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Risk Factors for Stroke in Adult Men

A Population-based Study

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Abstract
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In the last decades our knowledge concerning cardiovascular risk factors has grown rapidly through results from longitudinal studies. However, despite new treatment, in Western countries coronary heart disease remains the leading cause of death and stroke is still the leading cause of severe disability.

The studies reported in these papers examine the relationships between stroke/transient ischaemic attack (TIA) and a number of different factors measured on two different occasions in men born in Uppsala 1920-1924 and are epidemiological in their character.

The findings indicate that in addition to already established risk factors, indices of an unhealthy dietary fat intake and high serum lipoprotein(a) are independent predictors of stroke/TIA. Among different glucometabolic variables a low insulin sensitivity index derived from the euglycaemic insulin clamp and proinsulin carries a high predictive value for later stroke, independently of diabetes.

Moreover, cognitive test performance measured with Trail Making Test B at age 70 is a strong and independent predictor of brain infarction, indicating that the risk is already increased in the subclinical phase of milder cognitive dysfunction. Performance at a pre-stroke Trail Making Test is also of predictive value for mortality after first-ever stroke/TIA, but none of the studied pre-stroke variables or cognitive tests was found to be related to dependency after an event.

In summary these studies provide further knowledge about predictors of stroke and of mortality after first-ever stroke. They also indicate the possible importance of new markers of risk, such as the level of lipoprotein(a), profile of fatty acids in the diet, low insulin sensitivity derived from clamp investigations, level of proinsulin, and cognitive performance measured with Trail Making Tests.

Keywords: risk factor, stroke, TIA, lipoproteins, fatty acids, insulin resistance, proinsulin, clamp, cognitive function, epidemiology, Trail Making Test, stroke mortality, dependency

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”You are as old as your arteries” (Scott M Grundy, 1999)

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List of Papers

I. **Wiberg B, Sundström J, Ärnlöv J, Terént A, Vessby B, Zethelius B, Lind L.** Metabolic Risk Factors for Stroke and Transient Ischemic Attacks in Middle-Aged Men: A Community-Based Study with Long-Term Follow-Up. *Stroke*; 2006 Dec; 37: 2898-2903

II. **Wiberg B, Sundström J, Zethelius B, Lind L**. Insulin Sensitivity Measured by the Insulin Clamp and Proinsulin Levels as Predictors of Stroke. *Diabetologia*; 2009 Jan;52(1):90-6. Epub 2008 Oct 24

III. **Wiberg B, Lind L, Kilander L, Zethelius B, Sundelöf J, Sundström J.** Cognitive Function in Elderly Men and Risk of Stroke. *Neurology;* 2010 Feb 2;74(5):379-85

IV. **Wiberg B, Kilander L, Sundström J, Byberg L, Lind L.** Cognitive Function Prior to Stroke is a Risk Factor for Post-Stroke Mortality but Not Dependency**.** *Under review.*

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Contents

Abbreviations

AF	atrial fibrillation
Apo(a)	apolipoprotein(a)
ApoA1	apolipoprotein A1
ApoB	apolipoprotein B
ApoE	apolipoprotein E
ARIC	Atherosclerosis Risk in Communities
ASA	acetylsalicylic acid
AVM	arterio-venous malformations
BMI	body mass index
BI	brain infarction
BP	blood pressure
CDR	Cause of Death Register
CE	cholesterol ester
CI	confidence interval
CHD	coronary heart disease
CHF	congestive heart failure
CT	computed tomography
CRP	C-reactive protein
CV	coefficient of variation
CVD	cardiovascular disease
DBP	diastolic blood pressure
DSST	digit-symbol substitution test
ECG	electrocardiography/electrocardiogram
ECG-LVH	electrocardiographic left ventricular hypertrophy
EGIR	European Group for the Study of Insulin Resistance
FA	fatty acid
FAPC	fatty acid principal component
HDL	high-density lipoprotein
HR	hazard ratio
ICD	International Classification of Diseases
ICH	intracerebral haemorrhage
IRS	insulin resistance syndrome
IVGTT	intravenous glucose tolerance test
Lp(a)	lipoprotein(a)
LDL	low-density lipoprotein
LVH	left ventricular hypertrophy

M	glucose disposal
MAS	motoric assement scale
MI	myocardial infarction
M/I ratio	insulin sensitivity index
MMSE	mini-mental state examination
MRI	magnetic resonance imaging
NCEP	National Cholesterol Education Program
PYAR	person-years at risk
SAH	subarachnoid haemorrhage
SBP	systolic blood pressure
SHDR	Swedish Hospital Discharge Record
SD	standard deviation
SPMQS	short portable mental status questionnaire
TIA	transient ischaemic attack
Tg	triglyceride
TMT	Trail Making Test
ULSAM	Uppsala Longitudinal Study of Adult Men
WHO	World Health Organisation

Introduction

Let us start with the word epidemiology. If we translate it literally, it means"the study of that which befalls man". If we take it in parts, epi means "befalls" or "upon", demo means "man" or "people", and ology "the study of". In other words epidemiology is a quantitative study of the distribution and determinants of disease in a defined human population.

Regarding the studies forming the basis of this thesis, they are epidemiological in character and they address the relationship between stroke and a number of different factors measured on two different occasions in men born in Uppsala between 1920 and 1924.

The purpose of this research is to attempt to elucidate the aetiology of stroke disease with the ultimate aim of being able to prevent it (if we are fortunate) in some cases. And further to determine whether epidemiological data are consistent with current scientific knowledge and hypotheses. In these attempts it must be kept in mind that absolute proof of causality can only come from intervention studies.

Stroke

Historically the first depiction of stroke may not originate from Hippocrates but from the Chinese (475-221 B.C.). Hippocrates mentioned stroke in about 400 B.C., but it was the Roman physician Aurelius Celsus (25 B.C.-A.D. 50) who described "apoplexy" and discriminated it from "paralysis". The term "hemiplegia", which is still in use, was introduced by Paul of Aegina (A.D. 625-690). Gregor Nymman of Wittenberg (1594-1638) described the idea of interrupted circulation in the brain vessels as a cause of apoplexia. A Swiss physician, Johann Jakob Wepfer of Schaffhausen (1620-1695) stated that corpulent people and those with an irregular pulse, and in addition those whose face and hands are of bluish leaden colour, are at great risk of suffering a stroke. This could indicate that persons with hypertension or cardiac failure are vulnerable. Among the first to link atherosclerosis to apoplexy was Francis Bayle (1622-1709). The English physician William Heberden (1710-1801) noted that transient ischaemic attacks (TIA) often occur shortly before stroke. A description of "diseased and roughed" arteries or embolism from the heart causing neurological deficit is found in Allbutt's *System of Medicine* (1899).

In 1930, increased blood pressure was described as being connected to apoplexia and cerebral haemorrhage by Swartz and Goldinger.[1] When the horrors of World War 2 were fading, the world could turn towards other problems. Since cardiovascular diseases were the causes of half the deaths in the industrialised world in the middle of last century, these were a natural target; especially since many men in their younger middle-age, family supporters and at the peak of their working life, suddenly were struck without any warning.

With growing importance since 1949, the population-based Framingham Heart Study has played an immense role in identifying common factors that contribute to cardiovascular diseases. The concept of risk factors actually has its origin in that study and has been used since the beginning of the 1960s. I have been told that it was the Framingham Heart Study that inspired professor Bertil Hood to initiate and Dr Hans Hedstrand to perform baseline investigations in Uppsala 1970, later on named the Uppsala Longitudinal Study of Adult Men (ULSAM). Regarding stroke and risk factors, an important year is 1965, when Berkson and Stamler found an association between hypertension and stroke in an epidemiological study for the first time.[2]

Clinical aspects

Stroke is a common and serious disease, which causes human suffering and an economic burden on the health care system and the society. Together with heart disease it is the leading cause of death in persons over 15 years of age worldwide and the leading cause of severe disability in adults. According to Riks-Stroke (a national quality register for acute stroke), the approximate number of stroke events in in-patient care in Sweden is 30 000 annually.

According to World Health Organisation (WHO) criteria, stroke is defined as "rapidly developing clinical symptoms and/or focal and at times global loss of cerebral function, with symptoms lasting more than 24 hours or leading to death, with no apparent cause other than of vascular origin".

Subgroups of stroke consist of brain infarctions (BI) also called cerebral infarctions or ischaemic strokes, intracerebral haemorrhages (ICH) and subarachnoid haemorrhages (SAH).

TIA is thus not included in the definition, as the symptoms disappear less than 24 hours after the onset. However, the risk of stroke following TIA is as high as ten to twenty per cent in the first three months.[3, 4]

Stroke presents with a wide range of clinical manifestations, depending on the location and extent of the brain damage.

The typical signs of acute stroke and TIA are as follows:

- Sudden numbness or weakness of the face, arm or leg, especially on one side of the body
- Sudden confusion, difficulties in speaking or understanding (aphasia)
- Sudden visual impairment in one or both eyes
- Sudden difficulty in walking, dizziness, or loss of balance or coordination
- Sudden, severe headache with no known cause

The consequences of stroke can also be of a cognitive and perceptual nature and at first sight these are less obvious. Such symptoms are sometimes called "silent handicap" and may have a great impact on performances of activities of daily living (ADL) and quality of life.

Diagnostic tools

Virtually all patients in Sweden with symptoms suspected to indicate stroke or TIA undergo computed tomography (CT), a fast, painless and safe examination of the brain (Riks-Stroke, The National Stroke Register in Sweden, 2004). In Uppsala the use of CT started in the late 1970s, during the 80s it was mainly used by neurologists, and since the first years of the 90s it has been in more general use.

The sensitivity of CT for detecting bleeding is high. For haemorrhage it is nearly 100 % and for SAH it approaches 95 %. The CT sign of infarction often takes a while to develop and even large infarctions may be undetectable in the first hours. Hyperdensity (increased attenuation) is the first tomographic sign of developing infarction and most often represents an acute intramural thrombosis. However, the diagnosis is not dependent on the findings at tomography or other imaging, but it is clinically established after a neurological examination of the patient. A later CT image, usually after an hour or more, could show decreased attenuation (hypoattenuation).[5, 6]

Another method for diagnosing stroke is magnetic resonance imaging (MRI), which is more sensitive and demonstrates both the pathophysiology and the anatomy. This technique has rapidly developed during recent years and is used with different perfusions and diffusion-weighted imaging. MRI is not currently as widespread as CT for acute stroke diagnosis. The examination takes a longer time and is therefore more demanding for the emergency patient. In comparison with CT, MRI can detect abnormalities earlier, in some cases after only a few minutes, and with further development MRI might replace CT to an increasing extent in the future. MRI better detects

changes in the brainstem than CT and is often performed as a complementary examination after CT. In addition it is used to determine the origin of haemorrhages, especially to detect an underlying tumour or arterio-venous malformations.

To visualise the common carotid and vertebral arteries after stroke or TIA, B-mode ultrasound is used. This is able to detect atherosclerotic changes that might explain the attack. Many stroke patients undergo this examination and if a stenosis is seen, reaching a level of 50 % or more, the patient might be a candidate for surgical intervention to correct the narrowing, with the purpose of diminishing the risk of future stroke.

Brain infarction or ischaemic stroke

Brain (cerebral) infarction or ischaemic stroke is the most common type of stroke and is caused by a thrombus or embolus. In Sweden this origin accounts for about 85% of stroke cases (Socialstyrelsen, National Board of Health and Welfare, 1996). An embolus is a clot that is formed at another site, often in the left atrium of the heart or in the carotid arteries, and then follows the blood stream until the diameter of the vessel is too narrow for it to pass. Cardiac embolism is reported to account for 12 to 35 % of strokes, but these figures are probably underestimated. The most common source of cardiac embolism is atrial fibrillation, according to Riks-Stroke (2003), and other sources are mitral or aortic valve disease, endocarditis, cardiomyopathies and dyskinetic myocardial segments caused by myocardial infarction and ventricular aneurysms.

Atherosclerosis primarily affecting larger vessels is responsible for about 25 % of ischaemic strokes, a few per cent higher than are explained by atherosclerosis in small vessels causing lacunar infarcts in the deeper parts of the brain. These infarctions are small, by definition less than 15 mm. (Fig 1)

Coagulation disturbances as origins of stroke are rare[7] and only a few stroke patients are usually examined regarding hypercoagulation aetiologies.

Transient ischaemic attack

The usual TIA symptoms are the same as those of stroke. They occur rapidly but are only temporary, mostly lasting for only a few minutes, but by definition less than 24 hours. The usual cause of TIA is a blood clot temporarily clogging an artery. Most strokes are not preceded by TIA, but as many as 20 % of severe strokes are.[3] Prompt medical or surgical attentions to these symptoms can prevent a fatal or disabling stroke from occurring. This has lately been elucidated in several articles in the Swedish medical press.

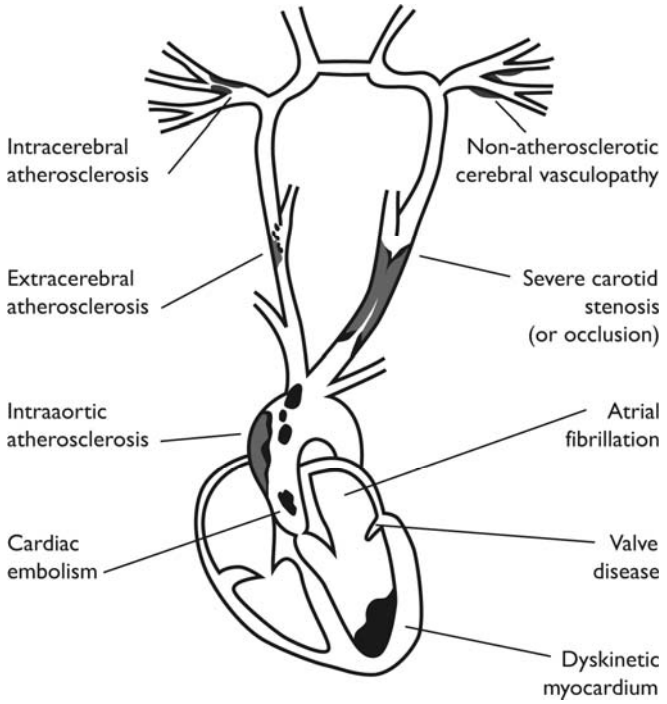

Figure 1. Aetiology of ischaemic stroke.

Haemorrhagic stroke

Haemorrhagic stroke consists of intracerebral haemorrhage (10 %) caused by rupture of a vessel in the brain or in the subarachnoid space on the surface of the brain, the latter called subarachnoid haemorrhage (5 %) (National Board of Health and Welfare, 1996). The cerebral bleedings have three major causes: arterial hypertension, ruptured arterio-venous malformations (AVMs) and in younger people certain drugs.[8] Subarachnoid haemorrhage (SAH) is most often caused by rupture of an intracranial aneurysm or AVM.[9]

Incidence

The number of stroke victims is increasing in many countries as a result of the changes in age structure in the Western world. Although there has been a trend towards decreasing mortality from stroke during the last twenty years, the prevalence is also increasing.[10]

According to a stroke register in Gothenburg, Sweden, in 1970 the incidence was estimated to be 150/100 000 population and year.[11]

Gender and Ethnic aspects

A frequently asked question about the ULSAM cohort is: why only men? And of course, if the cohort had been initiated today, both men and women would surely have been included, as in the PIVUS study that started in 2001 at the same department with the aim of investigating endothelial function and arterial compliance in 1000 subjects. In 1970 the situation was different, however. Cardiovascular diseases were considered to be mainly male afflictions, probably because men were victims at younger ages than women. The sudden event has often devastating consequences for the affected man, his family and the society.

Women are on average several years older than men when they suffer their first stroke. As women have a longer average lifetime however, the proportions of afflicted men and women are almost equal according to the stroke register in Gothenburg[11] Differences in risk factor profile between the sexes appear to be weak.[12]

It is well known that ethnicity plays an important role. The incidence among African-Americans is almost double that of white Americans, while Asian Americans, Native Americans and Hispanic Americans have stroke rates similar to those of white Americans.[13] In a recent study from Malmö the incidence of stroke was found to vary between immigrants from different regions, with a higher incidence among immigrants from Hungary and former Yugoslavia, but the same risk among those born in other Scandinavian countries, Germany, Chile, Poland and Czechoslovakia as for those born in Sweden.[14]

Cardiovascular risk factors

As evidence accumulates regarding the multifactor pathogenesis of vascular disease, there is a trend towards multimodal vascular prevention. The Framingham Heart Study and in Sweden the Gothenburg studies have played a key role in establishing these risk factors. Nevertheless, coronary heart disease (CHD) remains the leading cause of death in the Western world and it has been estimated by WHO that by 2010 cardiovascular disease will also be the leading cause of death in the developing countries.

Risk factors for stroke may be divided into modifiable and non-modifiable, the latter including age, sex, race and a family history of stroke. Most publications refer to risk factors for ischaemic heart disease in the broader context of atherosclerosis. Generally accepted risk factors are hypertension, smoking, diabetes, hyperlipidaemia, and physical inactivity. Other risk factors mentioned in the literature include the leucocyte count, lipoprotein(a), plasma fibrinogen, homocysteinaemia, and C-reactive protein. For stroke additional risk factors are atrial fibrillation (AF) and other cardiac diseases.

The association between risk factors and any-cause stroke varies between different types of stroke, although the different types share many of the conventional factors. Risk factors for stroke in the elderly may also differ from those in younger subjects, as congestive heart failure (CHF), AF and chronic nephropathy are known to increase in importance with age.[15]

The relation between a risk factor and a disease may be expressed as the *relative risk* (or *risk ratio*). In rare diseases the *odds ratio* is a good mode of appraisal. The risk ratio describes how the risk of having a disease changes if a certain factor is altered. If relative risks combine in a synergistic way, this indicates that each risk factor exerts its effect through different mechanisms.

Hypertension

This is the most important and most modifiable cerebrovascular risk factor[16] as confirmed by epidemiological data and by more recent intervention trials of primary and secondary prevention of stroke.[17, 18] It is also well known that the incidence of coronary heart disease (CHD) is higher in hypertensive than in normotensive patients even when the hypertension is treated.[19, 20] A suggested pathophysiological background is that hypertension promotes the development of atherosclerosis by causing impaired endothelial function and triggering acute plaque disruption.[21, 22] Hypertension has also been associated with impaired fibrinolytic activity and thereby with promotion of thrombus formation.[23]

Diabetes

Diabetic patients have an increased susceptibility to atherosclerosis and in particular have an increased prevalence of atherogenic risk factors, such as hypertension, obesity and abnormal blood lipids. Diabetes is associated with a two-to four-fold increase in the risk of ischaemic stroke[24] and also with a poorer stroke outcome and increased stroke mortality.[25] According to Risk-Stroke (2006), over 20 % of all stroke patients have diabetes.

Diabetes affects the central nervous system in different ways. It can lead to atherosclerosis in the large and small cerebral blood vessels, but it can also cause metabolic derangement in the brain tissue through prolonged hypoglycaemia, anoxia or ketoacidosis. Aggressive treatment of hypertension and dyslipidaemia has been shown to decrease the risk of stroke in diabetic patients.[26]

Dyslipidaemia

After a period with less focus on the lipid metabolism as an important risk factor for cardiovascular disease, studies in the 1990s have shed further light on the importance of dyslipidaemia. The Scandinavian Simvastatin Survival Study (4S) in 1994 demonstrated that hypercholesterolaemia is a strong risk

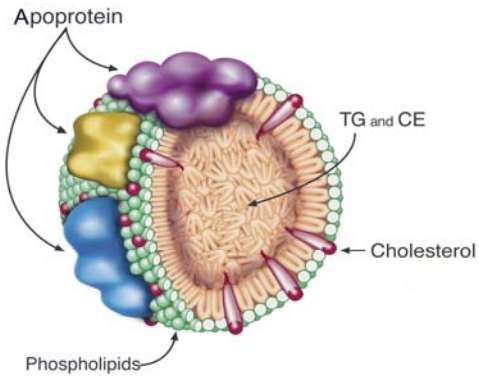

Figure 2. A lipoprotein particle. TG, triglyceride; CE, cholesterol ester

factor for CHD, especially the level of low-density lipoprotein (LDL)-cholesterol.[27] This together with new parameters for measuring lipid disorders has increased the interest in dyslipidaemia. Recently apolipoprotein B (apoB), apolipoprotein A1 (apoA1) and the ratio between apoB and apoA1 have shown predictive value in a Swedish study that has received much attention.[28] ApoB proved to be a stronger predictor than LDL-cholesterol, and apolipoproteins were also found to be important predictors of cardiac risk in subjects older than 70 years.

Lipoprotein(a), [Lp(a)] was discovered in 1963. It consists of an LDL-like particle and the specific apolipoprotein(a), which is covalently bound to the apoB of the LDL-like particle. Lp(a) plasma concentrations are highly inheritable and normally vary over one thousand-fold between individuals. The physiological function of Lp(a) is still unknown. As the structure is similar to plasminogen and it competes with plasminogen for its binding site, leading to reduced fibrinolysis, a function within the coagulation system seems reasonable. It is believed that because of its LDL cholesterol content, Lp(a) contributes to atherosclerosis.[29] Lp(a) cannot yet be regarded as a conventional, well established risk factor for cardiovascular disease (CVD), and its association with CVD seems complicated.[30]

Fatty acids

Dietary fat is mainly made up of triglycerides consisting of three individual fatty acids (FAs), each linked by an ester bond to a glycerol backbone. There are three major groups of fatty acids: saturated, monounsaturated and polyunsaturated (n-3 and n-6). Individual FAs have different biological effects dependent on their chain length, their degree of saturation and the isometric form. The more double bonds a fatty acid contains, the more unsaturated it is, although the FA chain will have a less regular shape. This gives unsaturated FA the ability to regulate metabolic processes in the cells; for example muscle cells with more unsaturated FAs in their membranes respond better to insulin, since the membrane is more “fluid” (Table 1).

Table 1. Fatty acids.

Saturated	Monounsaturated	Polyunsaturated
Myristic 14:0	Palmitoleic 16:1 n-7	Linoleic 18:2 n-6
Palmitic 16:0	Oleic 18:1 n-9	Gammalinolenic 18:3 n-6
Stearic 18:0		Alphalinolenic 18:3 n-3
		Dihomogamma-linolenic 20:3 n-6
		Arachidonic 20:4 n-6
		Eicosapentaenoic 20:5 n-3
		Docosahexaenoic 22.6 n-3

As a proxy for dietary fat quality, we investigated the serum fatty acid composition, which mainly reflects dietary fat quality over the past few weeks.[31, 32] A positive association between a diet high in saturated FAs, and the risk of CVD, and a negative association between a diet high in unsaturated fat and this risk have been documented,[33] but are not undisputed. Mechanisms that link dietary fatty acids with CVD have been found to be related to effects on blood cholesterol levels,[34-36] insulin resistance,[37] inflammation and endothelial dysfunction.[38] Palmitic (16.0) and palmitoleic (16:1) acids in particular seem to impair endothelial function.

The resting electrocardiogram (ECG)

In the history of electrocardiography there are two major landmarks: In 1887 Augustus Waller introduced ECG as a technique in humans and in 1901 Wilhelm Einthoven invented the electrocardiograph, for which he was later awarded the Nobel Prize. The resting ECG is the recording on the surface of the body of the heart's electrical activity. Although this is an old method, it continues to be the most widespread cardiovascular laboratory method and it is by interpreting the wave-formed pattern caused by the electrical activity that the most common heart disorders are diagnosed, such as myocardial infarction, cardiac ischaemia and arrhythmias. It has been widely used in epidemiological studies.

Left ventricular hypertrophy

An increased left ventricular mass has generally been thought to be the consequence of compensation by the ventricle for a haemodynamic stimulus, such as an increased demand for cardiac work. This increased mass is due to cardiomyocyte hypertrophy and is accompanied by proliferation and fibrosis.[39] According to the type of challenge to which the heart responds, the left ventricle changes either by thickening of the ventricular walls or by dilation. Both these cases may serve as compensation for augmented haemodynamic demands and improve heart function, as well as increasing the weight of the heart. The degree of left ventricular hypertrophy (LVH) was measured by ECG in our studies.

Metabolic syndrome

The existence of a syndrome involving important cardiovascular risk factors, including hypertension, glucose intolerance, hyperinsulinaemia, dyslipidaemia and obesity, was proposed by the Swede Eskil Kylin as early as in the 1920s (Kylin 1923). Later it has been called syndrome X, and as insulin resistance was suggested as the common link underlying these aberrations it has also been called the insulin resistance syndrome. Over the years several definitions and explanations have been suggested.

WHO has chosen the term metabolic syndrome since it is not fully clear whether insulin resistance is the key to all symptoms. Other symptoms have gradually been added to the syndrome, such as abdominal obesity, microalbuminuria, chronic subclinical inflammation, impaired fibrinolysis and left ventricular hypertrophy.

The most important definitions of the metabolic syndrome are the WHO definition,[40] the definition proposed by the European Group for the Study of Insulin Resistance (EGIR) and more recently a definition suggested from the Summary of the Third National Cholesterol Education Program (NCEP). They have much in common but also some differences. The WHO definition applies to both diabetic and non-diabetic persons, while that of EGIR applies only to non-diabetics. The most accepted definitions include the presence of insulin resistance or glucose intolerance together with two or more other components.

The prevalence, of course, depends on which definition is used. The lack of one single accepted definition leads to difficulties in comparing results between studies. The difficulty is partly due to the incongruence between a classification based on disease manifestations and phenotypes and the aetiological background. It is clear, however, that the syndrome increases in prevalence with age and that it is more common in men than in women.

Insulin resistance and insulin sensitivity

Impaired glucose regulation is the first abnormality leading to insulin resistance. Reduced insulin sensitivity or a finding of insulin resistance is common in type-2 diabetes mellitus but can also be found in non-diabetic subjects.[41] The term insulin sensitivity is generally used to define the ability of insulin to mediate glucose disposal in skeletal muscle, suppress lipolysis in the adipose tissue[42] and inhibit gluconeogenesis in the liver.[43] Insulin resistance is the condition when normal amounts of insulin are insufficient to produce a normal glucose uptake.

In normoglycaemic subjects a compensatory increase in pancreatic insulin secretion can maintain the blood glucose at a normal level, but this ability can decline over time. This resulting hyperglycaemia is explained by lower glucose uptake in skeletal muscles and less inhibition of hepatic glucose production, and it leads to increased levels of free fatty acids.[42]

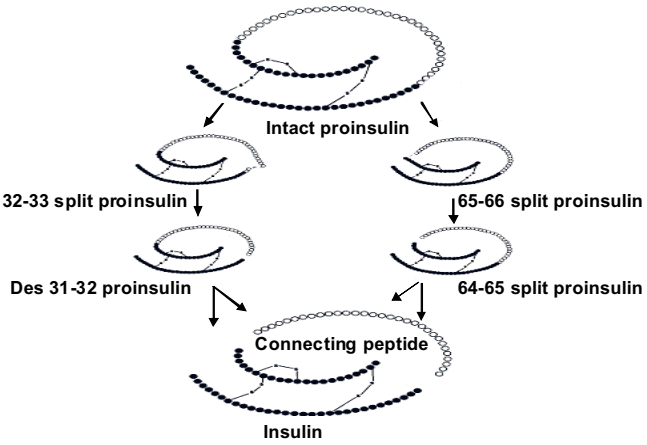

Figure 3. The conversion of intact proinsulin to insulin and connecting peptide.

The gold standard for measuring insulin sensitivity in skeletal muscle is the rather complicated hyperinsulinaemic euglycaemic clamp method. In epidemiological studies fasting serum insulin has often been used as a proxy.

Proinsulin and insulin

Proinsulin was first discovered in 1967.[44] The precursor of proinsulin, preproinsulin, is synthesised on the ribosomes in the endoplasmic reticulum in the beta cells in the pancreatic islets of Langerhans. By removal of a signal peptide, preproinsulin is then converted to proinsulin which is thereafter processed to insulin and connecting peptide (C-peptide) (Fig 3). As these processes are not perfect, partially converted split products will occur, of which 32-33 split proinsulin is the most dominant variant in plasma.[45] Hyperproinsulinaemia can be a sign of a primary reduction of the capacity for insulin secretion. An increased proportion of proinsulin in secretory granules may reflect a slower than normal rate of conversion from proinsulin to insulin.[46] Elevated concentrations of proinsulin have also been associated with insulin resistance, e.g. in oral and intravenous glucose tolerance tests or with the clamp method, and are also used as a marker of insulin resistance whereas the ratio between proinsulin and insulin is used as a marker of beta-cell function.

The elimination of both proinsulin and insulin takes place in the kidneys explaining why concentrations are increased in patients with renal failure.

Cognitive tests

Trail Making Tests (TMT) A and B are neuropsychological tests of visual attention and speed. The Taylor Number Series was the original form of the test, which consisted of connecting a series of numbers from 1 to 50. Later it was revised and its name was changed twice. In about 1944 it was given the

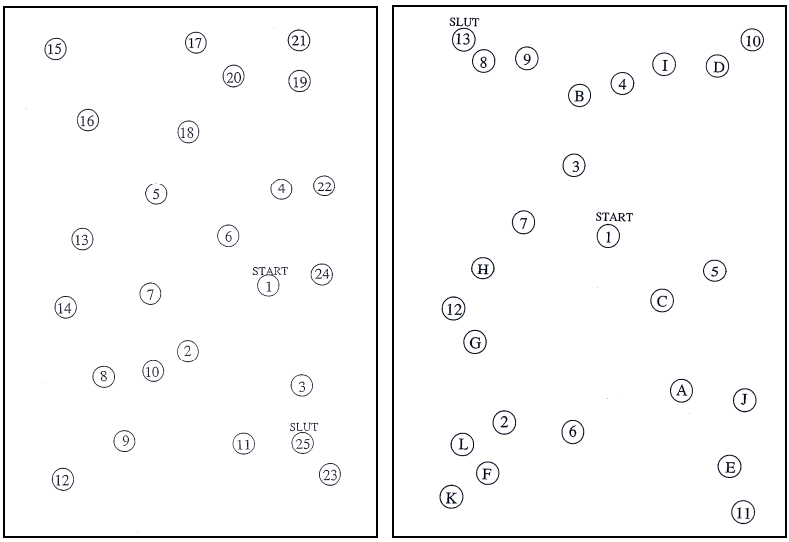

Figure 4. Trail Making Tests A and B.

present name and was used for assessing general intelligence (part of the Army Individual Test of General Ability), but has since become a common diagnostic tool in clinical settings, e .g in dementia screening. As both the tests are tests of speed, the examiner should stress the importance of time and efficiency.

Part A consists of encircled numbers from 1 to 25 randomly spread across a sheet of paper (Fig 4). It requires visual scanning, numeric sequencing and visuomotor speed. Part B is more complex and additionally requires visual-motor coordination and shift in organisation adequate enough to connect numbers and letters in an altering pattern. Trail Making Test B is especially used as a tool for identifying general frontal lobe dysfunction.

Mini-Mental State Examination (MMSE)[47] is a screening test for dementia and cognitive decline, the most widely used test for cognitive impairment both in clinical practice and in research. It takes about ten minutes and includes tests of orientation, memory, arithmetic skills, language use, comprehension and very basic motor skills.

Atherosclerosis

The word ‘atherosclerosis’ derives from the Greek “aterosclerose” and is a form of arteriosclerosis with atheromatous degeneration of bloodvessel walls. Arterio = artery, sclerosis = hardening, and athero = gruel. The atherosclerotic process passes through different morphological stages and is often a silent process, taking place over several decades. The first clinical manifesta-

tion is often dramatic, such as myocardial infarction, sudden death (death within one hour of onset of symptoms) or stroke.

Considerable effort has been devoted to attempts to detect the atherosclerotic process and its different pathways. The atherosclerotic lesion is considered to consist mainly of an accumulation of lipids, especially LDL, and inflammatory cells within the intima of the artery wall. This thickening in the intima can continue to develop into a fibrous plaque with a lipid core and a fibrous cap.[48] As a result of remodelling of the vessel, giving it an increasing diameter, the blood flow can be maintained and the patient can remain symptom-free. These plaques might rupture, some seeming more vulnerable to this than others, independently of the lesion size or degree of stenosis. Both external and internal conditions may be involved in this disruption leading to an acute event.

Insulin resistance may increase the risk for cardiovascular abnormalities, either through its effect on cardiovascular risk factors or by directly promoting atehrosclerosis. It has been claimed that a high degree of insulin sensitivity is associated with less atherosclerosis.

Aetiological factors and pathophysiology of stroke

Questions concerning aetiological factors in cardiovascular diseases have been extensively addressed and debated and are still receiving considerable attention.

Obesity, low physical activity and smoking, and also dietary factors inducing oxidative stress, all favour the development of insulin resistance.[49] A low birthweight has been associated with components of the metabolic syndrome[50, 51] and it has been hypothesised that this relationship is due to disturbances in cortisol activities.[52] It is also suggested that inflammatory markers such as C-reactive protein (CRP), fibrinogen and white blood cells may play a role, since the metabolic syndrome is correlated to increased concentrations of these markers. Thus a chronic inflammatory process is suggested as a triggering factor.

Other proposed pathophysiological factors underlying stroke are increased activity in the sympathetic nervous system[53] and defects in the actions of insulin receptors, insulin receptor substrates and glucose transport proteins, together with endothelial dysfunction causing changes in the function of the insulin receptors. In addition, the fatty acid compositions of the skeletal muscle membranes and the triglyceride contents in skeletal muscle and the liver have been proposed as underlying factors.

However, as non-obese, non-diabetic relatives of subjects with type-2 diabetes are more insulin resistant than non-diabetic controls, a genetic component is likely to exist. Genetic variations in enzyme actions involved in the lipid metabolism may also be of importance.

It is likely that the aetiology is multifactorial, including both environmental and genetic compounds.

Aims

The overall aim of these studies was to identify risk factors and predictors for incident fatal and non-fatal stroke or TIA, and for stroke subtypes, by analysing a comprehensive panel of variables and results from cognitive test performances. A further aim was to study the relations of these variables and cognitive test results to mortality and dependency after a first-ever stroke/TIA event.

The specific aims were:

Study I (Paper I). To investigate relations of baseline (ULSAM, age 50 years) lipo- and glucometabolic variables to increased risk for fatal and non-fatal stroke/TIA, independently of established risk factors during a follow-up of 32 years. Additionally, to investigate relations of these variables to subgroups of stroke, namely brain infarction, intracerebral haemorrhage and TIA.

Study II (Paper II). To determine whether proinsulin and insulin resistance in the ULSAM investigations at age 70 contributes to an increased risk of fatal and non-fatal stroke/TIA, independently of diabetes and established risk factors, during a follow up of 12 years. In addition, in the same cohort of men, to investigate relations of these variables to subgroups of stroke.

Study III (Paper III). To study the predictive power of cognitive test performance mainly related to subcortico-frontal pathways, for later occurrence of fatal or non-fatal stroke or transient ischemic attacks (TIA) and stroke subtypes. As in study II we used the findings at approximately age 70 as baseline. A further aim was to study the results of the composite screening test Mini Mental State Examination (MMSE), as stroke/TIA predictor.

Study IV (Paper IV). To determine whether the panel of variables measured in the ULSAM at age 70 had any predictive relation to mortality and dependency after a first-ever stroke/TIA event. Special interest was paid to the results of pre-stroke cognitive testing, as TMT-B was found to be a powerful predictor of brain infarction in study III. An additional aim was to validate the diagnosis obtained from the Cause of Death Register (CDR) och Swedish Hospital Discharge Record (SHDR) going through all medical records of those suffering a first-ever stroke/TIA during follow-up.

Subjects and methods

The ULSAM cohort

The Uppsala Longitudinal Study of Adult Men (www.pubcare.uu.se/ulsam) is a population-based study, which was initiated with the aim of identifying risk factors for cardiovascular disease. All men born in 1920-24 and residing in the county of Uppsala were invited at age 50 to participate in a health survey (in 1970-73), and 2,322 of 2,841 invited men agreed to be included (82%). All subjects gave informed consent and the local Ethics Committee of the Medical Faculty at Uppsala University has approved the studies on several occasions.

Between 1980 and 1984 the first re-examination took place and out of 2,130 eligible men who had participated in the first survey, 1,860 (87,5 %) attended. The third re-examination was at age 70. The ten-digit unique personal number was used to trace all eligible participants investigated at the first survey at age 50, and they were invited. This examination took place between 1991 and 1995. Out of 1,681 invited, 1,221 men participated (73 %) (Fig 5).

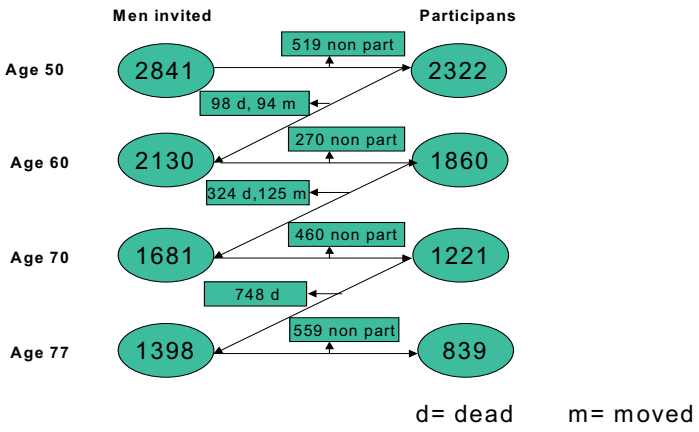

Figure 5. The ULSAM study.

A fourth, fifth and recently a sixth examination at ages 77, 82 and 88 have taken place. Those investigations are not included in the present studies. Studies I-IV were all based on data from the ULSAM, and were observational in nature.

Study participants

Study I

This study was based on the 2,322 men who participated in the baseline investigation at age 50. Nine subjects were excluded because of a history of stroke or TIA before the baseline examination.

Study II

Study II was based on the subjects who participated in the baseline examinations and in the re-investigation that took place 20 years later. Of the 1,681 available subjects invited to this re-investigation, 1,221 (73%) attended. Seventy subjects were excluded because of a history of stroke or TIA before the baseline examination, giving 1,151 participants in this study.

Study III

This study was based on the same participants that took part in study II and thereafter participated in subsequent testing of cognitive function (n=999), which was performed in 1993-96 and constituted the baseline in study III. Fifty-eight participants were excluded because of a previous history of stroke/TIA before the investigation at age 70 and another 11 because of a stroke/TIA between that examination and the cognitive testing rendering 930 participants eligible.

Study IV

Study IV was based on the same participants as in study III. Because of a stroke/TIA event before the investigation, 78 participants were excluded. Eight participants were excluded because of misdiagnosis, leaving 1135 eligible participants. In the subsequent testing of cognitive function, 930 took part and as 11 had a stroke/TIA in between the baseline examinations and the cognitive testing 919 were eligible.

Methods used in ULSAM

All investigations were carried out under standardised conditions and have been described in detail previously.[54] The investigations included a medical questionnaire and interview, blood sampling, anthropometric measurements and measurements of blood pressures.

Baseline investigations at age 50 years

Anthropometry

Height was measured to the nearest whole centimetre and weight to the nearest whole kilogram. Body mass index (BMI) was calculated as weight (in kg) divided by height squared.

Blood pressure and heart rate

Blood pressure (BP) was measured on the right arm after 10 minutes´ rest in the recumbent position and after another 2 minutes in the sitting position. Mercury manometers (Kifa Ercameter, wallmodel) were used. Systolic (SBP) and diastolic blood pressures (DBP) were read to the nearest 5 mmHg mark. DBP was recorded at the disappearance of the Korotkoff sounds (phase five). The blood pressure was taken either by a registered nurse or by a physician. The radial pulse rate was counted after 10 minutes´ rest before the BP measurement.

Glucose and glucose tolerance test

Blood glucose was measured by spectrophotometry using the glucose oxidase method. The intra-individual coefficient of variation (CV) for fasting plasma glucose was 2.9 %.

An intravenous glucose tolerance test (IVGTT) was performed and samples for determination of the blood glucose concentration were drawn before the start of the glucose injection.

Insulin and proinsulin

Concentrations of intact proinsulin and 32-33 split proinsulin were measured using the two-site immunometric assay technique (Sobey, 1989). Specific insulin concentrations were determined by means of the Access Immunoassay System (Beckman-Coulter), which uses a chemiluminescent immuno-

enzymatic assay. These analyses were carried out between 1995 and 1998 at the Department of Clinical Biochemistry, Addenbrooke's Hospital, Cambridge, UK, using plasma samples that had been stored frozen (at -70°C) since sampling.[55]

Serum lipids and apolipoproteins

Serum cholesterol and triglyceride (Tg) concentrations were measured in a Technicon Auto Analyzer type II in 1981-82 in serum samples that had been stored in liquid nitrogen since 1970-73. High-density lipoprotein (HDL) was assayed in the supernatant after precipitation with a heparin/manganese-chloride solution. LDL-cholesterol was calculated using Friedewald's formula: `LDL=serum cholesterol-HDL-(0.42 x serum Tg)`. The values presented are "Monarch adjusted", i.e. the values obtained were multiplied by a conversion factor to allow comparison with the Monarch method used at age 70.

Apolipoprotein(a) and ApoB were determined by a two-site immunoradiometric assay and ApoA1 by a competitive radioimmunoassay in 1988, with use of commercial kits from Pharmacia (Uppsala, Sweden), in samples that had been stored in liquid nitrogen since sampling.

Fatty acids

For analysis of the fatty acid composition of the serum cholesterol esters, lipids in serum were extracted with a hexane-isopropanol solution (1+4). Cholesterol esters were separated from the extract by thin layer chromatography before inter-esterification (acidic methanol at 85°C, 2 h), and free cholesterol liberated in the reaction was removed by an aluminium oxide column, to avoid contamination of the gas liquid chromatography column. The percentage composition of methylated fatty acids 14:0 to 22:6 was determined by gas chromatography.[56]

Principal components were determined, and only the first principal component was retained (eigenvalue 4.0) as a marker for the main variation in the fatty acid spectrum. Loadings (eigenvectors) of individual fatty acids on the fatty acid principal component (FAPC) are presented in Table 2. The interpretation of FAPCs in the present cohort has recently been described in detail.[57]

ECG and Minnesota codes

A standard 12-lead resting ECG was recorded at 50 mm/s and 10 mm/mV, including leads I, II, III, -aVR, aVL, aVF, and V1-6. The ECG machine was a direct-writing Mingograf 61 (Siemens-Elema Led, Solna, Sweden). The ECGs were classified according to the Minnesota Code (Blackburn, 1960) by two experienced physicians. The normal ECGs were first selected. The two physicians did the coding of the remaining ECGs independently. The final coding was not accepted until full agreement was reached.

Table 2. Relations of the individual serum cholesterol ester fatty acids to the fatty acid principal component (FAPC) at baseline

	FAPC eigenvectors
Myristic acid, 14:0	0.31
Palmitic acid, 16:0	0.36
Palmitoleic acid, 16:1	0.40
Stearic acid, 18:0	0.09
Oleic acid, 18:1	0.39
Linoleic acid, 18:2 n-6	-0.48
Gammalinolenic acid, 18:3 n-6	0.27
Alphalinolenic acid, 18:3 n-3	0.11
Dihomogammalinolenic acid, 20:3 n-6	0.20
Arachidonic acid, 20:4 n-6	0.17
Eicosapentaenoic acid, 20:5 n-3	0.25
Docosahexaenoic acid, 22:6 n-3	0.10

Diseases and drugs

Diseases were classified according to the code of the WHO International Classification of Diseases (ICD-8). The criteria used were that a disease should have given reason for a disability pension or led to inability to work for more than six months prior to the examination. The only exception was a serious disease, which was always recorded irrespective of its duration. Subjects with a disease requiring long-term medication were also recorded even if the participant was able to work. Overall 250 individuals (10.8%) were classified. Forty-three of them had a disability pension.

Drugs were classified in accordance with the then current list of pharmaceutical specialities available in Sweden (FASS 1974). Only daily drug consumers who were on long-term medication were included in the classification. Overall 224 of the participants (9.6%) were taking daily medication with one or more drugs. The most common drugs were antihypertensive agents.

Hypertension treatment was classified as treatment with diuretics, beta-adrenergic blocking agents, vasodilator drugs, sympathetic blocking drugs, hydralazines, or combined antihypertensive drugs. *Hypertension prevalence* was defined as a supine blood pressure of 140/90 or higher and/or treatment with antihypertensive drugs for hypertension.

Hyperlipidaemia prevalence was defined as serum cholesterol above 6.5 mmol/l and/or serum triglycerides above 2.3 mmol/l and/or hyperlipidaemia treatment.

The treatment groups had the following priority: 1) hypertension, 2) hyperlipidaemia, 3) glucose intolerance, and 4) normal.

Diabetes prevalence was defined according to the critera of the 1997 American Diabetes Association: (fasting blood [FB] glucose above or equal to 6.1 mmol/l) or anti-diabetic therapy.

Cardiovascular disease was defined as being hospitalised before the investigations at age 50 because of a heart attack or heart failure according to SHDR.

The metabolic syndrome

The metabolic syndrome was defined according to the National Cholesterol Education Program, with the slight modification that BMI was used instead of waist circumference.

Questionnaire and interview

A self-administered medical questionnaire was used to gather information regarding smoking habits, and cause of and age at death of parents.

Baseline investigations at 70 years

Anthropometry

Height was measured to the nearest whole centimetre, and body weight to the nearest 0.1 kg. BMI was calculated as the ratio of the weight (in kg) to height squared (kg/m^2). The waist and hip circumferences were measured in the supine position. The waist was measured midway between the lowest rib and the iliac crest and the hip circumference over the widest part. The waist/hip ratio was calculated.

Blood pressure

Blood pressure was measured in the right arm with the subject in the supine position after resting for 10 minutes, and then after he had been standing for 2 minutes. The values were recorded twice and to the nearest even figure. The mean of the two values is given for each blood pressure. The cuff size was 12x35 cm or 15x45 cm depending on the arm circumference. Systolic and diastolic blood pressures were defined as Korotkoff phases I and V, respectively.

Glucose

Plasma glucose in samples from an oral glucose tolerance test was measured by the glucose dehydrogenase method (Gluc-DH, Merck, Darmstadt, Germany). The intra-individual CV for fasting plasma glucose was 3.2%.

Insulin and proinsulin

Plasma insulin from the oral glucose tolerance test and the clamp study was measured by an enzymatic-immunological assay (Enzymmun, Boehringer Mannheim, Germany) performed in an ES300 automatic analyser (Boehringer Mannheim) and the result is given in mU/l. For conversion to pmol/l, multiply by 6.0.

The concentrations of intact proinsulin and 32-33 split proinsulin were determined with the same methodology as described on page 31.

Euglycaemic hyperinsulinaemic clamp

To estimate in vivo sensitivity to insulin the euglycaemic hyperinsulinaemic clamp technique described by De Fronco (1979), with slight modifications, was used. Basal samples were taken 40 minutes after cannulation. Semisynthetic regular human insulin was infused at a primary dose for the first 10 minutes and then as a continuous infusion (at 56 mU/min per body surface area (m^2), to maintain steady state hyperinsulinaemia.

The level of plasma glucose during the clamp study was maintained by measuring the plasma glucose every 5 minutes and adjusting the rate of infusion of a 20% glucose solution accordingly. The target plasma glucose level was 5.1 mmol/l. Plasma was immediately separated in a centrifuge and plasma glucose was assayed in duplicate in a glucose analyser. Steady-state plasma glucose, mean glucose and plasma insulin concentrations were calculated as the mean of all values obtained between the 60th and 120th minute of the clamp study.

The total amount of glucose infused serves as a measure of the subject's sensitivity to the prevailing plasma insulin concentrations.The glucose disposal (M) was calculated as the amount of glucose taken up during the last 60 minutes of the study and is given in mg/kg bw/min. The insulin sensitivity index (M/I ratio) is a measure of the tissue sensitivity to insulin expressed per unit insulin and was calculated by dividing M by the mean insulin (I) concentration during the same period of the clamp. M/I thus represents the amount of glucose metabolised per unit of plasma insulin and is given in mg/kg bw/min per mU/l of insulin multiplied by 100.

The calculation of the total body insulin sensitivity is based on the assumption that endogenous hepatic glucose production is entirely suppressed. It is known that under euglycaemic conditions almost 90% of this production is suppressed when the plasma insulin concentration is increased by 50 mU/l.

Lipids and lipoproteins

Cholesterol and triglyceride concentrations were measured in serum and in the isolated lipoprotein fractions by enzymatic techniques using IL Test Cholesterol Trinder's Method and IL Test Triglyceride Enzymatic-colorimetric Method for use in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA). HDL particles were separated by precipitation with magnesium chloride/phosphotungstate. LDL-cholesterol was calculated using Friedewald's formula.

The apolipoproteins were analysed in a random subsample of 550 men. ApoB and ApoA-l concentrations were determined by turbidimetry in a Monarch apparatus (Instrumentation Laboratories, Lexington, USA), using monospecifc polyclonal antibodies against apo B and A-l.

Apo(a**)** was measured by the Pharmacia Apo(a) RIA method (Pharmacia (a) RIA, Pharmacia Diagnostics AB, Uppsala, Sweden 1985).

Apolipoprotein E

Apolipoprotein (Apo) E was genotyped using a single-base primer extension assay with fluorescence polarization detection[58] using reagents from Perkin-Elmer.

Diseases

Hypertension treatment was defined as treatment with antihypertensive drugs. *Hypertension prevalence* was defined as a supine blood pressure of 140/90 mmHg or higher and/or treatment with antihypertensive drugs for hypertension. Treatment with these drugs for other reasons, e.g. cardiac failure, is thus not included in this definition.

Diabetes prevalence was defined according to the 1997 criteria of the American Diabetes Association: FB glucose above or equal to 6.1 mmol/l) or antidiabetic therapy.

Cardiovascular disease was defined as being hospitalised before the investigations at age 70 because of a heart attack or heart failure according to SHDR.

Questionnaires

Two self-administered optically readable questionnaires were used. One was a questionnaire on the general and medical background and the other concerned living conditions.

Smoking

During the clamp investigation, a nurse or a technician posed the question "Do you smoke?" to the participants.

Cognitive function tests

Three different cognitive tests were used. In TMT-A the test person is asked to draw lines with a pencil between numbers in the right order, as fast as possible. The score is equal to the time taken to complete the test, in seconds. The standard TMT-A consists of digits 1-25.

The second test was TMT-B and it consists of digits and letters (1-A-2-B – K-12-L-13). The maximum time set for TMT-B was 240 seconds. Long completion time is related to poor cognitive function. Part B of the TMT tests the same skills but is a better indicator as it places higher demands on attention, coordination, speed and flexibility.

The MMSE[47] is a screening test for dementia and cognitive decline, and is widely used both in clinical practice and in research. It has the advantage of being easy to administer, and has a high replicability. On the other hand, it is insensitive to minor cognitive dysfunction. The cognitive domains tested include orientation, registration, memory, attention, calculation, language, and constructional praxis, abilities related to cortical brain functions mainly

Points	0	+1	+2	+3	+4	+5	+6	+7	+8	+9	+10
Age	55-56	57-59	60-62	63-65	66-68	69-72	73-75	76-78	79-81	83-84	85
SBP-untrd	97-105	106-115	116-125	126-135	136-145	146-155	156-165	166-175	176-185	186-195	196-205
or SBP-trtd	97-105	106-112	113-117	118-123	124-129	130-135	136-142	143-150	151-161	162-176	177-205
Diabetes	No		Yes								
Cigarettes	No			Yes							
CVD	No				Yes						
AF	No				Yes						
LVH	No					Yes					

Figure 6. Score your stroke risk. Key: **SBP** = systolic blood pressure (score one line only, untreated or treated); **Diabetes** = history of diabetes; **Cigarettes** = smokes cigarettes; **CVD** (cardiovascular disease) = history of heart disease; **AF** = history of atrial fibrillation; **LVH** = diagnosis of left ventricular hypertrophy.

Worksheet developed from National Institute of Neurological Disorders and Stroke (NINDS)-supported work.

in the temporal and parietal lobes. The scores range from 0 (worst) to 30 (best). MMSE was added to the test battery after the start, and thus the number of participants is slightly smaller than in the other cognitive tests.

Education and social class

The coding of education was as follows: 1. Elementary school only (6 to 8 years), 2. Secondary school (12 years), 3. Three or more years of college or graduate exam. The coding of smoking, alcohol intake and social group classification was based on interview reports. The three conventional social classes were used and classified according to the Central Bureau of Statistics.

Framingham stroke risk score

In study III the Framingham stroke risk score was calculated according to its original description[59] (Fig 6).

Dependency

The medical records from the hospitalisation of those suffering a first-ever stroke or TIA during follow-up (n=217), were reviewed by one physician (BW). Those participants discovered to have had an earlier stroke/TIA event that was not found in the SHDR or found with a misdiagnosis in the discharge record were excluded.

Dependency was defined according to the destination of the patient on discharge after the hospitalisation (with or without rehabilitation), using three levels: 1) going home 2) to a nursing home 3) dead. Those already in a nursing home at the time for the event (n=4) were recoded as 2). Subjects tested (n=90, 61%) by a physiotherapist with the Motor Assessment Scale (MAS) during the hospital stay were noted and the results were used as per cent of possible maximum score in the dependency analyses.

Present investigations

Follow-up and outcome measures in study I

The subjects were followed up from the baseline investigation at age 50 (in 1970-73) to December 31, 2002. They had a median follow-up time of 29.3 years (range 0.04 to 32.7), contributing to 57,769 person-years at risk (PYAR).

Follow-up and outcome measures in study II

The subjects were followed up from the baseline investigation at age 70 (August 1991 - May 1995) to December 31, 2002. They had a median follow-up time of 8.8 years (range 0.01 to 11.4), contributing to 9,395 person PYAR.

Follow-up and outcome measures in study III

The subjects were followed up from the baseline investigation at age 70 (August 1991 - May 1995) to December 31, 2006. They had a median follow-up time of 11.1 years (range 0 to 13.6), contributing to 8,958 PYAR.

Follow-up and outcome measures in study IV

The subjects were followed up from the baseline investigation at age 70 (August 1991 - May 1995) to December 31, 2006. They had a median follow-up time after the event of 2.5 years (range 0 to 12.2), contributing to 566 PYAR.

Register data

The Cause of Death Register

The mortality data were obtained from the Cause of Death Register. This includes information on specific causes of death obtained from death certificates, collected by local parish registries. The register includes all deaths of persons registered in Sweden at the time of death; deaths occurring outside Sweden are thus also recorded for those persons. The register is updated

annually and contains the personal identification number, home town, underlying cause of death, nature of any injury, multiple causes of death, date of death, basis for statement of cause of death, sex and age. ICD-9 and 10 are used for classification of the causes of death.

The Hospital Discharge Record

Information on hospitalisation was obtained from the Swedish Hospital Discharge record (SHDR). The medical data in the register include main diagnosis, secondary diagnoses, external causes of injury, and poisoning and surgical procedures. The register is updated annually. One physician (BW) reviewed all medical records from the hospitalisation of those suffering a first-ever stroke/TIA after the investigations at age 70.

End-points

To determine the occurrence of end-points, two registries were used, the SHDR and CDR. Some participants experienced more than one of the specified end-points during the follow-up period and could appear in more than one category. Only the first occurrence of the end-point studied in a particular model merited censoring.

The end-points investigated in the studies I and II were, in order of priority:

1. Fatal or non-fatal stroke or TIA (ICD-9 codes 430-32 and 434-36, ICD-10 codes I60-I64, I66 and G45)
2. Fatal or non-fatal BI or TIA (ICD-9 codes 434-35, ICD-10 codes I63, I66 and G45)
3. Fatal or non-fatal intracerebral haemorrhage (ICD-9 codes 431-32, ICD-10 codes I61-62)

In study III the end-points were somewhat narrowed to get a more precise stroke/TIA evaluation and were, in order of priority:

1. Fatal or non-fatal stroke or TIA (ICD-9 codes 430-31 and 434-36, ICD-10 codes I60-61, I63.0-I63.5, I63.7-9, I64 and G45)
2. Fatal or non-fatal BI (ICD-9 code 434, ICD-10 codes I63.0-I63.5, I63.7-9)
3. Fatal or non-fatal intracerebral haemorrhage (ICD-9 code 431, ICD-10 codes I61).

In study IV we used the same end-points as in study III numbers one and two. In study IV only the first-ever stroke/TIA event was considered. Every discharge record was scrutinised and thereafter six participants were excluded because of misdiagnosis and another 15 because they had had an earlier stroke/TIA event not found at the first exclusion.

Statistical analysis

General analyses

Distributions were tested for normality by the Shapiro-Wilk W test and logarithmic transformation was performed when W was <0.95. The prognostic value of a one standard deviation (SD) difference in the continuous variables or transfer from one level to another of the dichotomous variables was investigated with Cox proportional hazard ratios. Two-tailed 95 % confidence intervals and p values were given. In the dependency analyses in study IV logistic regression analyses were used. The statistical software package Stata 6.0 (Stata Corporation, College Station, USA) was used in study I, and Stata 10.0 in studies II-IV.

Nelson-Aalen and Kaplan–Meier curves

Nelson-Aalen or Kaplan-Meier curves were used to confirm proportionality of hazards and to graphically describe risk over time.

Specific analyses

Study I

Two sets of models were investigated: one adjusting for treatment with anti-hypertensive, antidiabetic and lipid-lowering drugs; and the other adjusting additionally for hypertension, diabetes, the metabolic syndrome, serum cholesterol, smoking, and physical activity. We also estimated the incidence rates of the end-points by quartiles of the independent variables, to assess possible non-linear relationships. Two sets of models were investigated: one adjusting for treatment with anti-hypertensive, antidiabetic and lipid-lowering drugs; and the other adjusting additionally for hypertension, diabetes, the metabolic syndrome, serum cholesterol, smoking, and physical activity. The statistical power for detecting hazard ratios of 1.5 and 1.3 was 99.6 % and 80.1 %, respectively, assuming equally sized groups.

Study II

Three sets of models were examined in a hierarchical fashion: unadjusted; adjusted for diabetes at baseline; and adjusted for diabetes plus other established risk factors for stroke (hypertension, AF, ECG-LVH, serum cholesterol, and smoking) determined at baseline. Nelson-Aalen curves were used to confirm proportionality of hazards and to graphically describe risk over time.

Study III

Three sets of models were examined in a hierarchical fashion: unadjusted; adjusted for education and social group, and additionally, in a third group: SBP, hypertension treatment, serum cholesterol, diabetes mellitus, AF, ECG-LVH and current smoking. Secondary models were investigated with use of two sets of dichotomous MMSE and TMT-A and B variables with two different cut-offs: 1) worst quartile vs. other three quartiles, and 2) above vs. below median. Secondary models were also investigated by adding the Framingham Stroke Risk Score to models B.

Study IV

We examined three sets of models in a hierarchical fashion: adjusted for age at baseline: adjusted for age and cardiovascular drugs at baseline, and a third group with the following baseline covariates: age, education, social group, SBP, hypertension treatment, serum cholesterol, diabetes mellitus, AF, ECG-LVH and current smoking.

In the dependency analyses logistic regression analyses were used. In one model we adjusted for age and in a second model for age and the result from the Motor Assessment Scale in per cent, which was examined in the acute phase during the hospital stay and was available for 61 % of the participants suffering a stroke/TIA.

Discussion of methods

Selection bias: general aspects

Participation

The participation rate in study I was 82 % and the rate in studies II-IV was 73 %. Higher participation rates are rarely found in population-based studies. It is known that non-participants in cohort studies generally have more social and alcoholic problems and higher morbidity than participants.[60]

Mortality and morbidity analysis

By virtue of the official cause of death registry in Sweden, which includes all deaths of persons registered in Sweden at the time of death there was no loss of mortality data due to missing information.

Neither was there any loss of morbidity data, since all hospital admissions in the Uppsala health care region have been reported since 1965. Since 1984 the reporting to the SHDR has been mandatory in Sweden, and since 1987 it has covered all public, in-patient care in Sweden.

The study samples are also representative of the general population in Sweden regarding stroke incidence, according to data published by the Swed-

ish National Board of Health and Welfare (http://www.sos.se/sosmenye.htm). The accuracies of the CDR and SHDR have been shown to be high regarding stroke diagnosis.[61] Hospital discharge records were scrutinised in 50 random stroke cases in study I and a good coverage was found between the registered diagnosis and the actual event. In study IV all medical records of the participants suffering a first-ever stroke/TIA after the investigation at age 70 were examined. As a result six were excluded because of misdiagnosis and another 15 because they had had an earlier stroke/TIA that was not noted in SHDR.

Proinsulin

Owing to a freezer failure, proinsulin at age 50 was assayed in a random sample of only 1,335 out of a total of 2,322 subjects at that age.

The proinsulin analyses at age 50 were performed in samples that had been stored in liquid nitrogen for 15 years and thereafter stored frozen at minus 70°C. It cannot be ruled out that long-term storage may have influenced the absolute values of proinsulin. However, in that case all samples would have been affected in the same manner. Range specific deviations were not observed, using Bland-Altman plots.[55]

Smoking

Ex-smokers were classified as non-smokers regardless of how much they had smoked during their lifetime. In an earlier investigation of the ULSAM participants regarding the risk of fatal and non-fatal myocardial infarction (MI) ($p=0.52$) or total mortality ($p=0.56$) in non-smokers compared with ex-smokers, no differences were found.

End-points

The accuracy of the ICD diagnosis is high, especially since CT's sensitivity for bleeding is as high as nearly 100 %. CT has been in general use since the beginning of the 1990s in patients at the University Hospital, Uppsala presenting with symptoms of stroke or TIA. On examination of 217 medical records from subjects thought to have experienced a stroke/TIA after the investigation at age 70, it was found that at least 178 (91 %) had undergone a brain CT. In another two an autopsy had been performed.

Results and discussion

Study I

Results

A first hospitalisation for stroke/TIA occurred in 421 cases during follow-up. A first BI/TIA was experienced by 308 men and a first ICH by 86 men (Fig. 7).

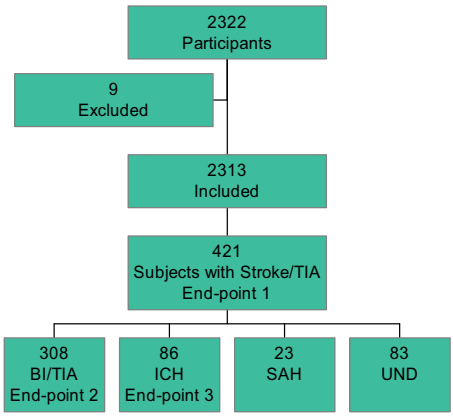

Figure 7. Description of end-points.

In univariate models BMI, systolic and diastolic blood pressures, use of antihypertensive medication, ECG-LVH, proinsulin, lipoprotein(a) and uric acid were all related to both the stroke/TIA and the BI/TIA outcomes. Smoking was associated with a higher risk for stroke/TIA but not for BI/TIA.

In models adjusting for treatment with antihypertensive, antidiabetic and lipid-lowering drugs, the variables SBP, DBP, ECG-LVH, proinsulin and Lp(a) were related to both the stroke/TIA and the BI/TIA outcomes. BMI and smoking were associated with a significantly elevated risk for stroke/TIA, but not for BI/TIA. Diastolic blood pressure, ECG-LVH and smoking were significant predictors of ICH.

When adjusting additionally for hypertension, diabetes, metabolic syndrome, serum cholesterol, atrial fibrillation, cardiovascular disease, smoking, and physical activity the significant predictors of stroke/TIA were all retained with the exception of BMI and proinsulin. In these models, SBP, ECG-LVH and Lp(a) were related to a significantly higher risk of stroke/TIA and BI/TIA.

The longitudinal relations of the levels of FAPC and Lp(a) to the incidence of stroke/TIA are presented in Figure 8a and b.

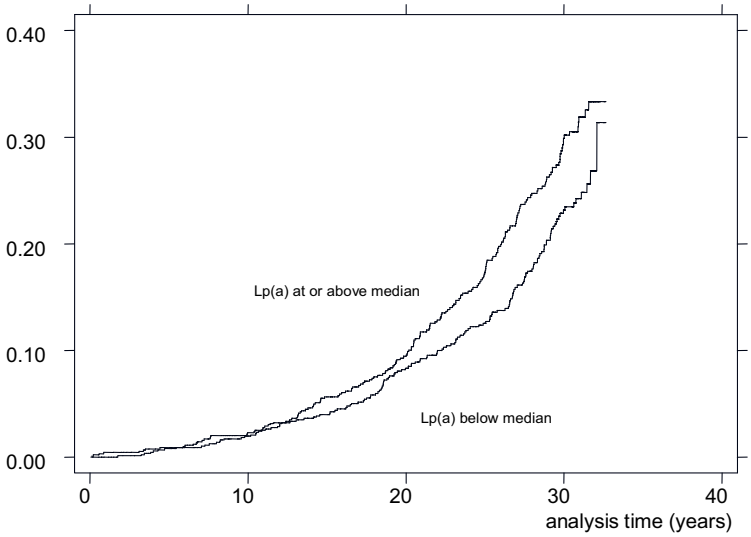

Figure 8a. Cumulative incidence of stroke/TIA by lipoprotein(a).

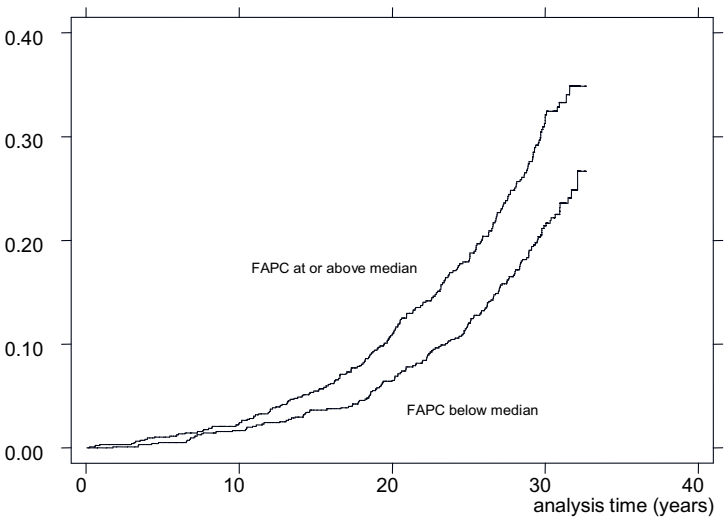

Figure 8b**.** Cumulative incidence of stroke/TIA by FAPC.

Fatty acids

In univariate models higher proportions of palmitic, palmitoleic and oleic acid were associated with an increased risk for stroke/TIA and BI/TIA, whereas a higher proportion of linoleic acid was protective against stroke/TIA and BI/TIA. A 1-SD increment in the FAPC was related to a 17 % increase in the risk for stroke/TIA, No significant relation between serum fatty acids and subsequent ICH was observed.

When additional adjustments were made for hypertension, diabetes, metabolic syndrome, serum cholesterol, atrial fibrillation, cardiovascular disease, smoking and physical activity, the fatty acid pattern and the FAPC followed essentially the same trend as in the more parsimonious models.

Discussion

An important finding in this study carrying new knowledge, is that an unhealthy dietary fat intake is a risk factor for stroke, indicating that modification of food habits may play a role in attempts to lower the stroke incidence. Another finding is that a high serum Lp(a) level predicts fatal and non-fatal stroke/TIA independently of established risk factors. Regarding glucometabolic variables, proinsulin showed the highest standardised hazard ratios.

As the cholesterol ester (CE) fatty acid composition in the serum is largely determined by the quality of the dietary fat ingested over the past few weeks,[31] it may be regarded as a proxy for dietary fat quality. Previous studies of the present cohort have shown that a fatty acid profile indicating a high dietary intake of saturated fats and a low intake of linoleic acid is related to insulin resistance,[62] and that it longitudinally predicts LVH[63] and myocardial infarction.[56] In the present study, the same pattern indicating a high dietary intake of saturated fats and a low intake of linoleic acid was related to the incidence of stroke/TIA. The fatty acid principal component from the factor analysis of all CE fatty acids had high positive loadings by shorter saturated fatty acids and a high negative loading by linoleic acid. The FAPC may therefore be perceived as a marker for dietary saturated vs. unsaturated fat.

Our observations confirm and extend those from two case-control studies.[64, 65] Nevertheless, intervention trials have not been unanimously able to confirm significant reductions in cardiovascular mortality or morbidity or in carotid artery disease when the diet was markedly enriched with linoleic or fish long-chain fatty acids.[66, 67] Linolenic acid has been found to be favourably related to some surrogate cardiovascular end-points and to lower platelet aggregation,[68] and to be cross-sectionally related to decreased carotid artery atherosclerosis,[69] and has been suggested as a promising target for neuroprotection after focal brain ischaemia.[70] In summary, the relations between fatty acid patterns and stroke incidence are to some extent still unclear.

A weak epidemiological correlation between hypercholesterolaemia and an increased risk for stroke has been demonstrated.[71] In the present study

none of the established lipid measures, or Apo A-1 and B, were predictors of stroke/TIA, which is also an important finding.

A large longitudinal study in the elderly indicated that Lp(a) was an independent risk factor for stroke,[72] as was also found in a population-based case-control study,[73] and it has been shown to be a predictor of combined cardiovascular events in a large cohort study.[74] In the present study we have confirmed these observations in a younger sample.

The relation of proinsulin to stroke has previously been pointed out in a case-control study.[75] Serum proinsulin should probably be regarded as a proxy for insulin resistance, as it is unlikely that proinsulin has metabolic effects of its own in the serum concentration ranges observed in population samples.[76] Insulin resistance, measured indirectly, has previously been shown to be prevalent among stroke/TIA patients.[77] However, intervention studies using proinsulin for glucose lowering in diabetic patients were all prematurely ended due to CVD side effects.[78]

Of the established risk factors for stroke for which we made adjustments in the present study, four have repeatedly been found in previous studies to play a major role, namely high blood pressure, LVH, smoking and atrial fibrillation.[79, 16, 80, 81] In our study we could again confirm that high blood pressure, ECG-LVH and smoking increase the risk for stroke/TIA and ICH.

Study II

Results

During follow-up there were 150 cases of stroke/TIA of which 89 were brain infarction and 27 were intracerebral haemorrage. TIA occurred in 34 cases (Fig 9).

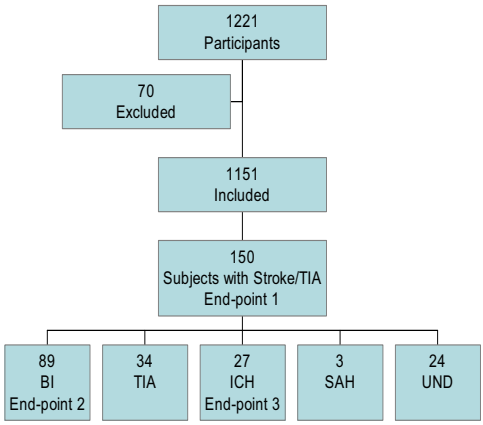

Figure 9**.** Description of end-points.

In univariate models, hypertension, ECG-LVH, atrial fibrillation, diabetes, plasma insulin, intact and split 32-33 proinsulin, and the M/I ratio were all associated with a subsequent stroke/TIA, whereas fasting plasma glucose, BMI, waist circumference, serum cholesterol and current smoking were not. A similar picture emerged when BI was used as the outcome variable, but none of the glucose and insulin variables were significant predictors of ICH. The predictive value of intact proinsulin and the M/I ratio for stroke/TIA was preserved when adjustment was made for diabetes, but not after adjustment for plasma insulin or split 32-33 proinsulin.

In multivariate models with additional adjustments for hypertension, atrial fibrillation, ECG-LVH, serum cholesterol and current smoking, intact proinsulin was retained as a significant independent predictor of stroke/TIA, but not of BI. No significant risk was related to the M/I ratio. None of these variables were significant predictors of ICH.

The longitudinal relation of intact proinsulin to incidence of stroke/TIA is presented in Figure 10.

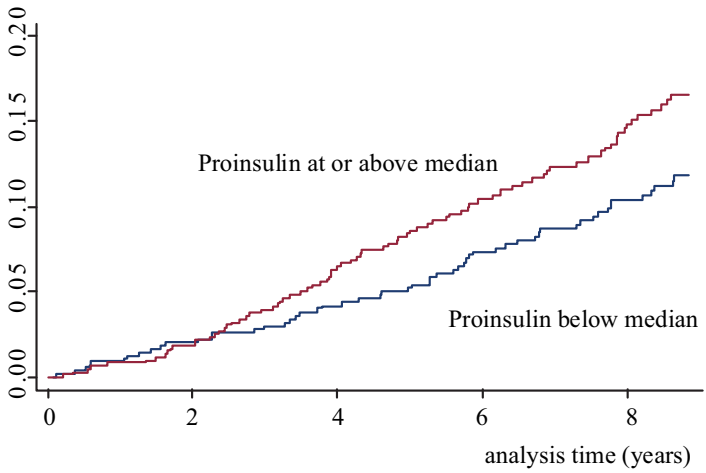

Figure 10. Cumulative incidence of stroke/TIA by proinsulin

Discussion

A low insulin sensitivity index derived from the euglycaemic insulin clamp, and elevated fasting intact proinsulin levels, predicted subsequent stroke/TIA over a follow-up period of 8.8 years independently of diabetes.

Intact proinsulin also preserved its predictive value after adjustment for other traditional risk factors. No other variable in the investigated panel, including true insulin and plasma glucose, which are usually measured on a routine basis in medical care, had the same predictive value. Proinsulin can serve as a good surrogate risk marker for insulin resistance, as a predictor of stroke/TIA.

The present finding that elevated intact proinsulin was possibly a stronger predictor of stroke/TIA than was true insulin in this elderly population extends our previous observations concernining the predictive power of proinsulin for myocardial infarction[82] and heart failure.[83] Our results may indicate that the risk of stroke/TIA is increased in the long subclinical phase of impaired glucose regulation that precedes clinically manifest diabetes, since an elevated proinsulin level is a predictor of diabetes development. Thus, proinsulin is a powerful predictor of stroke/TIA and was furthermore a significant predictor independently of diabetes and other established risk factors for stroke.

Defective insulin action as assessed by the euglycaemic insulin clamp is associated with a well-established cluster of abnormalities, consisting of type 2 diabetes mellitus, hypertension, dyslipidaemia and abdominal obesity, elevated plasma triglycerides, decreased HDL-cholesterol and microalbuminuria, which together constitute the insulin resistance syndrome.

The present study showed insulin resistance, as evaluated by this gold standard technique, to be a predictor of stroke/TIA independently of diabetes. However, when additional adjustments were made for all established risk factors for stroke, the M/I ratio was attenuated and was no longer significant (p=0.107). This further emphasises the difficulties in separating the different components of the metabolic syndrome in terms of independent risk predictors.

There are numerous possible explanations for the observed relation of proinsulin and insulin resistance to the incidence of stroke/TIA. First, high plasma proinsulin concentrations are associated with coronary artery atherosclerosis[84] and with an increased intima-media thickness in the common carotid artery.[85] Second, elevated plasma non-esterified fatty acids are known to aggravate the insulin-resistant state both in skeletal muscle and in the liver, and also to have direct vascular effects such as promotion of endothelial dysfunction[86, 87]and elevation of blood pressure.[88] Third, insulin resistance is related to an increased pressor response to angiotensin II (X) and has been found to enhance the stimulating effects of angiotensin II on cellular hypertrophy and collagen production[89] in individuals with hypertension. Fourth, a deranged microcirculation with vascular hypertrophy and rarefaction has been proposed as a central pathological feature in insulin resistance.[90]

To our knowledge, the ULSAM cohort is the largest single-centre population-based cohort that has been examined with the gold standard for measurement of insulin resistance, the euglycaemic insulin clamp method.

Study III

Results

During a median follow-up of 11.1 years (maximum 13.6 years), 166 cases of our primary end-point stroke/TIA occurred (incidence rate 18.5/1000 PYAR); 105 cases of BI and 24 cases of ICH occurred (incidence rates 11.4 and 2.5/1000 PYAR, respectively), (Fig. 11). During follow-up 364 men died.

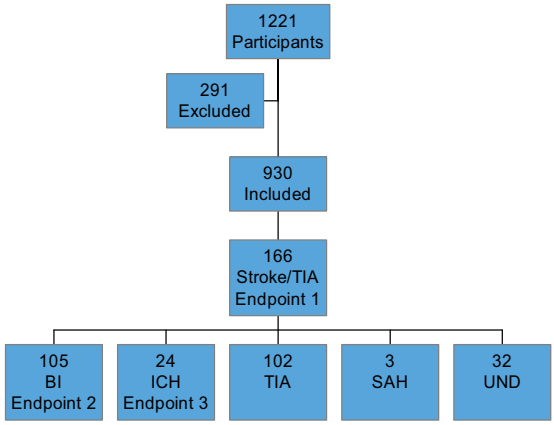

Figure 11. Description of end-points.

Modest associations between TMT-A results and stroke risk were observed. In unadjusted Cox proportional hazards models no associations between TMT-A results and stroke risk were seen. In multivariable-adjusted models longer TMT-A completion time was borderline statistically significantly associated with a higher risk of subsequent brain infarction, but not with risk of the broader primary end-point of stroke/TIA. No associations between TMT-A times and risk of intracerbral haemorrhage were observed.

Higher TMT-B times were weakly related to the primary end-point stroke/TIA but were consistently related to risk of subsequent brain infarction in all models. In multivariable-adjusted models, the risk of brain infarction was 48 per cent higher per SD longer TMT-B time. Adjusting for the same covariates, the risk of brain infarction increased gradually with higher quartiles of TMT-B, with a more than three-fold risk in the highest compared to the lowest (Fig.12).

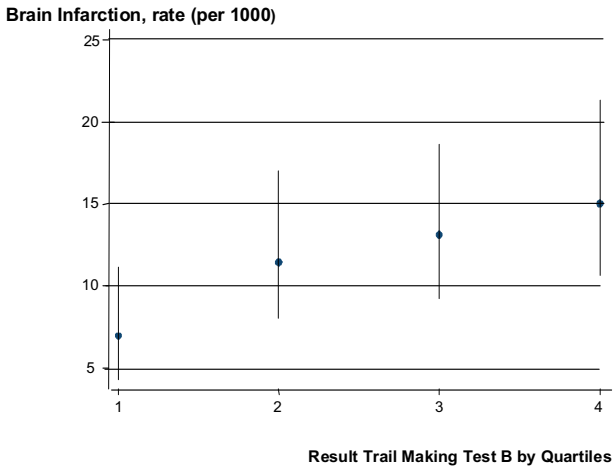

Figure 12. Incidence rate of brain infarction, rate (per 1000 person-years at risk) by quartiles of Trail Making Test B.

No significant associations of MMSE levels with risk of stroke were observed in any model for any of the investigated outcomes. Secondary analyses with additional adjustments for apolipoprotein E (ApoE), epsilon4 allele or alcohol in the multivariable model C did not affect the results.

Discussion

Cognitive function as measured by TMT-B strongly predicted subsequent brain infarction independently of education, social group and traditional stroke risk factors.[16, 79-81, 91, 92] This suggests that impaired executive function, reflecting sub cortico-frontal activities, is related to subsequent brain infarction.

The present observation of an association between cognitive function and subsequent risk of stroke/TIA is consistent with findings in previous longitudinal studies.[93, 94] Furthermore, stroke patients with dementia are at higher risk for long-term stroke recurrence compared with non-demented patients.[95] However, our study differs from the previous studies[93, 94] in terms of the cognitive tests used and regarding the cognitive level of the examined populations. To our knowledge, no other study has investigated the predictive values of TMTs A and B for subsequent stroke. The previous two studies demonstrated correlation between results from the brief dementia screening tests, the SPMSQ and the MMSE, and subsequent stroke, while we only detected a predictive value of results from TMTs A and B, but not from the MMSE.

One possible explanation for these differences is that the participants in our cohort had much better cognitive performance in general. The median follow-up time also differed between the studies: ours being 11.1 years and that of Ferruci et al 4.3 years. The study by Zhu et al. had a maximum follow-up of three years.

Subclinical cerebrovascular pathology may play a role in cognitive function[96] and cognitive deficit may hence be a manifestation of clinically unrecognised cerebrovascular disease. Multiple lines of research suggest associations between vascular risk factors and brain atrophy, white matter abnormalities and silent cerebral infarctions.[97-100] It is plausible that these vascular risk factors are the cause of brain lesions not yet expressed clinically. Other studies, in a setting assessing the relationship between stroke risk factors and cognitive function, have found a high cerebrovascular risk factor burden to be associated with worse cognitive function. Brady et al.[101] found that the stroke risk score predicted a 3-year decline in semantic verbal fluency, but no association between stroke and memory or visual-spatial function. They suggested that stroke risk factors might have a particularly damaging effect on frontally mediated cognitive function. In two other studies associations were found between cerebrovascular risk factor burden and a wide range of cognitive abilities.[102, 103]

Notable in this study, as the risk of brain infarction related to TMT-B score increased when adjustments were made for education and social factors and further in the multivariable-adjusted model is that the risk related to TMT-B seems to exist separately, beside the pathway of traditional stroke risk factors.

Our results indicate that the risk of brain infarction is increased already in the subclinical phase of cognitive function deficit, which may be an indicator of unrecognised cerebrovascular disease. TMT-B is an easily accessible cognitive test for clinical use. Further studies are warranted, to address the question whether treatment of cerebrovascular risk factors in patients with high TMT-B score may reduce the risk of stroke/TIA.

Study IV

Results

The mortality rate among the 155 cases of stroke/TIA was 148/1000 PYAR (84 deaths during 566 PYAR). The median follow-up after first-ever stroke/TIA was 2.5 years (maximum 12.2 years) and 22 subjects died within a month (14 %), 42 within a year (27 %) and 84 during the time of follow-up (54 %).

In Cox proportional hazard models adjusting for age at baseline, both ECG-LVH and diabetes prevalence at baseline were strongly related to death

after first-ever stroke. This was also found true when the subgroup of brain infarctions was analysed in all models, e.g. (HR 2.40 per SD, 95 % CI 1.24-4.66) for ECG-LVH, and (HR 2.01 per SD, 95 % CI 1.06-3.80) for diabetes prevalence after adjustment for age and traditional stroke risk factors. No other investigated variable showed any significant relation to mortality in the total stroke sample or in the subgroup of brain infarctions in these analyses.

Both Trail Making Tests A and B strongly predicted mortality, but no significant relation between MMSE scores and future mortality was seen. TMT-A and TMT-B were also constantly related to mortality in all models in analyses of the subgroup of brain infarctions. The results of Kaplan-Meier Survival Estimates for TMT A by tertiles are displayed in Figure 13.

In the dependency analyses the following were found: 130 stroke patients were discharged (for one patient data were missing), and 27 of these were regarded as dependent. However, neither TMT A nor B were significant predictors of dependency (OR 0.97 SE, 95 % CI 0.62-1.54, OR 1.08 SE, 95 % CI 0.91-1.76 respectively) following age adjustment, nor were the MMSE results.

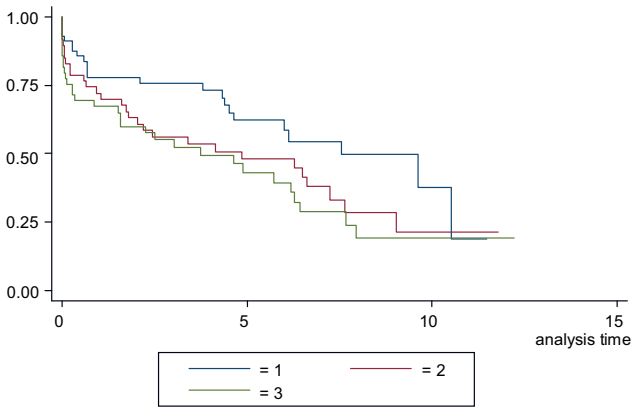

Figure 13. Kaplan-Meier survival estimates for Trail Making Test A in tertiles.

Discussion

Pre-stroke cognitive function in men free from stroke/TIA at baseline, measured by TMT A and B, predicted survival independently of education, social group and traditional stroke risk factors,[16, 79-81, 91, 92] whereas MMSE had no predictive power. None of the pre-stroke cognitive tests showed any consistent relation to dependency after first-ever stroke.

In a systematic review article validating prognostic models by Counsell et al., cardiac failure, level of consciousness and grade of impairment were predictors of survival after 30 days in stroke patients. Pre-stroke overt de-

mentia has previously been reported to be a predictor for initial stroke severity and for 28-day and one year case fatality.[104, 105] As poor TMT A and B results could reflect subclinical cognitive performance deficit, our results regarding survival point in the same direction. However, a previous finding that pre-stroke cognitive decline has a tendency to predict dependency[105] could not be confirmed in our study. This discrepancy is probably not due to limited power in the present study, since the hazard ratios did not even detect the slightest tendency towards an association.

The present study is unique in its kind, since a detailed cognitive function evaluation was performed up to 12 years before the stroke event. The present findings are therefore hardly comparable with studies evaluating cognitive function following stroke.

Cognitive deficit may be a manifestation of clinically unrealised cerebrovascular disease.[96] Biological ageing of the brain is partly attributable to ageing of the cerebrovascular circulation and effects of these vascular changes on the brain.[101, 106] Earlier cross-sectional studies on the ULSAM cohort have also demonstrated that high 24-hour BP, non-dipping, insulin resistance, and diabetes are all related to low cognitive function.[107] Our own earlier studies on this cohort indicated that the risk of brain infarction was increased in men with poor TMT B test results.[108]

Thus, TMTs A and B, which are easily accessible cognitive tests for clinical purposes, may not only be used as tools for identifying risk of stroke as stated in paper III, but may also be considered important predictors of post-stroke mortality.

General discussion

Our aim was to look for possible new risk factors and predictors for stroke. In study I, based on the investigations at age 50, indices of an unhealthy dietary fat intake and a high serum Lp(a) level predicted stroke/TIA independently of established risk factors. If these observations are confirmed in intervention studies, this may imply that modification of food habits may play a role in the attempts to lower stroke incidence.

There is no established way of decreasing the Lp(a) concentration by pharmacological treatment, but nicotinic acid (vitamin B3), acetylsalicylic acid (ASA) and fish oil supplements are thought to affect the levels. One obvious obstacle is the lack of standardisation of the measure and that the ethnic origin of the recipient has great importance.

In study II, based on the investigations at age 70, the concentration of plasma intact proinsulin and the insulin sensitivity index measured by the euglycemic insulin clamp were found to predict subsequent stroke/TIA, independently of diabetes and other traditional risk factors. We suggest that proinsulin, which in practice is a far easier measure than clamp, could serve as a good surrogate risk marker for insulin resistance in people who are not yet diabetic if the method became standardised.

In the third study, again based on the investigations at age 70, we looked at the cognitive test performances and found strong indications that the results from TMT B, reflecting subcortical frontal functions, were related to a later risk of brain infarction. We interpreted this as implying that the risk is increased already in the subclinical phase of cognitive function deficit, which may be an indicator of unrecognised cerebrovascular disease. The lack of association with the subgroup of haemorrhages could be explained by the small size (n=24) of that subgroup, which meant insufficient power. As TMT-B is an inexpensive and easily accessible cognitive test it could be used in the clinic as an additional tool in risk assessment. Intervention studies are warranted, to determine whether treatment of cerebrovascular risk factors in patients with a high TMT-B score may reduce the risk of stroke/TIA.

In the fourth study we took our earlier results further and examined if the question whether pre-stroke cognitive performance measured with TMT A and B at age 70 predicted post-stroke mortality and dependency. We found that both TMT A and B were strong predictors of mortality independently of education, socio-economic status or traditional risk factors. Thus, the tests

which can be used as tools for identifying the risk of stroke can also be considered important predictors of post-stroke mortality. However, we found no relation to dependency in any variable or test performance. In the fourth study we also validated the stroke/TIA diagnosis by going through the medical records.

Strengths and limitations

The strengths of the study include the large community-based sample, the long follow-up, the non-existent loss to follow-up, and the comprehensive characterisation of metabolic variables. The access to the registers CDR and SHDR in Sweden is an effective, reliable and time-saving advantage that is envied by epidemiologists in less fortunate circumstances. However, as we only examined men of similar age with the same ethnic background, the results may have limited generalisability to women and to other age and ethnic groups. If the cohort had been started up today, it would surely not have been confined to men, but in 1970 things looked different. Cardiovascular diseases were considered mainly a male affliction, probably because men were victims at younger ages then women.

Limitations of the study include possible misclassification of stroke, although the accuracy of the CDR and the SHDR is reported to be very high.[61] CT has been in general use since the beginning of the 1990s in patients at the University Hospital, Uppsala presenting with symptoms of stroke or TIA, especially since the sensitivity of the method for bleeding is as high as nearly 100 %. In study IV, scrutiny of 217 medical records from patients who had experienced a stroke/TIA revealed that as many as at least 178 (91 %) had undergone a brain CT.

The individual FFAs in study I were measured as proportions of the total FFAs in per cent and not as independent measures, which must be taken ad notam.

The present cohort has been re-examined a few times since baseline, risk factors have been treated, and the subjects may therefore have been healthier during follow-up than the average man. Follow-up data concerning medication, including antiplatelet therapy, over time were not available when the studies were undertaken. A PhD student is working on this issue at the moment. Furthermore, we have not taken into consideration dietary habits, and in general, food habits have changed during the last decades. A high circulating level of oleic acid in 1970 was a sign of high intake of fat in general, mainly from sources with a high content of saturated fat. Today when olive oil is in general use, a high intake can be related to an intake of more favourable dietary fat.

The predictive power of stroke risk factors during long follow-up periods

The assessment of the ability of different risk factors to predict stroke morbidity and mortality over a long follow-up period was based on a single measurement at baseline. The risk factor status in an individual might of course change over time and the length of follow-up may thus influence the predictive power. For instance smoking, alcohol habits and other life-style factors could have changed considerably during the follow-up period as well as pharmacological treatment.

Future Perspectives

The ULSAM cohort is a rich source for exploring risk factors and predictors for CVD. The investigators have managed to get the participants to return repeatedly to a great degree and the examinations have been broad and extensive, especially the ages of 50 and 70. Many new studies are possible concerning risk factors and stroke end-points in ULSAM. For example, studies of variables in the mineral metabolism are motivated since plasma parathyroid hormone levels were recently found to predict cardiovascular mortality in the community and a study of stroke end-points can easily be accomplished. Stroke recurrence in relation to pre-stroke variables was not investigated in this project and could be a logical sequel. Further studies on TMT A and B are tempting, as they were found to carry important weight as predictors.

Since 2003 the ULSAM study has been part of a project comprising genetic studies, named “Genetic and environmental determinants of common disease”. This has opened up a new field with possibilities of obtaining new knowledge of variations in the genome and an opportunity to relate these to conditions and diseases such as stroke. The research on ULSAM has been very active over the years, and is likely to continue to be so, and research on stroke predictors is an important part.

The most important question to be answered first, however, is whether more efforts should be devoted to studies of risk factors. Or would our patients and the community be better off if we united our attempts to treat what we have found and try to promote a healthier lifestyle and well-treated risk factors instead?

An intervention study is especially warranted on the basis of our results to investigate whether treatment of cerebrovascular risk factors in patients with a high TMT-B score may reduce the risk of stroke/TIA. That is something for the future.

Conclusions

II. The profile of fatty acids in cholesterol esters was found to be an independent predictor of stroke/TIA, indicating that an unhealthy dietary fat intake plays a role as a stroke risk factor. A high serum Lp(a) level predicted stroke/TIA independently of established risk factors, and regarding glucometabolic variables, proinsulin showed the highest standardised hazard ratios in middle-aged men followed for up to 32 years.

II. A low insulin sensitivity index derived from the euglycaemic insulin clamp and elevated fasting intact proinsulin levels predicted subsequent stroke/TIA over a follow-up period of 8.8 years independently of diabetes. Further, intact proinsulin preserved its predictive value after adjustment for other traditional risk factors. Proinsulin can serve as a good surrogate risk marker for insulin resistance as a predictor of stroke/TIA.

III. Mildly impaired cognitive performance as identified by Trail Making Test B, which reflects functions related to subcortico-frontal pathways, was a strong and independent predictor of subsequent brain infarction over a follow-up period of up to 12 years. Results from MMSE showed no association with later stroke/TIA.

IV. Cognitive performance as measured by Trail Making Tests A and B before stroke was associated with long-term risk of mortality, but not dependency, after first-ever stroke or TIA. Results from MMSE were found to be not associated with mortality or dependency. Pre-stroke ECG-LVH and diabetes prevalence were strongly related to death after first-ever stroke but had no predictive value for dependency.

Sammanfattning på svenska (Summary in Swedish)

Allmän bakgrund

Stroke (slaganfall) är en ofta allvarlig sjukdom som drabbar uppemot 30 000 människor i Sverige varje år. Stroke är samlingsnamnet på de plötsliga symptom som uppstår när hjärnans nervvävnad skadas på grund av hämmad blodtillförsel. Ofta kommer slaganfallet som en plötslig förlamning eller känselnedsättning i ena sidan av kroppen, som talsvårigheter eller att man inte ser en viss del av synfältet. Hur exakt symtomen ter sig beror på var i hjärnan skadan sitter.

Om symtomen är övergående, högst 24 timmar (oftast bara några minuter) kallas det TIA, transitorisk ischemisk attack. Detta är en allvarlig förvarning och påvisar att risken är ökad för att personen senare kan få en stroke. Medelåldern vid insjuknandet i stroke är 73 år för män, 77 år för kvinnor.

Skadan kan ha flera olika orsaker. Det vanligaste (85%) är en blodpropp i något blodkärl (hjärninfarkt), eller en bristning i något kärl (15%) inne i hjärnan eller på hjärnans yta (hjärnblödning eller subarachnoidalblödning). Bakom stroke ligger ofta en långvarig process av åderförfettning (ateroskleros). Sedan 1960-talet har vår kunskap om riskfaktorer för hjärtkärlsjukdom ökat kraftigt. Mycket tack vare s.k. longitudinella studier d v s studier där man följer en grupp personer under en lång tid och gör upprepade mätningar på dessa.

ULSAM

I Uppsala har en sådan studie pågått sedan 1970. ULSAM (Uppsala Longitudinal Study of Adult Men). Den började med att alla 50-åriga män födda i Uppsala 1920-24 inbjöds att delta i en hälsoundersökning vars målsättning var att upptäcka riskfaktorer för kardiovaskulär sjukdom och identifiera högriskindivider för intervention. Uppslutningen var god. Av de 2841 som inbjöds att delta kom 2322 (82%). Därefter har deltagarna inbjudits att delta i fyra ytterligare undersökningar vid ungefärlig ålder 60, 70, 77 och 84 år och en ytterligare undersökning vid 88 års ålder har nyligen avslutats. Det unika med ULSAM-kohorten är dels den långa uppföljningstiden (mer än 30 år) och den noggranna karaktäriseringen av glukos- och lipidmetabolismen som har gjorts vid både 50 och 70 års ålder.

Arbete I

I vårt första arbete har vi utgått ifrån 50-årsdata och studerat de i materialet som drabbats av stroke eller TIA fram t o m 2002. Antalet uppgick till ca 420 stycken. Vi har undersökt riskfaktorer och fann, förutom de traditionella riskfaktorerna, ett samband mellan halten av olika fettsyror och risken att senare utveckla stroke/TIA. En hög halt av mättade fettsyror ökade risken men en hög halt av den fleromättade fettsyran linolensyra däremot verkade utgöra ett skydd mot att insjukna i stroke. Hög halt av blodfettet lipoprotein(a)och hög halt av proinsulin visade sig också vara en riskfaktor.

Däremot sågs t ex ingen ökad risk relation till serumkolesterol eller andra blodfettsvariabler som ingår i det batteri som vanligen kontrolleras i rutinsjukvård i samband med stroke/TIA insjuknande.

Arbete II

Vårt andra arbete utgår ifrån 70-årsdata. Av de 1151 män som genomgick undersökning vid 70 års ålder insjuknade 150 i stroke eller TIA under en uppföljningstid på tolv år. Tonvikten i dessa analyser är lagd på glukosmetabolism, där särskilt resultaten avseende insulinkänslighet mätt med s.k. Euglucemic Insulin Clamp och nivå av proinsulin har studerats. Då clamp är en relativt komplicerad undersökning med glukos- och insulininfusioner i flera timmar och proinsulin visade sig vara minst lika bra som mått på insulinkänslighet, är detta att föredra i klinisk praxis.

Arbete III

Även detta arbete utgår ifrån 70-årsdata och gäller kognitiv funktion som prediktor för stroke. Av de 1151 män som genomgick undersökning vid 70 års ålder testades 999 med tre kognitiva tester som alla gjordes med penna och papper; Mini Mental State Exam som är ett screeningtest för demens och Trail Making Test (TMT) A och B som framför allt anses testa färdigheter som är relaterade till vitsubstanen i frontalloberna. Man anser att små kärlskador som kommer med ökande ålder och särskilt om individen har högt blodtryck och diabetes, särskilt drabbar detta område. Av de tre tester som gjordes visar TMT-B mycket intressant resultat som prediktor för hjärninfarkt. Man ser där en 48 % ökad risk per standardavvikelse utifrån resultatet från TMT-B. Den fjärdedelen av deltagarna som hade de sämsta resultaten hade en mer än tre gånger ökad risk att drabbas av hjärninfarkt jämfört med dem som hörde till fjärdedelen med bäst resultat.

Arbete IV

Har nivån på olika variabler och resultatet från kognitiva tester mätta före det första strokeinsjuknandet något att berätta om risken att dö och graden av beroende efter att man väl drabbats? Detta undersökte vi i det fjärde arbetet. Svaret blev att diagnosen diabetes mellitus och EKG-förändringar talande för vänsterkammarförstoring var ett ogynnsamt tecken vad gäller överlevnaden efter förstagångsinsjuknande i stroke/TIA. Likaså var ett sämre resultat på de kognitiva testerna Trail Making Test A och B klart ofördelaktigt. Däremot fann vi ingen koppling mellan någon av de uppmätta variablerna eller resultaten från de tre kognitiva testerna vad gäller graden av beroende efter insjuknande.

I detta arbete gjordes också en genomgång av alla journaler från dem som drabbats av förstagångsstroke/TIA efter 70-årsundersökningen (n=217).

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