres than a control group of normotensive men. Furthermore, the capillarisation was less pronounced. Capillarisation was related to insulin sensitivity and to blood flow changes during hyperinsulinaemia, and FFA concentrations also played an important role in determining the vasodilatory response to hyperinsulinaemia. We demonstrated a relationship between heart rate at age 50 and low capillary density at age 70 as well as between heart rate at 50 and development of hypertension during the following 20 years. This indicates a possible influence of high sympathetic tone being involved in both structural changes in skeletal muscle and hypertension.

Extensive echocardiographic investigations were performed in nearly 600 men. These data have now been studied with regard to metabolic characteristics of dif-

ferent morphologic measures. Several characteristics of the metabolic syndrome and insulin resistance itself correlated to the left ventricular relative wall thickness, in contrast to the findings for left ventricular hypertrophy, with which only 24-h blood pressure and heart rate were correlated. Skeletal muscle insulin resistance may thus be important for development of increased relative wall thickness, as supported from the study with positron emission tomography.

In this study population, it is interesting that insulin resistance and proinsulin concentrations are significant predictors also for coronary heart disease and left ventricular systolic dysfunction. Some of the effect on coronary heart disease may be mediated by PAI-1 activity, which is strongly correlated to insulin resistance. A deleterious effect of insulin resistance also on small vessels may explain the correlation between cognitive function and insulin resistance/diabetes mellitus.

The metabolic consequences of antihypertensive medication have been extensively described in the original publications and will not be commented in detail here. The increased insulin resistance of beta-blockers and diuretics most likely explains the increased risk for diabetes mellitus associated with use of these drugs, as high doses were used in the 1970s. This was recently confirmed in a large study for beta-blockers but not for diuretics (13). The doses used in the study are not provided in the report, but it seems likely that they were lower for diuretics in the study than in the 1970s.

Much of our data (in co-operation with the London School of Hygiene and Tropical Medicine, London, UK) on the relationship between low birth weight and risk for diabetes confirms David Barker's findings (3-5). However, we have demonstrated that this increased risk is mediated by an effect of birth weight on insulin resistance and not on insulin secretion. In our population, it seems that only part of the syndrome is related to birth weight, in particular those metabolic variables that are related to cortisol effects.

The effect of candidate genes for insulin resistance has been studied in this cohort in co-operation with Steno Diabetes Centre in Copenhagen. This project is ongoing. The ULSAM population supplements some of the younger populations of Caucasian origin in the Steno studies.

In summary, these studies on insulin resistance and diabetes are based on a prospective cohort study of medium size. The population is small for genetic studies, but can supplement studies in younger populations in order to describe the different gene-environment interactions at different ages. Most of the findings described here are the preliminary results for some of our Ph.D. students, whose dissertations will soon be providing more in-depth analysis of the ULSAM data. A list of publications and more detailed information on the cohort can be found at the web-site www.pubcare.uu.se/ulsam.

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Address for correspondence:
Hans Lithell, MD, PhD
Department of Public Health and Caring Sciences/Geriatrics
Uppsala University
Box 609
SE-751 25 Uppsala
Sweden