SUSANNE FONVILLE

NEWLY-DIAGNOSED DISTURBED GLUCOSE METABOLISM AFTER TIA OR STROKE

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NEWLY-DIAGNOSED DISTURBED GLUCOSE METABOLISM AFTER TIA OR STROKE

Nieuw-gediagnosticeerde gestoord glucose metabolisme na een TIA of beroerte

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CONTENTS

CHAPTER 1

GENERAL INTRODUCTION

Epidemiology and etiology of stroke

Stroke is the third cause of death and the first cause of disability in developed countries. The incidence of stroke rises with increasing age and as the population is aging, the incidence of stroke is expected to further increase. In The Netherlands, over 40.000 patients are admitted for acute stroke annually and about 9.000 of them die [1]. Hence, more accurate prevention of stroke is needed.
Strokes are either ischemic or hemorrhagic, and imaging of the brain can distinguish between these two subtypes. Ischemic strokes account for about 80% of all strokes, and are caused by a transient or permanent reduction of cerebral blood flow caused by occlusion of a cerebral artery or arteriole [2]. The causes of ischemic stroke are divers, and can be divided in large artery disease, intracranial small vessel disease, cardioembolism, and other causes [2, 3]. An atheromatous plaque in the large and medium-sized arteries complicated by thrombosis and embolism is the most common cause of ischemic stroke [2].

Treatment of ischemic stroke

The treatment of ischemic stroke consists of acute treatment and subsequently secondary stroke prevention. The acute treatment of ischemic stroke involves intravenous recombinant tissue-plasminogen activator (rt-PA), antiplatelet therapy and stroke unit care [4-6].
Following acute treatment, all efforts are aimed at preventing recurrent ischemic stroke in these patients. The risk is about 5% in the first 2 days and 17% in the first 3 months [7]. Also, the risk of myocardial infarction and other cardiovascular complications is increased after a TIA or stroke [2].
Secondary stroke prevention is focused on discovery and treatment of risk factors for stroke and other vascular diseases. The current secondary stroke prevention can be divided into pharmacological treatment, lifestyle modification and surgical interventions.
Despite of all available treatment methods, secondary stroke prevention is still not perfect. It therefore remains a challenge to discover new treatable risk factors.

Glucose metabolism in secondary stroke prevention

Type 2 diabetes mellitus is an increasing problem in the Western world. It leads to a reduced life expectancy and increased risk for cardiovascular disease, including stroke. The prevalence of pre-diabetes is increasing as well. Pre-diabetes is an intermediate metabolic state between normal glucose metabolism and diabetes mellitus, representing a high risk, of up to 70%, of developing diabetes mellitus in the future [8, 9]. It is still controversial whether pre-diabetes should be considered a risk factor for (recurrent) stroke and whether treatment is necessary.
Hyperglycemia after stroke is common, also in non-diabetic patients [10, 11]. This hyperglycemia may be transient, known as stress hyperglycemia, or persistent reflecting undiagnosed abnormal glucose metabolism [12, 13]. Differentiation between transient and persistent hyperglycemia is important to identify patients with an increased risk of recurrent stroke.
Several methods are known to identify people with (pre-)diabetes, including fasting plasma glucose levels, 2-hour post-load glucose levels and glycosylated hemoglobin levels [8]. However, it is unknown what the best detection method is and what the timing of the measurement should be, taking the acute phase effect into account.

Atherosclerosis might be one of the underlying mechanisms of the increased risk of recurrent stroke in patients with (pre-) diabetes. However, there is no consensus whether pre-diabetes is associated with atherosclerosis as well.
Both non-pharmacologic and pharmacologic interventions are possible treatment strategies in prevention of progression to type 2 diabetes in pre-diabetic patients and they have been found to be almost equally effective [14, 15]. Treatment of impaired glucose tolerance after TIA or stroke with metformin is safe and likely to improve glucose tolerance, but leads to frequent side effects and discontinuation of the treatment [16]. However, the effect of these treatments on preventing (recurrent) cardiovascular events is still the subject of several trials [17, 18].

Aims and outline of the thesis

The aim of this thesis is to investigate the role of newly-diagnosed disturbed glucose metabolism (pre-diabetes or newly-diagnosed diabetes mellitus) as a potential treatable risk factor in the secondary prevention after TIA or stroke.
In **Chapter 2**, I give a general overview of the literature concerning the prevalence, risk and clinical management of pre-diabetes in patients with stroke or TIA. In **Chapter 3.1** the scope of the problem pre-diabetes in stroke patients and the best detection method will be discussed with data of the Erasmus Stroke Study, an ongoing registry of all neurovascular patients in the Erasmus MC University Medical Center. We present a prediction model for persistent impaired glucose tolerance after TIA or ischemic stroke in **Chapter 3.2**. In **Chapter 4**, I focus on atherosclerosis, as this is the most common cause of ischemic stroke. In **Chapter 4.1**, I investigate whether newly-diagnosed disturbed glucose metabolism in patients with a recent TIA or ischemic stroke is associated with atherosclerosis. In **Chapter 4.2**, we study the associations between genes for metabolic traits such as glucose and lipids, and atherosclerosis in patients with ischemic stroke. In **Chapter 4.3**, I investigate another potential new risk factor for (recurrent) stroke, nocturnal dipping blood pressure patterns, and its association with atherosclerosis.
Chapter 5 explores the treatment of disturbed glucose metabolism after ischemic stroke or TIA, and describes the protocol of the ongoing MAAS trial that focuses on the feasibility, safety and effects of treatment of impaired glucose tolerance after TIA or ischemic stroke.
In **Chapter 6**, I discuss methodological aspects and implications of the studies described in this thesis. Finally, the results of all studies are summarized in **Chapter 7**.

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CHAPTER 2

PRE-DIABETES IN PATIENTS WITH STROKE OR TIA

CHAPTER 2.1

PRE-DIABETES IN PATIENTS WITH STROKE OR TIA: PREVALENCE, RISK AND CLINICAL MANAGEMENT

AUTHORS
Susanne Fonville [1]
Adrienne A.M. Zandbergen [2]
Peter J. Koudstaal [1]
Heleen M. den Hertog [1,3]

1
DEPARTMENT OF NEUROLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

2
DEPARTMENT OF INTERNAL MEDICINE
IKAZIA HOSPITAL
Rotterdam, The Netherlands

3
DEPARTMENT OF NEUROLOGY
MEDISCH SPECTRUM TWENTE
Enschede, The Netherlands

ACCEPTED IN CEREBROVASCULAR DISEASES 2014

Abstract

BACKGROUND

The prevalence of diabetes is emerging worldwide and is an important modifiable risk factor for stroke. People with pre-diabetes, an intermediate metabolic state between normal glucose metabolism and diabetes, have a tenfold increased risk of developing diabetes compared to those with normal glucose metabolism. Pre-diabetes is comprised of impaired fasting glucose and/or impaired glucose tolerance and/or disturbed glycosylated hemoglobin levels. Pre-diabetes is highly prevalent in non-diabetic patients with TIA or ischemic stroke, and nearly doubles their risk of stroke. This offers new options for secondary stroke prevention.

SUMMARY

Several detection methods exist for identifying (pre-) diabetes, including fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels. The concordance between these tests is not 100% and they seem to be complementary. Screening for (pre-) diabetes after stroke with fasting plasma glucose levels alone is insufficient, and 2-hour post-load glucose and/or glycosylated hemoglobin levels should be determined as well. The prevalence of pre-diabetes in previously non-diabetic patients with a recent TIA or stroke ranges from 23-53%. This high prevalence in the acute phase after stroke can be transient or persistent, representing undiagnosed abnormal glucose metabolism.
Impaired fasting glucose and impaired glucose tolerance have different pathophysiological mechanisms, including respectively hepatic insulin resistance and muscle insulin resistance. Pre-diabetes seems to be a modest predictor for stroke, but doubles the risk for recurrent stroke. The relation between pre-diabetes after stroke and functional outcome is still unknown. However, it is most likely, that pre-diabetes is a risk factor for poor clinical outcome after stroke.
There is a growing recognition that patients with pre-diabetes should be treated more aggressively. Both lifestyle and pharmacologic interventions are possible treatment strategies. They are at least equally effective in preventing progression to diabetes. Lifestyle changes are difficult to maintain over a long period. The evidence of pharmacological interventions on stroke or other cardiovascular disease is limited though, and is still subject of several clinical trials.

CONCLUSIONS

As the prevalence of pre-diabetes is growing rapidly, pre-diabetes might become one of the most important modifiable therapeutic targets in both primary and secondary prevention.

Introduction

Type 2 diabetes mellitus is an increasing problem in the Western world and is accompanied by a reduced life expectancy. Pre-diabetes is an intermediate metabolic state between normal glucose metabolism and type 2 diabetes, representing a high risk of developing type 2 diabetes in the future [1, 2]. Up to 70% of the patients with pre-diabetes may develop type 2 diabetes [1]. Pre-diabetes comprises impaired fasting glucose and/or impaired glucose tolerance and/or impaired glycosylated hemoglobin [1, 2]. The risk of developing type 2 diabetes is approximately 0.7% per year in normoglycemic individuals, whereas patients with impaired fasting glucose or impaired glucose tolerance have a yearly risk of 5-10% [1]. The transition from pre-diabetes to type 2 diabetes usually takes several years, but may also be more rapid [1].

The estimated worldwide prevalence in 2010 of impaired glucose tolerance was 7.9%. In Europe, the prevalence was even higher, 8.9%. The prevalence of impaired fasting glucose is estimated on 5% but the use of 2 different criteria (from the American Diabetes Association and the World Health Organization) hampers the comparison of various studies [3].

Patients with pre-diabetes do not only have an increased risk of type 2 diabetes, but also of cardiovascular diseases, including stroke and recurrent stroke [4-6]. There is a growing recognition that patients with pre-diabetes should be treated more aggressively. Both lifestyle modification and antidiabetic drugs lower the risk of developing type 2 diabetes [7, 8]. However, the effect of these treatments on preventing cardiovascular events is still the subject of several trials [9, 10].

This review provides an updated overview of pre-diabetes in patients with stroke or TIA. We discuss methods of identification and the prevalence of pre-diabetes in stroke patients. We also explore the pathophysiology of pre-diabetes in stroke patients, the association with (recurrent) cardiovascular events, and the impact on functional outcome and therapeutic options.

Identification of pre-diabetes

Several methods are known to identify people with pre-diabetes, including fasting plasma glucose levels, 2-hour post-load glucose levels and glycosylated hemoglobin levels. The cut-off values for the different methods are shown in Table 1.

Fasting plasma glucose levels can be used to diagnose impaired fasting glucose. After overnight fasting (at least 8 hours), fasting plasma glucose levels are measured. The definition of impaired fasting glucose however, is not clear. The American Diabetes Association reduced the lower cut-off point of the definition of impaired fasting glucose in 2003 to 5.6mmol/L, but the World Health Organization preserved the previous cut-off point of 6.1mmol/L [1, 2]. Both definitions are used in stroke related trials.

An oral glucose tolerance test can be performed to detect impaired glucose tolerance. After overnight fasting, a solution of 75 grams glucose in 150mL water is ingested by the patient. Two hours after ingestion, the 2-hour post-load glucose levels are measured [11]. Unlike impaired fasting glucose, the American Diabetes Association and World Health Organization agree on the definition of impaired glucose tolerance [1, 2].

Glycosylated hemoglobin levels are mostly used as a marker of chronic hyperglycemia in the evaluation of known diabetic patients. The glucose levels of the previous 2-3 months are reflected in this marker. However, recently, the American Diabetes Association, the International Diabetes Federation, and the European Association for the study of Diabetes published a report in which the use of glycosylated hemoglobin levels was recommended for the diagnosis of (pre-) diabetes [2]. The advantage of glycosylated hemoglobin levels over fasting plasma glucose and 2-hour post-load glucose in identifying pre-diabetes in patients with a recent stroke, is that it remains unaffected by the acute phase reaction [2].

The concordance between these three tests is not 100% [12]. Furthermore, several studies showed that the use of fasting plasma glucose levels alone is insufficient to detect (pre-) diabetes. Both 2-hour post-load glucose and glycosylated hemoglobin levels diagnose patients with (pre-) diabetes otherwise undetected [13-18].

TABLE 1 - CUT-OFF VALUES OF THE DIFFERENT GLUCOSE TESTS ACCORDING TO THE AMERICAN DIABETES ASSOCIATION [2].

	Normal glucose metabolism	Pre-diabetes	Diabetes mellitus
Fasting plasma glucose (mmol/L)	<5.6*	5.6-6.9*	≥7.0
2-hour post-load glucose (mmol/L)	<7.8	7.8-11.0	≥11.1
Glycosylated hemoglobin (mmol/mol)	<39	39-47	≥48

* according to the World Health Organization respectively <6.1mmol/L and 6.1-6.9mmol/L [1]

Prevalence of pre-diabetes in patients with stroke

The prevalence of pre-diabetes in previously non-diabetic patients with a recent ischemic stroke or TIA is on average 37% (range 29-53%) in the acute phase (within 3 months after the event) and 32% (range 23-46%) in the post-acute phase (≥3 months after the event), which is clearly higher than in the overall population [13-16, 18-25]. Several studies assessed the prevalence of pre-diabetes based on fasting plasma glucose levels and/or 2-hour post-load glucose levels in stroke patients [13-16, 19-24], 1 study assessed the prevalence based on glycosylated hemoglobin levels [25] and only 1 study assessed the prevalence based on all three detection methods [18]. However, inclusion criteria, definition of disturbed glucose metabolism, ethnicity and time between event and glucose measurement differed among these studies, making it difficult to compare them (Table 2).

Most studies were performed in patients with ischemic stroke [15, 16, 19-21, 23-25]. Others did not differentiate between patients with ischemic stroke, intracerebral hemorrhage or a TIA [13, 14, 22]. Studies were performed in different regions of the world including China [16, 20, 21], Japan [15], South Africa [24], USA [13, 19, 25] and Europe [14, 18, 22, 23]. The prevalence of diabetes, and also of pre-diabetes, is influenced by ethnicity [26] and this factor should therefore be taken into account when considering the prevalence of pre-diabetes in stroke patients. Time from the event to glucose level assessment also differed between the studies. Some assessed glucose levels in the acute phase (<2 weeks after the event) [14-16, 18, 20, 23-25], others in the chronic stroke phase (>3 months after the event) [13, 19, 21, 22]. Only 3 studies repeated the glucose measurement after 3 months and this revealed that 22-44% of the patients had persistent pre-diabetes [20, 23, 24]. No predictors for persistent pre-diabetes are known.

TABLE 2 - OVERVIEW OF STUDIES ASSESSING THE PREVALENCE OF PRE-DIABETES IN STROKE PATIENTS.

Study	Region	Event	Glucose assessment	Time between event and glucose level assessment	Number of previously non-diabetic patients	Normal glucose metabolism n (%)	Pre-diabetes n (%)	Newly-diagnosed diabetes n (%)
GLUCOSE ASSESSMENT IN THE ACUTE PHASE (< 3 months after event)								
Vancheri[23]	Europe	IS	OGTT	7 days	96	15 (16)	37 (39)	44 (46)
Matz[14]	Europe	IS & TIA & ICH	FPG & OGTT	7-10 days	190	94 (49)	57 (30)	39 (21)
Urabe[15]	Japan	IS	FPG & OGTT	≥2 weeks	113	42 (37)	43 (38)	28 (25)
Dave[24]	South Africa	IS	FPG & OGTT	2-3 days	107	42 (39)	39 (36)	26 (24)
Jia[20]	China	IS	FPG & OGTT	14 days	110	37 (34)	32 (29)	41 (37)
Jia[16]	China	IS	FPG & OGTT	14 days	1793	706 (39)	556 (31)	531 (30)
Huisa[25]	USA	IS	A1C	On admission	166	53 (32)	88 (53)	25 (15)
Fonville[18]	Europe	IS & TIA & ICH	FPG & OGTT & A1C	4 days (IQR 3-11)	700	147 (21)	365 (52)	188 (27)
TOTAL					3275	1136 (35)	1217 (37)	922 (28)
GLUCOSE ASSESSMENT IN THE POST-ACUTE PHASE (≥ 3 months after event)								
Lam[21]	China	IS	OGTT	3-6 months	111	64 (58)	26 (23)	21 (19)
Gray[22]	Europe	IS & ICH	OGTT	12 weeks	62	26 (42)	23 (37)	13 (21)
Kernan[13]	USA	IS & TIA	FPG & OGTT	105 days (range 24-180)	98	44 (45)	30 (31)	24 (24)
Vancheri[23]	Europe	IS	OGTT	3 months	96	34 (35)	26 (27)	36 (38)
Ivey[19]	USA	IS	FPG & OGTT	>6 months	80	30 (38)	37 (46)	13 (16)
Dave[24]	South Africa	IS	FPG & OGTT	3 months	44	26 (59)	12 (27)	6 (14)
Jia[20]	China	IS	FPG & OGTT	3 months	107	42 (39)	37 (35)	28 (26)
TOTAL					598	266 (44)	191 (32)	141 (24)

IS = ischemic stroke, ICH = intracerebral hemorrhage, TIA = transient ischemic attack,
OGTT = oral glucose tolerance test, FPG = fasting plasma glucose, A1C = glycosylated hemoglobin.

Pathophysiology of pre-diabetes after stroke

PATHOPHYSIOLOGY OF HYPERGLYCEMIA IN ACUTE ISCHEMIC STROKE

Hyperglycemia is often present in patients with stroke. Up to 40% of these patients have no history of diabetes [27, 28]. This hyperglycemia can be transient, or persistent, reflecting undiagnosed abnormal glucose metabolism [29, 30]. Serious illness, like stroke, can result in an acute stress reaction that involves stimulation of the hypothalamus-pituitary-adrenal-axis (HPA-axis) resulting in a release of catecholamines, cortisol and glucagon. This release results in insulin resistance, glycogenolysis, gluconeogenesis, proteolysis, and lipolysis [29, 30]. Furthermore, there is substantial evidence that stroke can induce hyperglycemia indirectly by activation of an inflammatory reaction [29-31].

PATHOPHYSIOLOGY OF PRE-DIABETES

Impaired fasting glucose and impaired glucose tolerance do not share the same pathophysiological mechanisms. In individuals with normal glucose metabolism, ingested glucose uptake occurs in insulin-insensitive tissues, like brain and erythrocytes. The endogenous glucose production takes primarily place in the liver. Uptake and production of glucose are complementary: fasting plasma glucose levels are mainly dependent on glucose production, which is regulated by the plasma insulin and glucagon concentrations. After glucose ingestion, insulin secretion is promoted by the increased plasma glucose levels. Subsequently, glucose production is suppressed and glucose uptake, primarily by muscle, is stimulated, to remain normoglycemic [26].

Impaired fasting glucose reflects hepatic insulin resistance and normal muscle insulin sensitivity. Patients with impaired glucose tolerance on the other hand, have (near) normal hepatic insulin sensitivity, but display muscle insulin resistance. Insulin secretion is impaired in both impaired fasting glucose and impaired glucose tolerance, but in different ways. Patients with impaired fasting glucose have a decreased early-phase insulin response to oral glucose whereas patients with impaired glucose tolerance have deficiencies in both early- and late-phase insulin responses. Patients with both impaired fasting glucose and impaired glucose tolerance show both hepatic and muscle insulin resistance and decreased both early- and late-phase insulin responses to oral glucose, which might explain the higher risk in these patients in developing diabetes compared with patients with impaired fasting glucose or impaired glucose tolerance alone [26, 32]. There is evidence that insulin sensitivity is impaired after ischemic stroke, possibly due to decreased hepatic insulin receptor expression and up-regulation of gluconeogenesis, which can lead to impaired glucose tolerance [31].

Patients with impaired fasting glucose or impaired glucose tolerance and glucose levels in the higher range have an increased risk compared with patients with glucose levels in the lower range. This indicates that pre-diabetes should not be considered as a distinct clinical entity, but rather that glucose levels should be regarded as a continuum with increasing levels representing increasing risk of developing diabetes.

Association between pre-diabetes and (recurrent) stroke

Pre-diabetes is considered a risk factor for developing (recurrent) ischemic stroke. Several studies have shown the association between both fasting plasma glucose and 2-hour post-load glucose levels on the one hand, and risk of cardiovascular disease or ischemic stroke on the other. Most studies indicate that 2-hour post-load glucose levels are a stronger predictor of stroke than impaired fasting glucose [33-36].

Recently, two meta-analyses assessed the association between pre-diabetes and respectively cardiovascular disease and stroke [4, 5]. The effects of impaired fasting glucose and/or impaired glucose tolerance on cardiovascular or stroke risk were modest. However, the changing definitions of impaired fasting glucose and different statistical assessments (hazard ratio vs. relative risk vs. odds ratio) over the years make it difficult to compare the results of the studies used in these meta-analyses.

Few studies have investigated the risk of recurrent stroke. Vermeer et al. assessed the association between random plasma glucose levels and recurrent stroke in patients with a TIA or minor ischemic stroke in the previous 3 months. Patients with non-fasting glucose levels in the range of impaired glucose tolerance had a nearly twofold increased risk of recurrent stroke compared to those with normal glucose levels (adjusted hazard ratio (aHR) 1.8 (95%-CI 1.1-3.0)). Patients with non-fasting glucose levels in the diabetes range (11.1mmol/L or higher) had nearly a threefold increased risk (aHR 2.8 (95%-CI 1.9-4.1)). No associations were found between glucose levels and risk of myocardial infarction or cardiac death, however [6].

Several studies have assessed the risk of recurrent cardiovascular events in patients with pre-diabetes and a myocardial infarction, and have shown an increased risk of recurrent cardiovascular disease with adjusted hazard ratios ranging from 2.2 to 4.2 [37, 38]. Also 2-hour post-load glucose levels predict cardiovascular events in patients with myocardial infarction without known pre-existent diabetes [39]. However, they did not differentiate between myocardial infarction and ischemic stroke in outcome assessment.

Influence on outcome after stroke

Diabetes is associated with unfavorable functional outcome and with slightly increased case fatality after stroke [40]. The effect of post-stroke hyperglycemia or stress-hyperglycemia on functional outcome after stroke in non-diabetic patients has been a subject of many studies [27, 28]. Acute hyperglycemia in both diabetic and non-diabetic patients is not only associated with mortality, but also with unfavorable functional outcome (modified Rankin Scale (mRS) ≥2 [41]) at 3 months after the event. The pooled relative risk (95%-CI) for in-hospital or 30-day mortality is 3.07 (2.50-3.79) and for poor functional outcome is 1.41 (1.16-1.73) in non-diabetic patients with stress hyperglycemia [27]. Different cut-off points are used to define hyperglycemia in these studies, ranging from 6.0 to 8.0mmol/L [27]. However, glucose level as a continuous variable is associated with functional outcome as well [42]. Also the functional outcome after treatment with intravenous tPA is influenced by hyperglycemia and the presence of diabetes [43-45].

All these studies, however, have studied the relationship between glucose levels on admission or random glucose levels rather than the fasting plasma glucose and/or 2-hour post-load glucose and/or glycosylated hemoglobin levels with outcome. Only 1 recent study has assessed the association between pre-diabetes and functional outcome after 30 days, with an adjusted odds ratio for poor functional outcome (mRS 2-6) of 1.9 (95% CI 0.8-5.0) [46]. Nevertheless, it is most likely, but still unproven, that patients with pre-diabetes have a risk of poor functional outcome somewhere between the risk of patients with normal glucose metabolism and of patients with diabetes.

Treatment strategies

The prevention of type 2 diabetes in pre-diabetic patients has been the subject of many large randomized clinical trials. Both non-pharmacologic and pharmacologic interventions are possible treatment strategies.

NON-PHARMACOLOGIC INTERVENTIONS

Non-pharmacologic interventions comprise lifestyle intervention. Extending lifestyle advices by means of individualized diet and regular exercise with intensive counseling sessions clearly reduces the progression to type 2 diabetes by 33-58% compared to those receiving standard lifestyle advice [7, 47-49]. One study compared lifestyle intervention with metformin treatment. The incidence of new-onset type 2 diabetes was reduced by 58% (95%-CI 48-66%, *p*-value <0.001) in patients receiving lifestyle intervention and 31% (95%-CI 17-43%, *p*-value <0.001) in patients randomized to metformin compared with patients with placebo. The incidence of type 2 diabetes was also significantly lower in the lifestyle intervention group compared with the metformin group (*p*-value <0.001) [7]. However, no significant effect of lifestyle intervention on cardiovascular disease was found [50-52].

PHARMACOLOGIC INTERVENTIONS

Different classes of anti-diabetic drugs to prevent progression to type 2 diabetes in patients with pre-diabetes have been studied in randomized clinical trials. Biguanides (metformin) [7, 52-54], alpha-glucosidase inhibitors (acarbose) [55, 56], and glitazones (rosiglitazone and pioglitazone) [53, 57, 58] all significantly decrease the risk of type 2 diabetes, with hazard ratios ranging from 0.31 to 0.75.
Not only antidiabetic drugs, but also inhibitors of the renin-angiotensin system have been studied on their effects on the glucose homeostasis [59, 60]. For example, the angiotensin receptor antagonist valsartan reduced the risk for type 2 diabetes (HR (95%-CI) 0.38 (0.33-0.44)) [59].

Intensive glycemic control is important to reduce the risk on microvascular and neuropathic complications in patients with diabetes mellitus. However there is few data supporting the benefits of intensive glycemic control in reducing the risk of cardiovascular events in diabetic patients with previous cardiovascular disease, including stroke [61-63]. It is suggested that patients with a shorter duration of diabetes or lower glycosylated hemoglobin levels at entry might benefit of this intensive glucose control [63]. Therefore, we think that pre-diabetes is a more interesting starting point to initiate treatment than advanced diabetes mellitus.

The few studies on the effects of glucose-lowering pharmacological interventions on cardiovascular events in patients with pre-diabetes are not conclusive [9, 10], but a recent meta-analysis suggests that any intervention (either non-pharmacologic and/or pharmacologic) reduce the risk for fatal and non-fatal stroke (HR (95%-CI) 0.76 (0.58-0.99)), compared to no intervention [9].

Few studies have investigated the effect of pharmacological treatment in pre-diabetic patients with TIA or stroke. However, pioglitazone seemed to improve insulin sensitivity in non-diabetic patients with impaired insulin sensitivity [58] and treatment with metformin improved glucose tolerance in pre-diabetic patients with recent TIA or stroke [54]. No randomized clinical trials have been published on the effect of non-pharmacologic and pharmacologic interventions on the risk of recurrent stroke. The ongoing **M**etformin and sit**A**gliptin in patients with imp**A**ired glucose tolerance and a recent TIA or minor ischemic **S**troke (MAAS) trial (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=3196) is a phase II trial investigating the feasibility and safety of both metformin and sitagliptin in patients with impaired glucose tolerance after TIA or ischemic stroke in preparation of a phase III trial to investigate the effect on the incidence of recurrent stroke. Furthermore, the **I**nsulin **R**esistance **I**ntervention after **S**troke (IRIS) trial (http://clinicaltrials.gov/ct2/show/NCT00091949) is an ongoing phase III trial on the effect of treatment with thiazolidinedione drugs on recurrent stroke in patients with a recent TIA or ischemic stroke and insulin resistance.

The current guidelines for the prevention of recurrent stroke recommend treating patients with diabetes mellitus according to the existing guidelines of the American Diabetes Association [62], and not with intensive glycemic control [61]. Unfortunately, detection and detection methods of newly-diagnosed diabetes mellitus and pre-diabetes, and the treatment of pre-diabetes are not mentioned in the current guidelines.

In conclusion, lifestyle intervention in patients with pre-diabetes seems to be at least an equally effective treatment strategy in preventing type 2 diabetes as drug therapy [7, 8]. It remains difficult to maintain lifestyle changes over a longer period and a challenge for each patient. Therefore, pharmacologic interventions might be a good supplement to these lifestyle interventions in preventing diabetes [64]. Furthermore, pre-diabetes seems to be more interesting than advanced diabetes to start treatment to prevent recurrent stroke.

Conclusions

Up to 53% of the non-diabetic patients with a recent ischemic stroke or TIA have pre-diabetes, which is clearly more than in the community.

Different screening methods are available. To detect all patients with an increased risk for diabetes and recurrent stroke, it is advisable to assess 2-hour post-load glucose and glycosylated hemoglobin levels besides fasting plasma glucose levels. However, as more than 50% of the patients will return to normal glucose metabolism within 3 months after the stroke, it is necessary to repeat the test in order to identify the patients with the highest risk both of developing diabetes and recurrent strokes.

Pre-diabetes increases the risk of cardiovascular disease and recurrent ischemic stroke, making this an important target for both primary and secondary prevention. Future studies should show whether pre-diabetes also affects functional outcome after stroke. Lifestyle interventions are at least equally effective as pharmacologic interventions in preventing progression to type 2 diabetes, but more difficult to carry out. The effect of these interventions on the risk of (recurrent) cardiovascular disease is still unclear, but is more promising than these interventions in patients with advanced diabetes.

As the prevalence of pre-diabetes is growing rapidly, pre-diabetes might become one of the most important modifiable therapeutic targets in both primary and secondary prevention. We therefore recommend that the routine screening of newly-diagnosed diabetes mellitus and pre-diabetes with fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels, and the treatment of pre-diabetes should be included into future updating of the guidelines for the prevention of recurrent stroke.

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CHAPTER 3

PREVALENCE AND DIAGNOSIS OF DISTURBED GLUCOSE METABOLISM AFTER TIA OR STROKE

CHAPTER 3.1

PREVALENCE OF PRE-DIABETES AND NEWLY-DIAGNOSED DIABETES IN PATIENTS WITH A TIA OR STROKE

AUTHORS

Susanne Fonville [1]
Adrienne A.M. Zandbergen [2]
Sarah E. Vermeer [3]
Diederik W.J. Dippel [1]
Peter J. Koudstaal [1]
Heleen M. den Hertog [1,4]

1
DEPARTMENT OF NEUROLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

2
DEPARTMENT OF INTERNAL MEDICINE
IKAZIA HOSPITAL
Rotterdam, The Netherlands

3
DEPARTMENT OF NEUROLOGY
RIJNSTATE HOSPITAL
Arnhem, The Netherlands

4
DEPARTMENT OF NEUROLOGY
MEDISCH SPECTRUM TWENTE
Enschede, The Netherlands

CEREBROVASCULAR DISEASES 2013; 36: 283-289

Abstract

BACKGROUND

Patients with transient ischemic attack (TIA) or stroke and pre-diabetes or newly-diagnosed diabetes are at high risk of recurrent stroke or cardiovascular events. This underlines the importance of accurate screening for impaired glucose metabolism in clinical practice. Fasting plasma glucose levels are currently the most commonly measured glycaemic parameter to detect pre-diabetes or diabetes, however 2-hour post-load glucose and glycosylated hemoglobin levels can be used as well. We assessed the prevalence of pre-diabetes and newly-diagnosed diabetes with different screening methods, including fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels in consecutive patients with recent TIA, ischemic stroke or intracerebral hemorrhage admitted to the stroke unit or visiting the specialized TIA outpatient clinic in the Erasmus MC University Medical Center.

METHODS

We measured fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels in 269 patients with a TIA, 374 with ischemic stroke and 57 with intracerebral hemorrhage all without a history of diabetes mellitus. Pre-diabetes was defined as fasting plasma glucose levels of 5.6-6.9 mmol/L and/or 2-hour post-load glucose levels of 7.8-11.1 mmol/L and/or glycosylated hemoglobin levels of 5.7-6.4%. Newly-diagnosed diabetes was defined as fasting plasma glucose levels of ≥7.0mmol/L and/or 2-hour post-load levels of ≥11.1mmol/L and/or glycosylated hemoglobin levels of ≥6.5%. The diagnosis was based on a one-time measurement.

RESULTS

Based on fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels combined, 365 patients (52%) were identified as pre-diabetics and 188 (27%) as having newly-diagnosed diabetes. Patients with intracerebral hemorrhage had more often newly-diagnosed diabetes compared with patients with an ischemic stroke or TIA (respectively 27 (47%) and 161 (25%), p-value <0.001); the prevalence of pre-diabetes was similar.
Newly-diagnosed diabetes was identified more frequently with 2-hour post-load glucose levels (n=162 (23%)) than with fasting plasma glucose (n=49 (7%)) or glycosylated hemoglobin levels (36 (5%)). About one third of the patients with normal fasting glucose levels, has impaired glucose tolerance or impaired glycosylated hemoglobin levels.

CONCLUSIONS

Pre-diabetes and newly-diagnosed diabetes are highly prevalent in patients with TIA or stroke. The majority of these patients would not have been identified with fasting plasma glucose levels alone. Both 2-hour post-load glucose and glycosylated hemoglobin levels identify more patients with disturbed glucose metabolism.

Introduction

Type 2 diabetes is a well-known risk factor for stroke and is associated with unfavorable outcome after stroke [1, 2]. Pre-diabetes, an intermediate metabolic state between normal glucose metabolism and type 2 diabetes, comprises the same metabolic problems as type 2 diabetes [3, 4]. Not only have patients with pre-diabetes an increased risk of developing diabetes, but they also have an increased risk of stroke and other cardiovascular disease [4-6]. Furthermore, pre-diabetes nearly doubles the risk of recurrent stroke in patients with transient ischemic attack (TIA) or stroke [7]. Hence, pre-diabetes might be a therapeutic target for secondary prevention.

Previous studies have shown that about one third of patients with TIA or ischemic stroke and no history of diabetes have pre-diabetes and more than one quarter have newly-diagnosed diabetes [8-16]. About one third of the non-diabetic patients with intracerebral hemorrhage has pre-diabetes, but newly-diagnosed diabetes is less common [11, 13, 17].

Pre-diabetes and newly-diagnosed diabetes can be detected with either fasting plasma glucose levels, or an oral glucose tolerance test or glycosylated hemoglobin [4]. Recently, the American Diabetes Association recommended the additional use of glycosylated hemoglobin levels to diagnose diabetes [18]. At present, there is no consensus on using one in preference to the other. In patients with stroke or TIA, fasting plasma glucose is currently the most commonly measured glycaemic parameter to detect pre-diabetes and/or diabetes [19].

We aimed to assess the prevalence of pre-diabetes and newly-diagnosed diabetes with different screening methods, including fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels in a large population with recent TIA, ischemic stroke or intracerebral hemorrhage.

Materials and methods

STUDY POPULATION

Patients were derived from the Erasmus Stroke Study, a prospective registry aimed at collecting clinical information and blood samples of all patients with neurovascular diseases admitted to the Erasmus MC University Medical Center Rotterdam, The Netherlands, since December 2005 [20]. We evaluated all consecutive patients with TIA, ischemic stroke or intracerebral hemorrhage admitted between November 2007 and December 2010 without a history of diabetes mellitus in whom we assessed fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels. Patients missing one of the glucose tests were excluded. Written informed consent was obtained from all patients signed by the participants or a first-degree relative, as approved by the Institutional Ethics Committee.

PATIENT CHARACTERISTICS

We recorded data on vascular history and risk factors, event characteristics, body mass index (BMI), mean arterial blood pressure and laboratory assessments including lipid profile and renal function. Stroke subtype was classified with the Trial of Org 10710 in Acute Stroke Treatment (TOAST) classification [21]. Stroke severity was assessed by means of the National Institutes of Health Stroke Scale (NIHSS) score [22].

TABLE 1 - PATIENT CHARACTERISTICS OF TIA OR STROKE PATIENTS WITH NORMAL GLUCOSE METABOLISM, PRE-DIABETES AND NEWLY-DIAGNOSED DIABETES

	Normal glucose metabolism [a] (n=147)	Pre-diabetes [b] (n=365)	*P*-value	Newly-diagnosed diabetes [c] (n=188)	*P*-value
Age (yrs), mean (SD)	56 (15)	62 (14)	<0.001	67 (13)	<0.001
Male, n (%)	69 (47)	214 (59)	0.016	97 (52)	0.398
Caucasian, n (%)	116 (82)	295 (85)	0.440	148 (82)	0.986
Vascular risk factors					
Obesity [d], n (%) (missing in 254pt)	45 (49)	142 (60)	0.079	78 (66)	0.019
Current smoking, n (%)	57 (39)	113 (31)	0.089	40 (21)	<0.001
Alcohol abuse [e], n (%)	3 (2)	18 (5)	0.136	10 (5)	0.123
Hypertension [f], n (%)	49 (33)	189 (52)	<0.001	115 (61)	<0.001
Hypercholesterolemia [g], n (%)	31 (21)	111 (30)	0.033	62 (33)	0.016
Atrial fibrillation, n (%)	7 (5)	27 (7)	0.279	23 (12)	0.017
Medical history					
Stroke or TIA, n (%)	37 (25)	80 (22)	0.428	50 (27)	0.768
Ischemic heart disease, n (%)	15 (10)	63 (17)	0.044	32 (17)	0.075
Stroke subtype					
Intracerebral hemorrhage, n (%)	4 (3)	26 (7)	0.055	27 (14)	<0.001
Ischemic event, n (%)	143 (97)	339 (93)		161 (86)	
TIA, n (%)	63 (44)	155 (46)	0.737	51 (32)	0.026
Stroke severity					
Total NIHSS score, median (IQR) [h]	2 (1-5)	2 (1-5)	0.456	4 (2-8)	<0.001
Ischemic stroke/TIA etiology					
Large artery atherosclerosis, n (%)	16 (11)	55 (16)	0.230	29 (18)	0.027
Cardio-embolism, n (%)	14 (10)	47 (14)		32 (20)	
Small vessel occlusion, n (%)	33 (23)	60 (18)		29 (18)	
Other determined etiology, n (%)	6 (4)	19 (6)		4 (3)	
Undetermined etiology, n (%)	74 (52)	156 (46)		66 (41)	
Physical examination					
MAP datascope (mmHg), mean (SD)	91 (12)	92 (12)	0.495	95 (13)	0.005
Laboratory results					
Glucose on admission (mmol/L), mean (SD)	5.9 (1.4)	6.4 (1.4)	0.001	7.2 (2.3)	<0.001
Fasting glucose (mmol/L), mean (SD)	5.0 (0.4)	5.5 (0.6)	<0.001	6.3 (1.7)	<0.001
2-hour post-load glucose (mmol/L), mean (SD)	6.2 (1.2)	8.2 (1.8)	<0.001	13.1 (3.9)	<0.001
Glycosylated hemoglobin (%), mean (SD)	5.4 (0.2)	5.7 (0.3)	<0.001	6.1 (0.9)	<0.001
Total cholesterol (mmol/L), mean (SD)	5.1 (1.1)	5.1 (1.2)	0.672	4.9 (1.2)	0.230
Triglycerides (mmol/L), mean (SD)	1.27 (0.72)	1.49 (0.78)	<0.001	1.53 (0.95)	0.005
HDL (mmol/L), mean (SD)	1.36 (0.43)	1.34 (0.42)	0.550	1.31 (0.42)	0.239
LDL (mmol/L), mean (SD)	3.26 (0.98)	3.24 (1.02)	0.819	3.04 (1.02)	0.051
GFR ≤60ml/min, n (%)	13 (9)	56 (16)	0.051	43 (24)	0.001
Time between event and glucose assessment (days), median (IQR)	5 (3-14)	4 (2-12)	0.149	4 (2-8)	0.014
Discharge assessment					
Discharge to home, n (%) [h]	69 (87)	160 (79)	0.100	76 (56)	<0.001

a. defined as fasting plasma glucose level <5.6mmol/L and 2-hour post-load glucose <7.8mmol/L and glycosylated hemoglobin <5.7%
b. defined as fasting plasma glucose level 5.6-6.9mmol/L and/or 2-hour post-load glucose 7.8-11.0mmol/L and/or glycosylated hemoglobin 5.7-6.4%
c. defined as fasting plasma glucose level ≥7.0mmol/L and/or 2-hour post-load glucose ≥11.1mmol/L and/or glycosylated hemoglobin ≥6.5%
d. defined as BMI ≥25kg/m^2
e. defined as >4 units/day
f. defined as use of antihypertensive drugs on admission
g. defined as use of statins on admission
h. in patients with ischemic stroke or intracerebral hemorrhage only

GLUCOSE ASSESSMENTS

In all patients, fasting plasma glucose and glycosylated hemoglobin levels were assessed on the 2nd or 3rd day of admission or when the patient visited the specialized TIA outpatient clinic. On the same day, an oral glucose tolerance test (OGTT) was performed according to the World Health Organization protocol [23]. After overnight fasting, patients drank a solution of 75 grams glucose in 150 mL water, and 2-hour post-load glucose levels were assessed. Pre-diabetes was defined as a fasting plasma glucose level of 5.6 to 6.9 mmol/L or 2-hour post-load glucose level of 7.8 to 11.0 mmol/L or glycosylated hemoglobin level of 5.7-6.4%, according to the American Diabetes Association criteria [4]. Newly-diagnosed diabetes was defined as fasting plasma glucose level of 7.0mmol/L or higher or 2-hour post-load glucose level of 11.1 mmol/L or higher or glycosylated hemoglobin level of 6.5% or higher [4].

STATISTICAL ANALYSES

Statistical analyses were performed with Stata/SE 12.0 for Windows (Statacorp, College Station, Texas). We compared patient characteristics between glucose groups, with normal glucose metabolism as a reference. Categorical variables were tested with the χ^2-test and continuous variables with Student's t test. Non-normal distributed variables were compared with Wilcoxon rank sum test. *P*-values of less than 0.05 were considered statistically significant.

Results

Between November 2007 and December 2010, 927 patients were included in the Erasmus Stroke Study. Of these, 146 (16%) were excluded because of pre-existent diabetes. In 79 (9%) patients, either fasting plasma glucose or 2-hour post-load glucose or glycosylated hemoglobin levels were not available, because of vomiting, dysphagia, or moribund condition. In two patients (0.2%) the glucose assessment was performed more than 1 year after the event. This left 700 patients for analysis. The excluded non-diabetic patients were comparable in sex, age, vascular risk factors and medical history, but more often had an intracerebral hemorrhage than the included patients (respectively 14 (17%) and 57 (8%), *p*-value 0.007). No adverse events due to the OGTT were reported.

In the total study population, 365 patients (52%) had pre-diabetes and 188 (27%) had newly-diagnosed diabetes based on all three detection methods combined. The prevalence of pre-diabetes and newly-diagnosed diabetes found with different detection methods per event is shown in Figure 1.

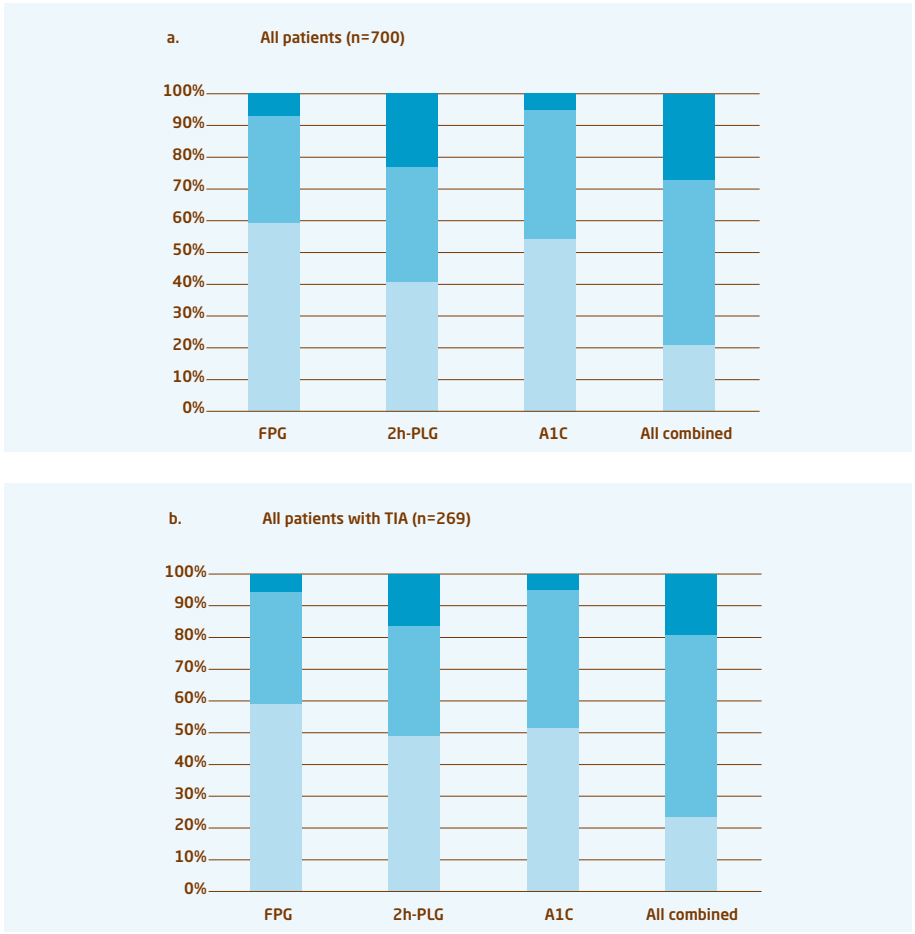

Figure 1 – Prevalence of normal glucose metabolism (light blue), pre-diabetes (medium blue) and newly-diagnosed diabetes (dark blue), based on fasting plasma glucose (FPG), 2-hour post-load glucose (2-h PLG) and glycosylated hemoglobin (A1C) levels and all detection methods combined, in all patients (a), patients with TIA (b), patients with ischemic stroke (c), and patients with intracerebral hemorrhage (d).

Patients with pre-diabetes and newly-diagnosed diabetes were older, more often had hypertension, hypercholesterolemia, disturbed renal dysfunction, and higher triglycerides. Patients with newly-diagnosed diabetes also more often had obesity, atrial fibrillation, less often smoked, and had more severe strokes. Patients with ischemic stroke or intracerebral hemorrhage and newly-diagnosed diabetes were significantly less often discharged to home than patients with normal glucose metabolism (see Table 1).

Based on respectively fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels, pre-diabetes was present in 235 (34%), 253 (36%) and 284 (41%) patients. Newly-diagnosed diabetes was identified more frequently with 2-hour post-load glucose levels (162 (23%)) than with fasting plasma glucose (49 (7%)) or glycosylated hemoglobin levels (36 (5%)).

As shown in Figure 2, of the 416 patients with normal fasting plasma glucose levels, 151 (36%) had impaired glucose tolerance and 136 (33%) had glycosylated hemoglobin levels in the pre-diabetic range. Also, 59 (14%) had 2-hour post-load glucose levels in the diabetic range and 3 (1%) had glycosylated hemoglobin levels in the diabetic range. Furthermore, of the 235 patients with impaired fasting glucose, 71 (30%) and 15 (6%) had respectively 2-hour post-load glucose levels and glycosylated hemoglobin levels in the diabetic range.

Patients with intracerebral hemorrhage had more often newly-diagnosed diabetes compared with patients with an ischemic stroke or TIA (respectively 27 (47%) and 161 (25%), *p*-value <0.001); the prevalence of pre-diabetes was similar. Also, patients with an ischemic stroke had more often newly-diagnosed diabetes compared with patients with a TIA (respectively 110 (29%) vs. 51 (19%), *p*-value 0.026). The prevalence of pre-diabetes was similar in these patients as well.

Median time from symptom onset to glucose assessment was 4 days (interquartile range (IQR) 3-11). This was shorter in patients with pre-diabetes or newly-diagnosed diabetes than

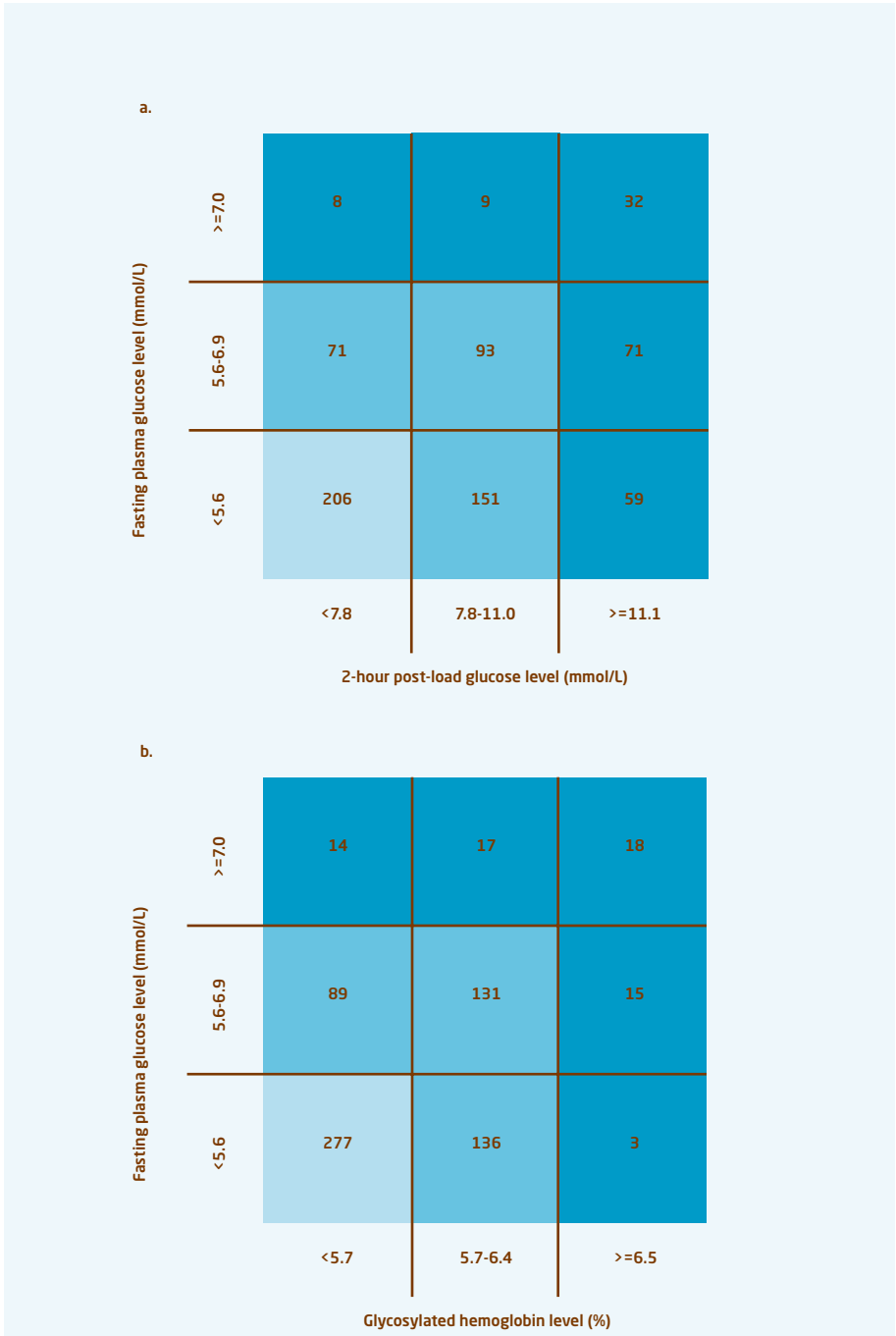

Figure 2 - Number of patients within the different ranges based on fasting plasma glucose levels and respectively 2-hour post-load glucose (a) and glycosylated hemoglobin (b) levels. Normal range (light blue), pre-diabetic range (medium blue) and diabetic range (dark blue).

in patients with normal glucose metabolism. The time between symptom onset and glucose assessment was significantly larger in those that visited the outpatient clinic. Moreover, the prevalence of newly-diagnosed diabetes was lower in patients that visited the outpatient clinic than the patients admitted to the stroke unit, while the proportion of patients with pre-diabetes was larger (data not shown).

Discussion

We found that pre-diabetes and newly-diagnosed diabetes were often present in patients with recent TIA, ischemic stroke or intracerebral hemorrhage, and were underdiagnosed on the basis of fasting plasma glucose levels alone.

Of the 416 patients with normal fasting plasma glucose levels, 151 (36%) had 2-hour post-load glucose levels in the pre-diabetic range and 59 (14%) in the diabetic range. Also, 136 patients (33%) had glycosylated hemoglobin levels in the pre-diabetic range and 3 (1%) in the diabetic range. Of the 235 patients with fasting plasma glucose levels in the pre-diabetic range, 71 (30%) had 2-hour post-load glucose levels in the diabetic range and 15 (6%) had glycosylated hemoglobin levels in the diabetic range. Pre-diabetes was more often identified with both 2-hour post-load glucose and glycosylated hemoglobin levels than with fasting plasma glucose levels alone. Newly-diagnosed diabetes was more often diagnosed with 2-hour post-load glucose levels than with both fasting plasma glucose and glycosylated hemoglobin alone.

Previous studies found a similar prevalence of pre-diabetes and newly-diagnosed diabetes diagnosed with both fasting plasma glucose and 2-hour post-load glucose levels [8-14, 16] and of vascular risk factors in these patients [11, 12, 14].

The added value of the OGTT to detect pre-diabetes and newly-diagnosed diabetes in stroke patients without a history of type 2 diabetes has been advocated before [9, 11-13, 15, 16], but this compromises only one large study among Chinese stroke patients [13] and several smaller studies with either European, American or Japanese patients [9, 11, 12, 16]. To our knowledge, this is the first study to investigate the added value of OGTT in a large sample of European stroke patients. Only 1 study assessed the prevalence of pre-diabetes based on glycosylated hemoglobin levels [24].

Our study has some limitations. Exclusion of patients lacking fasting plasma glucose, 2-hour post-load glucose and/or glycosylated hemoglobin levels and the small percentage of patients with an intracerebral hemorrhage might affect generalizability. However, no significant differences in sex, age, vascular risk factors and medical history existed between in- and excluded patients. Furthermore, the excluded patients had more often an intracerebral hemorrhage or were in a moribund state. As these patients are shown to have more often newly-diagnosed diabetes, we expect that our observation is an underestimation of the true prevalence.

Second, the time between the event and the glucose assessment was significantly shorter in patients with newly-diagnosed diabetes than in patients with normal glucose metabolism and patients visiting the specialized TIA outpatient clinic also less often had newly-diagnosed diabetes. Impaired glucose metabolism in patients with stroke may be transient, reflecting an

acute stress response, or persistent, representing undiagnosed impaired glucose metabolism [25]. Hence, this might have resulted in an overestimation of patients with impaired glucose metabolism. Re-assessment of the fasting plasma glucose, 2-hour post-load glucose and/or glycosylated hemoglobin levels in the chronic stroke phase might help to differentiate between transient hyperglycemia and persistent impaired glucose metabolism. On the other hand, glycosylated hemoglobin levels, which are much less influenced by acute stress responses, were in concordance suggesting pre-existent disturbed glucose metabolism.

Third, the diagnostic tests were not repeated to rule out laboratory error. Therefore, the prevalence of pre-diabetes and newly diagnosed diabetes might be overestimated in our study.

To date, assessment of fasting plasma glucose levels still dominate screening of disturbed glucose metabolism in patients with TIA or stroke, as the oral glucose tolerance test is more time-consuming and inconvenient than fasting plasma glucose. Assessment of glycosylated hemoglobin has recently been advocated as a diagnostic tool of diabetes [18]. Measurement of glycosylated hemoglobin does not require a fasting sample, has much less intra-individual variation, and is less influenced by the events related to acute stroke. However, in our study using plasma glycosylated hemoglobin to screen for diabetes resulted in fewer patients with newly-diagnosed diabetes than with the oral glucose tolerance test.

There is a growing recognition that patients with pre-diabetes should be treated more aggressively, as these patients do not only have an increased risk of developing diabetes, but also of ischemic stroke and other cardiovascular events [5, 6]. Pharmacologic and lifestyle interventions reduce the rate of progression to type 2 diabetes and the risk for stroke [26, 27]. Lifestyle interventions, however, are often difficult to carry out successfully, and life style advice needs to be reinforced on a regular basis. Whether pharmacologic or lifestyle intervention will reduce the risk of stroke and other cardiovascular events in stroke patients with pre-diabetes is not known yet.

In conclusion, 79% of patients with TIA or stroke and without a history of diabetes mellitus have either pre-diabetes or newly-diagnosed diabetes. Without the 2-hour post-load glucose and glycosylated hemoglobin levels, the majority of these patients would not have been identified. Hence, our study provides a rationale for the use of the OGTT and glycosylated hemoglobin levels as a part of standard care in patients with TIA or stroke besides fasting plasma glucose.

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CHAPTER 3.2

OCCURRENCE AND PREDICTORS OF PERSISTENT IMPAIRED GLUCOSE TOLERANCE AFTER ACUTE ISCHEMIC STROKE OR TRANSIENT ISCHEMIC ATTACK

AUTHORS
Susanne Fonville [1]
Heleen M. den Hertog [1,2]
Adrienne A.M. Zandbergen [3]
Peter J. Koudstaal [2]
Hester F. Lingsma [4]

1
DEPARTMENT OF NEUROLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

2
DEPARTMENT OF NEUROLOGY
MEDISCH SPECTRUM TWENTE
Enschede, The Netherlands

3
DEPARTMENT OF INTERNAL MEDICINE
IKAZIA HOSPITAL
Rotterdam, The Netherlands

4
DEPARTMENT OF PUBLIC HEALTH
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

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Abstract

BACKGROUND

Impaired glucose tolerance is often present in patients with a transient ischemic attack (TIA) or ischemic stroke and doubles the risk of recurrent stroke. This impaired glucose tolerance can be transient, reflecting an acute stress response, or persistent, representing undiagnosed impaired glucose metabolism possibly requiring treatment. We aimed to assess the occurrence of persistent impaired glucose tolerance after stroke or TIA and to develop a prediction model to identify patients at risk of persistent impaired glucose tolerance.

METHODS

Patients admitted to the stroke unit or TIA clinic of the Erasmus MC University Medical Center with ischemic stroke or TIA and impaired glucose tolerance (2-hour post-load glucose level of 7.8-11.0mmol/L) were consecutively enrolled between July 2009 and June 2012. The oral glucose tolerance test (OGTT) was repeated after 3 months and patients were classified as having transient impaired glucose tolerance or persistent impaired glucose tolerance. We developed a prediction model by means of a multivariable logistic regression model. We calculated the area under the Receiver Operating Characteristic curve (AUC) to quantify the performance of the model and the internal validity by bootstrapping.

RESULTS

Of the 101 patients included 53 (52%) had persistent impaired glucose tolerance or progression to diabetes. These patients were older and more often had hypertension and used statins. A prediction model including age, current smoking, statin use, triglyceride, hypertension, previous ischemic cardiovascular disease, body mass index (BMI), and fasting plasma glucose accurately predicted persistent impaired glucose tolerance (bootstrapped AUC 0.777), with statin use and fasting plasma glucose as the most important predictors.

CONCLUSIONS

Half of the patients with impaired glucose tolerance after TIA or ischemic stroke have persistent impaired glucose tolerance. We provide a prediction model to identify patients at risk for persistent impaired glucose tolerance, with statin use, triglyceride and fasting plasma glucose as the most important predictors, which, after external validation, might be used to optimize secondary prevention.

Introduction

Impaired glucose tolerance is an intermediate metabolic state between normal glucose tolerance and diabetes mellitus, and is present in more than one third of the patients with TIA or ischemic stroke [1-10]. This impaired glucose tolerance can be transient, reflecting an acute stress response, or persistent, representing undiagnosed impaired glucose metabolism [11, 12]. Studies have found that impaired glucose tolerance is still present after 3 months in 26-71% of the patients [5-7]. In patients with TIA or ischemic stroke, impaired glucose tolerance nearly doubles the risk of recurrent stroke [13]. It is therefore important to identify patients with persistent impaired glucose tolerance as they might benefit from long-term lifestyle intervention and/or treatment with glucose-lowering agents [14-16].
We aimed to assess the occurrence of persistent impaired glucose tolerance in non-diabetic patients with ischemic stroke or TIA. We developed a prediction model to identify patients at risk of persistent impaired glucose tolerance, based on clinical predictors available at the time of admission.

Methods

STUDY POPULATION

Patients were derived from the Erasmus Stroke Study, a prospective registry that started in 2005 and collects clinical information and blood samples of all patients with neurovascular diseases admitted to Erasmus MC University Medical Center Rotterdam, The Netherlands. We prospectively studied all consecutive patients with ischemic stroke or TIA and impaired glucose tolerance (2-hour post-load glucose levels between 7.8 mmol/L and 11.0 mmol/L) admitted to the stroke unit or visiting our specialized TIA clinic between July 2009 and June 2012 within 2 weeks after symptom onset. Patients with pre-existent diabetes and patients with 2-hour post-load glucose levels of 11.1mmol/L or higher (indicating newly-diagnosed diabetes mellitus) were excluded. Written informed consent was obtained from all patients signed by the participants or a first-degree relative, as approved by the Institutional Ethics Committee.

CLINICAL DATA

Demographic data, vascular history and risk factors including statin use, laboratory assessments including lipid profile, and data on event characteristics were collected. Stroke severity was assessed with the National Institutes Health Stroke Scale (NIHSS) score. Stroke subtype was classified with the Trial of ORG 10172 in Acute Stroke Therapy (TOAST) classification [17].

GLUCOSE ASSESSMENTS

In all patients, fasting plasma glucose and glycosylated hemoglobin levels were assessed on the 2nd or 3rd day of admission or when visiting the outpatient clinic, as part of standard clinical care. On the same day, an oral glucose tolerance test (OGTT) was performed according to the World Health Organization protocol [18]. After overnight fasting, patients drank a solution of 75 grams glucose in 150mL water, and 2-hour post-load glucose levels were assessed. Impaired glucose tolerance was defined as 2-hour post-load glucose levels between 7.8 and 11.0mmol/L. Patients with fasting plasma glucose levels of 7.0mmol/L or higher were diagnosed with diabetes and therefore excluded.

TABLE 1 - PATIENT CHARACTERISTICS COMPARED BETWEEN PATIENTS WITH TRANSIENT AND PERSISTENT IMPAIRED GLUCOSE TOLERANCE

Patient characteristics	Transient impaired glucose tolerance (n=48)	Persistent impaired glucose tolerance (n=53)	*P*-value
Age (yrs), mean (SD)	56 (14)	63 (14)	0.017
Male, n (%)	31 (65)	29 (55)	0.313
Caucasian, n (%)	43 (90)	45 (85)	0.483
Vascular risk factors			
Current smoking, n (%)	18 (38)	11 (21)	0.063
BMI (kg/m^2)*, mean (SD)	26.6 (4.0)	27.6 (5.4)	0.305
Hypertension**, n (%)	17 (35)	35 (66)	0.002
Statin use, n (%)	6 (13)	21 (40)	0.002
Atrial fibrillation, n (%)	5 (10)	6 (11)	0.884
Vascular medical history			
Ischemic cardiovascular disease, n (%)	8 (17)	16 (30)	0.111
Event characteristics			
TIA, n (%)	17 (35)	20 (38)	0.809
Total NIHSS score, median (IQR)†	3 (1-6)	2 (1-4)	0.232
TOAST classification			0.885
Large artery disease, n (%)	6 (13)	5 (10)	
Cardio-embolism, n (%)	6 (13)	8 (15)	
Small vessel disease, n (%)	17 (35)	15 (28)	
Other, n (%)	2 (4)	2 (4)	
Undetermined, n (%)	17 (35)	23 (43)	
Glucose assessments during admission/visiting TIA clinic			
Glucose on admission (mmol/L), mean (SD)	6.3 (1.3)	6.7 (1.6)	0.207
Fasting plasma glucose (mmol/L), mean (SD)	5.4 (0.5)	5.5 (0.6)	0.135
2-hour post-load glucose (mmol/L), mean (SD)	8.9 (0.8)	9.2 (0.9)	0.165
Glycosylated hemoglobin (mmol/mol)/ (%), mean (SD)	37 (3)/ 5.5 (0.3)	39 (4)/ 5.7 (0.4)	0.027
Days between event and OGTT, median (IQR)	3 (2-5)	2 (2-5)	0.333
Laboratory assessments during admission/visiting TIA clinic			
Total cholesterol (mmol/L), mean (SD)	5.5 (1.1)	5.0 (1.2)	0.058
Triglycerides (mmol/L), mean (SD)	1.68 (0.81)	1.43 (0.58)	0.151
HDL (mmol/L), mean (SD)	1.32 (0.45)	1.37 (0.43)	0.611
LDL (mmol/L), mean (SD)	3.56 (1.04)	3.16 (1.07)	0.059

* missing in 16 patients
** defined as the use of antihypertensive drugs prior to the event
† in patients with ischemic stroke only

OUTCOME

At 3 months all patients were invited to visit the outpatient clinic and were asked to undergo a second OGTT. The OGTT was repeated and based on the results patients were classified as having transient impaired glucose tolerance (2-hour post-load glucose level of less than 7.8mmol/L), persistent impaired glucose tolerance (2-hour post-load glucose level between 7.8 and 11.0mmol/L) or progression to diabetes (2-hour post-load glucose level of 11.1mmol/L or over) [1].

STATISTICAL ANALYSIS

Statistical analyses were performed with Stata/SE 12.1 for Windows (Statacorp, College Station, Texas). Missing variables were imputed with single imputation using the baseline characteristics and the outcome variable. We compared clinical variables between glucose groups, with transient impaired glucose tolerance as a reference. Patients with persistent impaired glucose tolerance or progression to diabetes were grouped together as persistent disturbed glucose tolerance because of the small sample size. The differences between the glucose groups in categorical variables were tested with the Chi^2-test and continuous variables with Student's t test. Non-normal distributed variables were compared with Wilcoxon sum rank test. *P*-values of less than 0.05 were considered statistically significant.

MODEL DEVELOPMENT

Possible predictors of persistent impaired glucose tolerance included known risk factors for developing diabetes and other risk factors according to previous literature: age, sex, ethnicity, current smoking, statin use, lipids (triglycerides, low-density lipoprotein (LDL), and high-density lipoprotein (HDL)), hypertension, previous ischemic cardiovascular disease, (paroxysmal) atrial fibrillation, body mass index (BMI), TIA vs. ischemic stroke, large artery atherosclerosis, fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels [5-7, 19]. We graphically assessed the shape of relationships between continuous predictors and persistent impaired glucose tolerance with restricted cubic spline functions. When the shape was non-linear, the variable was transformed according to the observed relation. All potential predictors were tested in univariable logistic regression analysis and those with *p*-value ≤0.2 were further analyzed in a multivariable logistic regression model, except for the lipid variables. As the lipid variables are expected to be correlated to statin use, they were first added separately and subsequently simultaneously to the model. This resulted in the basic model to which the glucose levels were added to assess their additional prognostic value. Based on the -2 log likelihood of the models and the *p*-values of the corresponding Chi^2, the best prediction model was chosen. The discriminative ability of this model (area under the Receiver Operating Characteristic curve (AUC)) was calculated. The AUC ranges from 0.5 for non-informative to 1.0 for perfect models. The internal validity of the model was assessed by means of bootstrapping techniques, resulting in an internally validated AUC, which represents the expected performance of the model in future patients.

Results

STUDY POPULATION

Between July 2009 and June 2012 1176 patients with a TIA or ischemic stroke were admitted to the stroke unit or visited the outpatient clinic, and 236 of them had previously been diagnosed with diabetes mellitus. Of the 940 non-diabetic patients 191 (20%) did not have an initial oral

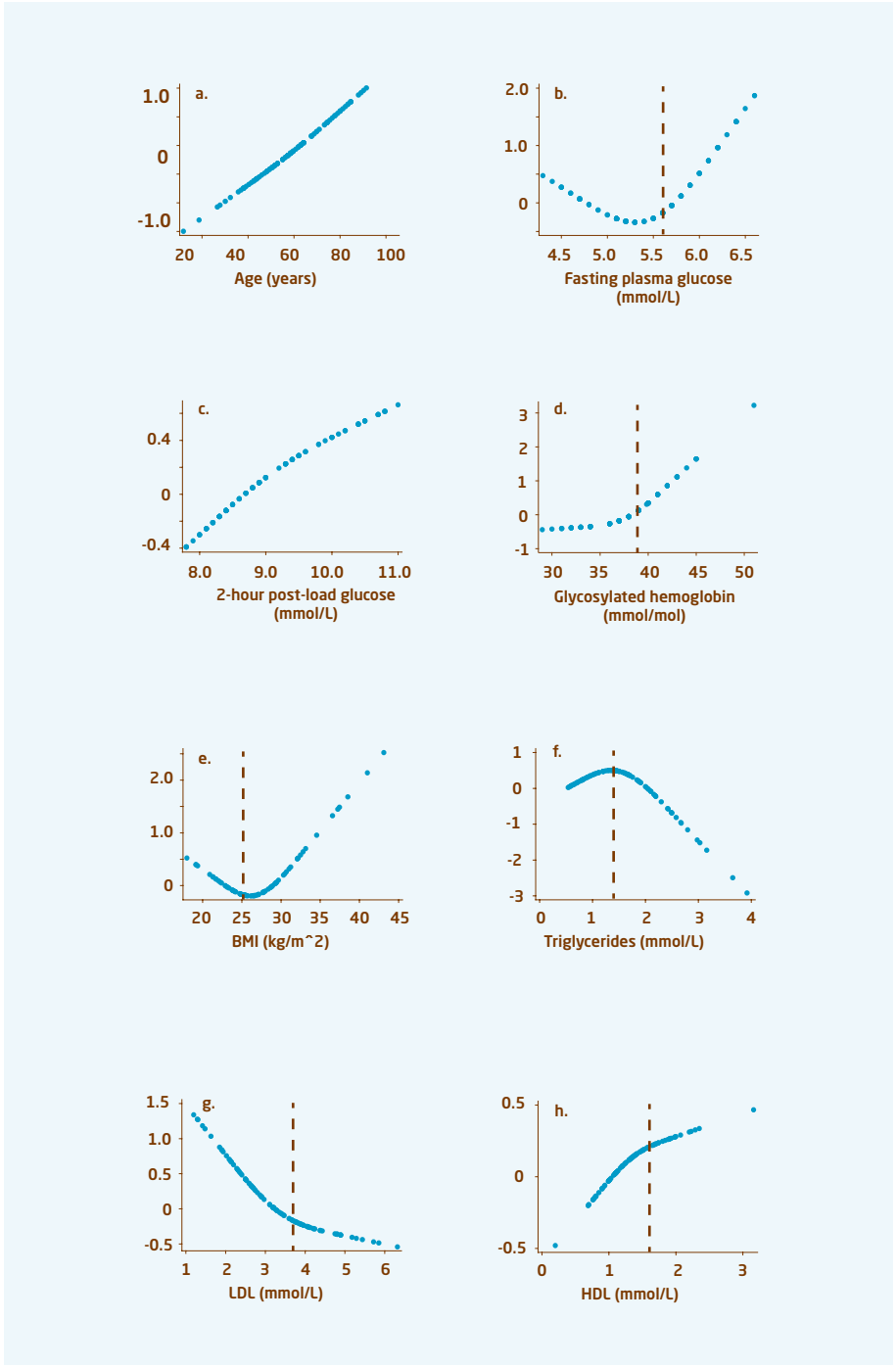

Figure 1 - Shape of relations between persistent impaired glucose tolerance and the continuous variables with the corresponding cut-off points (dashed line): a. age, b. fasting plasma glucose (≥5.6mmol/L), c. 2-hour post-load glucose, d. glycosylated hemoglobin (≥39mmol/mol), e. BMI (≥25kg/m²), f. triglycerides (≥1.30mmol/L), g. LDL (≤3.70mmol/L), h. HDL (≤1.60mmol/L). Linear predictor (log odds) is shown on the y-axis.

glucose tolerance test, 357 (38%) had 2-hour post-load glucose levels in the normal range, 245 patients (26%) had impaired glucose tolerance, and 147 (16%) had 2-hour post-load glucose levels in the diabetic range. Of the 245 patients with initially impaired glucose tolerance, 98 patients (40%) did not have a repeat OGTT, 31 (13%) had the initial oral glucose tolerance test more than 2 weeks after the event, and 15 (6%) were excluded because they did not have a fasting plasma glucose or it was ≥7.0mmol/L. The remaining 101 patients with initially impaired glucose tolerance and a repeat OGTT were analyzed in this study. The OGTT was not repeated due to follow-up in another institution (general practitioner, other hospital or nursing home of residence) (16% of all patients), test failed or refused by patient (5%), no show (8%), event before follow-up (deceased or recurrent stroke) (4%) or lost-to-follow-up (7%). Patients who did not undergo a second OGTT compared to those who did were significantly older (66 ±15 vs. 60 ±15 years, *p*-value 0.010), and were less often discharged to home (37% vs. 79%, *p*-value <0.001).

OCCURRENCE OF PERSISTENT IMPAIRED GLUCOSE TOLERANCE

Of the 101 patients, 47 (47%) had persistent impaired glucose tolerance at 3 months and 6 (6%) had progressed to diabetes. The patient characteristics are shown in Table 1. Patients with persistent disturbed glucose tolerance were older and had more often hypertension and statin use compared to those with transient impaired glucose tolerance. Glycosylated hemoglobin levels were significantly higher in these patients.

TABLE 2 - SELECTION OF PREDICTORS FOR PERSISTENT DISTURBED GLUCOSE TOLERANCE

Possible predictors	OR (95%-CI)	*p*-value
Age (per 10 years)	1.40 (1.05-1.88)	0.020
Male sex	0.66 (0.30-1.48)	0.314
Caucasian	0.65 (0.20-2.16)	0.485
Current smoking	0.44 (0.18-1.06)	0.066
BMI (per kg/m²)(if <25kg/m², 25kg/m² was used)*	1.13 (1.00-1.28)	0.054
Hypertension	3.55 (1.56-8.05)	0.002
Statin use	4.59 (1.66-12.70)	0.003
Atrial fibrillation	1.10 (0.31-3.86)	0.884
Previous ischemic cardiovascular disease	2.16 (0.83-5.64)	0.115
Ischemic stroke vs. TIA	0.90 (0.40-2.04)	0.809
Large artery atherosclerosis vs. other causes	0.73 (0.21-2.56)	0.622
Triglycerides (per mmol/L)(if <1.30mmol/L, 1.30mmol/L was used)*	0.38 (0.17-0.86)	0.021
LDL (per mmol/L) (if ≥3.70mmol/L, 3.70mmol/L was used)*	0.53 (0.29-0.95)	0.033
HDL (per mmol/L) (if ≥1.60mmol/L, 1.60mmol/L was used)*	1.89 (0.50-7.19)	0.351
Fasting plasma glucose (per mmol/L) (if <5.6mmol/L, 5.6mmol/L was used)*	6.37 (1.16-35.08)	0.033
2-hour post-load glucose (per mmol/L)	1.40 (0.87-2.23)	0.165
Glycosylated hemoglobin (per mmol/mol) (if <39mmol/L, 39mmol/L was used)*	1.31 (0.98-1.76)	0.064

* cut-off point based on Figure 1

Possible predictors with p-value ≤0.2 were included in the multivariable logistic regression analysis.

TABLE 3 - SELECTION OF THE BEST BASIC MODEL AND THE ADDED VALUE OF GLUCOSE TESTS TO THE MODEL

Model	Variables	Chi²	*p*-value
1	Age, current smoking, hypertension, previous ischemic cardiovascular disease, BMI, and statin use	21.40	Reference
Addition of lipid variables to model			
2	Model 1 + triglycerides (per mmol/L)†	25.48	0.043*
3	Model 1 + LDL (per mmol/L)††	23.84	0.118*
4	Model 1 + triglycerides + LDL	26.64	0.073*
Added value of glucose test to model			
5	Model 2 + fasting plasma glucose (per mmol/L)‡	30.56	0.024**
6	Model 2 + 2-hour post-load glucose (per mmol/L)	26.22	0.390**
7	Model 2 + glycosylated hemoglobin (per mmol/mol)‡‡	29.06	0.059**

* Compared to model 1
** Compared to model 2
† if <1.30mmol/L, 1.30mmol/L was used
†† if ≥1.60mmol/L, 1.60mmol/L was used
‡ if fasting plasma glucose <5.6mmol/L, 5.6mmol/L was used
‡‡ if glycosylated hemoglobin <39mmol/mol, 39mmol/mol was used

PREDICTION MODEL

The shape of relations between persistent impaired glucose tolerance and the continuous variables with the corresponding cut-off points are shown in Figure 1. Age and 2-hour post-load glucose levels had a linear relation with persistent impaired glucose tolerance and were analyzed as such. Glycosylated hemoglobin showed a constant risk <39mmol/mol (<5.7%) and values below that cut-off were truncated. The other non-linear variables (triglycerides, LDL, HDL, BMI, and fasting plasma glucose) were all truncated in the same way as glycosylated hemoglobin, with the corresponding cut-off points shown in Figure 1.

Based on the results of the univariable regression analysis (Table 2), the variables age, current smoking, hypertension, previous cardiovascular disease, BMI, statin use, triglycerides, LDL, fasting plasma glucose, 2-hour post-load glucose, and glycosylated hemoglobin levels were selected for the prediction model. All variables were further analyzed in a multivariable logistic regression model, except for the three glucose variables. The lipid variables (triglycerides and LDL) were added separately to select the basic model. Adding triglycerides (if <1,30mmol/L, 1,30mmol/L was used; model 2) improved the model significantly (*p*-value 0.043). However, addition of LDL or both triglycerides and LDL to the model (model 3-4) did not improve the model significantly (Table 3), so model 2 was chosen as basic model.

Addition of fasting plasma glucose (if <5.6mmol/L, 5.6mmol/L was used; model 5) to the basic model significantly improved the model (*p*-value 0.024). The basic model was not improved after entering 2-hour post-load glucose (model 6, *p*-value 0.390) or glycosylated hemoglobin levels (model 7, *p*-value 0.059) (Table 3). Model 5 was therefore chosen as the final prediction model, with a good discriminative disability of 0.805. The internally validated AUC was 0.777, indicating good performance. The strongest predictors in the model were the presence of statin use (adjusted OR (95% CI) 4.74 (1.24-18.01)), triglycerides (adjusted OR (95% CI) 0.39 (0.16-0.96)) and fasting plasma glucose (adjusted OR (95% CI) 8.97 (1.19-67.70)). The risk of persistent impaired glucose tolerance after TIA or stroke for individual patients can be calculated with the formula shown under Table 4.

TABLE 4 - FINAL PREDICTION MODEL TO ASSESS THE RISK OF PERSISTENT DISTURBED GLUCOSE TOLERANCE

Predictor	aOR (95% CI)
Age (per 10 year)	1.18 (0.79-1.77)
Current smoking	0.74 (0.23-2.37)
Statin use	4.74 (1.24-18.01)
Triglycerides (per mmol/L)	0.39 (0.16-0.96)
Hypertension	2.14 (0.76-6.01)
Previous ischemic cardiovascular disease	0.48 (0.12-2.03)
BMI (per kg/m²)	1.12 (0.98-1.28)
Fasting plasma glucose (per mmol/L)	8.97 (1.19-67.70)

Risk of persistent disturbed glucose tolerance: exp(linear predictor)/(1+exp(linear predictor)). Linear predictor = 15.607 + 0.017 x age - 0.308 x current smoking + 1.55 x statin use - 0.934 x triglycerides + 0.763 x hypertension - 0.726 x previous ischemic cardiovascular disease + 0.115 x BMI + 2.194 x fasting plasma glucose. If triglycerides <1.30mmol/L, use 1.30mmol/L, if BMI <25kg/m², use 25kg/m² and if fasting plasma glucose <5.6mmol/L, use 5.6mmol/L.
AUC = 0.805, bootstrapped mean AUC = 0.777.

Discussion

This study showed that approximately half of the patients with a TIA or ischemic stroke and impaired glucose tolerance had persistent disturbed glucose tolerance after 3 months. We developed a prediction model that accurately predicts persistent impaired glucose tolerance (bootstrapped AUC 0.777) using age, current smoking, hypertension, previous ischemic cardiovascular disease, BMI, statin use, triglycerides, and fasting plasma glucose, clinical variables readily available on admission. The strongest predictors for persistent impaired glucose metabolism at three months were statin use, triglycerides, and fasting plasma glucose.

To our knowledge, only 3 studies repeated the oral glucose tolerance test 3 months after discharge to investigate the persistence of disturbed glucose tolerance after an ischemic stroke [5-7]. Persistent disturbed glucose tolerance was present in 26-71% patients, with 4-42% progressing to diabetes [5-7]. Two of these studies found that 2-hour post-load glucose level was a predictor for persistent disturbed glucose tolerance [6, 7], which is in contrast to our results. This may be due to the small sample size and different statistical analysis methods of these studies. Impaired fasting glucose and impaired glucose tolerance have different pathophysiological mechanisms: hepatic versus muscle insulin resistance. However, they often co-exist indicating more advanced disease and an even higher risk in developing diabetes compared to patients with impaired fasting glucose or impaired glucose tolerance alone [20, 21]. Dyslipidemia is often present in patients with impaired fasting glucose and/or impaired glucose tolerance. This corresponds with the importance of fasting plasma glucose, triglyceride levels and the presence of statin use in predicting persistent impaired glucose tolerance [19].

Strengths of our study are the prospective design and the relative large number of cases. Our study has also some limitations. The OGTT was not repeated in 49% of the patients. As these patients were older, and were less often discharged to home, this affects generalizability of our findings. Since we included not all patients with a TIA or ischemic stroke admitted to our

hospital in our study, we are not able to estimate the prevalence of persistent impaired glucose tolerance in the total stroke population. However, based on data from our center we estimate that approximately 20% of all patients has pre-existing diabetes. From our study we have learned that 33% of the non-diabetic patients has impaired glucose tolerance, and about half had persistent impaired glucose tolerance or progression to diabetes.
Furthermore, this was an observational study and was thus hampered by important missing data. We used imputation to overcome the problem of missing values, which is preferred over complete case-analysis [22]. Of course, our model should be externally validated in an independent comparable population before its use in clinical practise can be recommended.

Our study confirms the hypothesis that disturbed glucose metabolism in the acute phase is not only due to a stress reaction, but might also indicate underlying undiagnosed disturbed glucose metabolism. As impaired glucose tolerance is often present after TIA or stroke, predicting the persistence of impaired glucose tolerance is very important. These patients do not only have an increased risk for developing diabetes but also an increased risk for recurrent events. Intensive lifestyle intervention and/or anti-diabetic drug therapy can be started directly after the TIA or stroke in the patients with high risk for persistent impaired glucose tolerance in the context of secondary stroke prevention. The treatment of impaired glucose tolerance after TIA or stroke is still subject of several trials. The **M**etformin and sit**A**gliptin in patients with imp**A**ired glucose tolerance and a recent TIA or minor ischemic **S**troke (MAAS) trial (http://www.trialregister.nl/trialreg/admin/rctview.asp?TC=3196) is a phase II trial currently investigating the feasibility and safety of both metformin and sitagliptin in patients with impaired glucose tolerance after TIA or ischemic stroke. The **I**nsulin **R**esistance **I**ntervention after **S**troke (IRIS) trial (http://clinicaltrials.gov/ct2/show/NCT00091949) is a phase III trial investigating the effect of treatment with thiazolidinedione drugs on the occurrence of recurrent stroke in patients with a recent TIA or ischemic stroke and insulin resistance.

Conclusions

To sum up, half of the patients with impaired glucose tolerance after TIA or ischemic stroke have persistent impaired glucose tolerance. We provide a prediction model to identify patients at risk for persistent impaired glucose tolerance, with statin use, triglyceride and fasting plasma glucose as the most important predictors, which, after external validation, might be used to optimize secondary prevention.

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CHAPTER 4

GLUCOSE METABOLISM AND NOCTURNAL BLOOD PRESSURE PATTERNS AS VASCULAR RISK FACTORS OF ATHEROSCLEROSIS

CHAPTER 4.1

NEWLY-DIAGNOSED DISTURBED GLUCOSE METABOLISM IS ASSOCIATED WITH ATHEROSCLEROSIS IN PATIENTS WITH TIA OR ISCHEMIC STROKE

AUTHORS

Susanne Fonville [1]
Anouk C. van Dijk [1,2]
Thaira Zadi [2]
Evita G. van den Herik [1]
Hester F. Lingsma [3]
Peter J. Koudstaal [1]
Aad van der Lugt [2]
Heleen M. den Hertog [1,4]

1
DEPARTMENT OF NEUROLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

2
DEPARTMENT OF RADIOLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

3
DEPARTMENT OF PUBLIC HEALTH
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

4
DEPARTMENT OF NEUROLOGY
MEDISCH SPECTRUM TWENTE
Enschede, The Netherlands

SUBMITTED

Abstract

BACKGROUND

Newly-diagnosed disturbed glucose metabolism is highly prevalent in non-diabetic patients with transient ischemic attack (TIA) or ischemic stroke, and increases the risk of recurrent stroke. Diabetes mellitus is associated with atherosclerosis. We aimed to assess whether newly-diagnosed disturbed glucose metabolism is associated with atherosclerosis as well.

METHODS

Patients with a recent TIA or ischemic stroke were classified in three groups based on glucose levels and use of antidiabetic drugs. Pre-existent diabetes mellitus was defined as the use of antidiabetic drugs prior to the event. Newly-diagnosed disturbed glucose metabolism was defined as two or more disturbed glucose tests: fasting plasma glucose level ≥5.6mmol/L, 2-hour post-load glucose level ≥7.8mmol/L, and/or glycosylated hemoglobin level ≥39mmol/mol.
We used CT-angiography to assess stenosis in the carotid artery bifurcations and calcification volume in the aortic arch, carotid bifurcations and intracranial carotid arteries. The relation between glucose groups and measures of atherosclerosis was expressed as odds ratios and beta coefficients with corresponding 95% confidence interval (CI), adjusted for potential confounders.

RESULTS

Of the 1217 patients, 384 (32%) had newly-diagnosed disturbed glucose metabolism, and 210 (17%) had pre-existent diabetes mellitus. Newly-diagnosed disturbed glucose metabolism was independently associated with stenosis ≥50% (aOR (95%CI) 1.15 (1.04-2.20)). Pre-existent diabetes mellitus was associated with stenosis ≥50% (aOR (95%CI) 1.65 (1.06-2.58)), and calcification volume in all regions (adjusted beta coefficient (95%CI) 0.49 (0.18-0.81), 0.50 (0.22-0.79) and 0.57 (0.25-0.88) for aortic arch, carotid bifurcations and intracranial carotid arteries respectively).

CONCLUSION

Our study shows that newly-diagnosed disturbed glucose metabolism in patients with a recent TIA or ischemic stroke is associated with more severe extra- and intracranial atherosclerosis, similar to pre-existent diabetes mellitus.

Introduction

Diabetes mellitus is a well-known risk factor for first stroke and stroke recurrence [1]. Pre-diabetes is a metabolic state with a high risk of developing diabetes mellitus in the future [2, 3] and comprises impaired fasting glucose and/or impaired glucose tolerance and/or disturbed glycosylated hemoglobin levels [3, 4]. Pre-diabetes and newly-diagnosed diabetes mellitus are highly prevalent (up to 79%) in patients with an ischemic stroke or transient ischemic attack (TIA) without known diabetes mellitus prior to the event [5-9] and is also associated with an increased risk of cardiovascular disease and recurrent stroke [10-12].

Patients with diabetes mellitus are highly susceptible for atherosclerosis, which is reflected in increased carotid intima media thickness (CIMT) [13-15] and severe calcification in the aortic arch and carotid arteries [16, 17]. However, it is still unsure whether the association between diabetes mellitus and calcification volume is similar between men and women [16-20]. More severe atherosclerosis is presumed to be the main underlying mechanism of the increased risk of recurrent stroke in patients with diabetes mellitus. However, there is no consensus whether pre-diabetes is associated with atherosclerosis as well [13-15, 21, 22].

Several methods are available to assess carotid atherosclerosis. Duplex ultrasound, CT or MR angiography and conventional angiography are all used for evaluation of degree of carotid artery stenosis [23]. CT angiography allows the measurement of not only severity of stenosis, but also calcification volume in different vessel beds, which gives more information about the severity of atherosclerosis in comparison with the assessment of CIMT by duplex ultrasound [16, 24].
To the best of our knowledge, there are no studies on the association between newly-diagnosed disturbed glucose metabolism (pre-diabetes or newly-diagnosed diabetes mellitus) and atherosclerosis in patients with TIA or ischemic stroke. We therefore aimed to assess whether newly-diagnosed disturbed glucose metabolism, determined with fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels is associated with craniocervical atherosclerosis in patients with TIA or ischemic stroke compared with patients with pre-existent diabetes mellitus or normal glucose metabolism.

Methods

STUDY POPULATION

Patients were derived from the Erasmus Stroke Study, an ongoing registry of patients with cerebrovascular diseases treated at the Erasmus MC University Medical Center Rotterdam. All consecutive patients with a clinical diagnosis of acute ischemic stroke or TIA between December 2005 and October 2010 were included. Patients without a CT-angiography and/or missing glucose assessment were excluded. Our study was approved by the Institutional Ethics Committee and written informed consent was obtained from all participants.
Ischemic stroke was defined as a focal neurological deficit of sudden onset of presumed vascular origin, lasting 24 hours or more, with brain imaging showing typical signs of brain infarction or no abnormalities. The diagnosis of TIA was defined as a focal neurologic deficit of sudden onset lasting less than 24 hours and with no signs of recent infarction on CT-scan.

CLINICAL DATA

Demographic data, vascular history (previous TIA or ischemic stroke and previous ischemic heart disease among others) and risk factors (current smoking and body mass index (BMI) among others), laboratory assessments, and data on event characteristics were collected. Hypertension was defined as the use of antihypertensive drugs prior to the event and dyslipidemia was defined as the use of cholesterol lowering drugs prior to the event. Stroke subtype was classified with the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria [25]. Stroke severity was assessed by means of the National Institutes of Health Stroke Scale (NIHSS) score [26].

GLUCOSE ASSESSMENT

Pre-existent diabetes mellitus was defined as the use of oral and/or parenteral antidiabetic drugs prior to the TIA or ischemic stroke. In all patients, fasting plasma glucose and glycosylated hemoglobin levels were assessed on the 2nd or 3rd day of admission or when the patient visited the outpatient clinic in case of TIA patients who were not hospitalized. Subsequently, an oral glucose tolerance test (OGTT) was performed according to the World Health Organization protocol in all patients without pre-existing diabetes mellitus [27]. After overnight fasting, patients drank a solution of 75 grams glucose in 150 mL water, and 2-hour post-load glucose levels were assessed. Newly-diagnosed disturbed glucose metabolism was defined as 2 or more disturbed glucose tests according to the American Diabetes Association: fasting plasma glucose level ≥5.6mmol/L, 2-hour post-load glucose level ≥7.8mmol/L, or glycosylated hemoglobin level ≥39mmol/mol [3].

CT ACQUISITION AND ANALYSIS

CT Angiography (CTA) of the carotid artery was performed with a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Sensation 64, Definition, Definition AS+ or Definition Flash, Siemens Medical Solutions, Erlangen, Germany) using a standardized optimized contrast-enhanced CTA protocol (120 kVp, 180-200 mAs, collimation 16 x 0.75 mm; 32 x 2 x 0.6 mm; 64 x 2 x 0.6 mm, pitch < 1). The scan range extended from the ascending aorta to the intracranial circulation (3 cm above the sella turcica). All patients received 80 ml of contrast agent (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, UK), followed by 45 ml saline bolus chaser, both at an injection rate of 4 or 5 ml/s. Image reconstructions were made with field of view of 120 mm, matrix size 512 × 512, slice thickness 0.75 or 1.0 mm, increment 0.4 – 1.0 mm and with an intermediate reconstruction algorithm.

STENOSIS

The severity of stenosis in carotid bifurcations (within 3 cm proximal and distal of the bifurcation) was assessed with the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [28]. A stenosis of 50% or more was considered significant. An intraclass correlation coefficient (ICC) for the degree of stenosis was based on the ratings of 3 independent observers (S.F., A.v.D. & E.v.d.H.) on 50 CT examinations: 0.96-0.99.

VOLUME OF CALCIFICATIONS

Dedicated commercially available software (Syngo CalciumScoring, Siemens) was used to semi-automatically quantify calcifications in the aortic arch and both extracranial carotid arteries, expressed as calcification volume in mm^3. The aortic arch was defined as the origin of the aortic arch (the image in which the ascending and descending aorta merge into the inner curvature of the aortic arch) to the first 1 cm of the common carotid arteries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. The carotid arteries were scored within 3 cm proximal and distal of the bifurcation. A threshold of 600 Hounsfield units (HU) was used to differentiate calcifications from contrast material in the lumen. A detailed description of the measurement is provided elsewhere [16].

A custom-made plug-in for the freely available software ImageJ (Rasband, National Institute of Mental Health, Bethesda, MD, available at http://rsb.info.nih.gov/ij) was used for quantification of intracranial internal carotid artery calcifications. The intracranial internal carotid artery comprised the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery. Due to the close relationship of calcium in the arterial wall to the skull base, the previous mentioned semi-automatic tool could not be used. With the plug-in, it is possible to draw regions of interest in axial multidetector (MD) CTA images and to calculate automatically the total number of pixels above a predefined threshold. The volume of the intracranial calcifications (in mm^3) was calculated as the product of the number of pixels above the threshold (600 HU), the pixel size, and the increment [29].

ICC for extracranial and intracranial calcifications was assessed, based on the ratings of respectively 3 independent observers (S.F., A.v.D. & T.Z.) on 35 CT examinations (ICC 1.00) and the ratings of 2 independent observers (S.F. & T.Z.) on 40 CT examinations (ICC 0.99).

STATISTICAL ANALYSIS

The analysis was carried out with STATA 12.1 statistical package (Statacorp, College Station, Texas). Missing variables were imputed with single imputation using relevant baseline patient characteristics and the outcome variable. We compared clinical and radiological data among glucose groups, with normal glucose metabolism as a reference. In the analysis with severity of stenosis, we used the highest degree of stenosis in both carotid artery bifurcations. In the analysis with calcification as continuous measure, we used natural log-transformed values and added 1.0 mm^3 to the non-transformed values to deal with participants with a calcium score of zero. Calcification volumes of the left and right side were summed for the extra- and intracranial carotid arteries separately.

We performed logistic and linear analyses to study the relation between glucose metabolism on the one hand and atherosclerosis on the other. Adjustments were made with a multivariable logistic and linear regression model that included age, sex, hypertension, dyslipidemia, previous TIA or ischemic stroke, previous ischemic heart disease, current smoking, and BMI.

Based on the previous conflicting results between men and women, we repeated these analyses in men and women separately. Furthermore, we tested the difference in the effect of glucose on atherosclerosis between men and women with a test for interaction.

P-values of less than 0.05 were considered statistically significant.

Results

Between December 2005 and October 2010, 1611 consecutive patients with a TIA or ischemic stroke were included in the Erasmus Stroke Study. Of these patients, 205 (13%) were excluded because no glucose assessment was performed and 189 patients (12%) were excluded because no CTA was done because of contrast allergy, renal failure, or because a duplex ultrasound or CTA was performed in another hospital. In the remaining 1217 patients, the mean age was 62 years (SD 14), and 642 (53%) were men. Men were more often current smokers (34% vs. 27%, *p*-value 0.004), and more often had dyslipidemia (37% vs. 30%, *p*-value 0.023) and previous ischemic heart disease (24% vs. 11%, *p*-value <0.001) than women. Other vascular risk factors were comparable between the two sexes.

Of the 1217 patients, 623 patients (51%) had normal glucose metabolism, 384 (32%) had newly-diagnosed disturbed glucose metabolism, and 210 (17%) had pre-existent diabetes mellitus. Patients with newly-diagnosed disturbed glucose metabolism or pre-existent diabetes mellitus were older, more often had hypertension, dyslipidemia, previous TIA or ischemic stroke, previous ischemic heart disease, were less often current smokers, and had a higher BMI. These patients were more often diagnosed with ischemic stroke, and the ischemic strokes were more severe. Large artery disease according to the TOAST criteria was more often present in patients with newly-diagnosed disturbed glucose metabolism or pre-existent diabetes mellitus. The demographic and clinical characteristics of the patients are shown in Table 1.

ATHEROSCLEROSIS

Patients with newly-diagnosed disturbed glucose metabolism or pre-existent diabetes mellitus more often had a carotid artery stenosis ≥50%, and higher calcification volumes in aortic arch, carotid bifurcations, and intracranial carotid arteries, compared to patients with normal glucose metabolism (Figure and Table 2).

After adjusting for confounders, pre-existent diabetes mellitus was associated with stenosis ≥50%, and calcification volume in all regions. Also, newly-diagnosed disturbed glucose metabolism was associated with stenosis ≥50% (Table 3).

Both men and women with newly-diagnosed disturbed glucose metabolism or pre-existent diabetes mellitus had higher calcification volumes in aortic arch, carotid bifurcations, and intracranial carotid arteries, compared to patients with normal glucose metabolism (Table 2). Stenosis ≥50% was more often present in men with newly-diagnosed disturbed glucose metabolism or pre-existent diabetes mellitus, compared to those with normal glucose metabolism, but not in women (Table 2). The prevalence of carotid artery stenosis ≥50% and the calcification volumes in all regions was higher in men with newly-diagnosed disturbed glucose metabolism or pre-existent diabetes mellitus than in women, except for calcification volume in the aortic arch. We found different associations among men and women. After adjusting for confounders, in men pre-existent diabetes mellitus was associated with stenosis ≥50%, and calcification volume in all regions. Newly-diagnosed disturbed glucose metabolism was also associated with stenosis ≥50% and calcification volume in the intracranial carotid arteries in men. In women, no significant associations were found, except for an association between pre-existent diabetes mellitus and calcification volume in the intracranial arteries (Table 3). These findings were not confirmed by the test for interaction, which showed no significant difference in the effect of glucose on atherosclerosis between men and women (*p*-values >0.05, data not shown).

Discussion

Our study shows that newly-diagnosed disturbed glucose metabolism in patients with a recent TIA or ischemic stroke is associated with more severe extra- and intracranial atherosclerosis. The association equaled that of pre-existent diabetes mellitus and appeared to be stronger in men than in women. The latter was not confirmed, however, by the test for interaction.

In line with previous studies, we found that pre-existent diabetes mellitus is associated with atherosclerosis in patients with TIA or ischemic stroke [13-16, 30, 31]. Furthermore, we found that newly-diagnosed disturbed glucose metabolism after TIA or ischemic stroke is associated with atherosclerosis. Most previous studies that assessed the association between glucose

TABLE 1 - PATIENT CHARACTERISTICS PER GLUCOSE METABOLISM GROUP

	Normal glucose metabolism N=623	Newly-diagnosed disturbed glucose metabolism N=384	Pre-existent diabetes mellitus N=210	*p*-value
Demographic data				
Age (yrs), mean (SD)	59 (14)	66 (13)	65 (12)	<0.001
Men, n (%)	316 (51)	202 (53)	124 (59)	0.112
Caucasian, n (%)	492 (82)	296 (82)	123 (63)	<0.001
Risk factors				
Hypertension*, n (%)	263 (42)	233 (61)	153 (73)	<0.001
Dyslipidemia**, n (%)	153 (25)	131 (34)	126 (60)	<0.001
Atrial fibrillation, n (%)	29 (5)	30 (8)	17 (8)	0.063
Current smoking, n (%)	228 (37)	99 (26)	48 (23)	<0.001
BMI (kg/m^2)†, mean (SD)	26 (3)	27 (4)	28 (4)	<0.001
Medical history				
TIA/ischemic stroke, n (%)	139 (22)	102 (27)	77 (37)	<0.001
Ischemic heart disease, n (%)	82 (13)	69 (18)	65 (31)	<0.001
Event characteristics				
TIA, n (%)	287 (46)	133 (35)	69 (33)	<0.001
NIHSS‡, median (IQR)	2 (1-5)	3 (1-6)	3 (2-5)	0.006
TOAST classification				<0.001
Large artery disease, n (%)	82 (13)	94 (25)	39 (19)	
Cardio-embolism, n (%)	68 (11)	51 (13)	30 (14)	
Small vessel disease, n (%)	131 (21)	62 (16)	50 (24)	
Other determined disease, n (%)	47 (8)	12 (3)	7 (3)	
Undetermined disease, n (%)	295 (47)	165 (43)	84 (40)	
Glucose assessment				
Fasting plasma glucose (mmol/L), mean (SD)	4.9 (0.6)	6.2 (1.4)	7.9 (3.0)	<0.001
2-hour post-load glucose (mmol/L), mean (SD)	7.5 (2.4)	11.2 (3.6)	NA	<0.001
Glycosylated hemoglobin (mmol/mol), mean (SD)	36 (4)	43 (8)	58 (17)	<0.001

* defined as the use of antihypertensive drugs prior to the ischemic stroke/TIA
** defined as the use of statins prior to the ischemic stroke/TIA
† missing in 490 patients, imputated BMI used
‡ in patients with ischemic stroke only

levels and atherosclerosis were performed in population-based studies and measured CIMT with duplex ultrasound instead of stenosis degree and calcification volume with CTA. We used degree of stenosis and calcification volumes assessed with CTA as a proxy for atherosclerosis. Carotid artery stenosis is a well-known and important risk factor for ischemic stroke and a stenosis of 50% or more is therefore a good marker for atherosclerosis [32]. Furthermore, in large population-based studies calcification volume in aortic arch and extracranial carotid arteries was not only associated with vascular risk factors, but also with the presence of (silent) cerebral infarcts on MRI and prevalent stroke [16, 29, 33]. Also, diabetes mellitus and glucose levels are significantly associated with calcification growth in patients with a TIA or ischemic stroke.[34] However, the

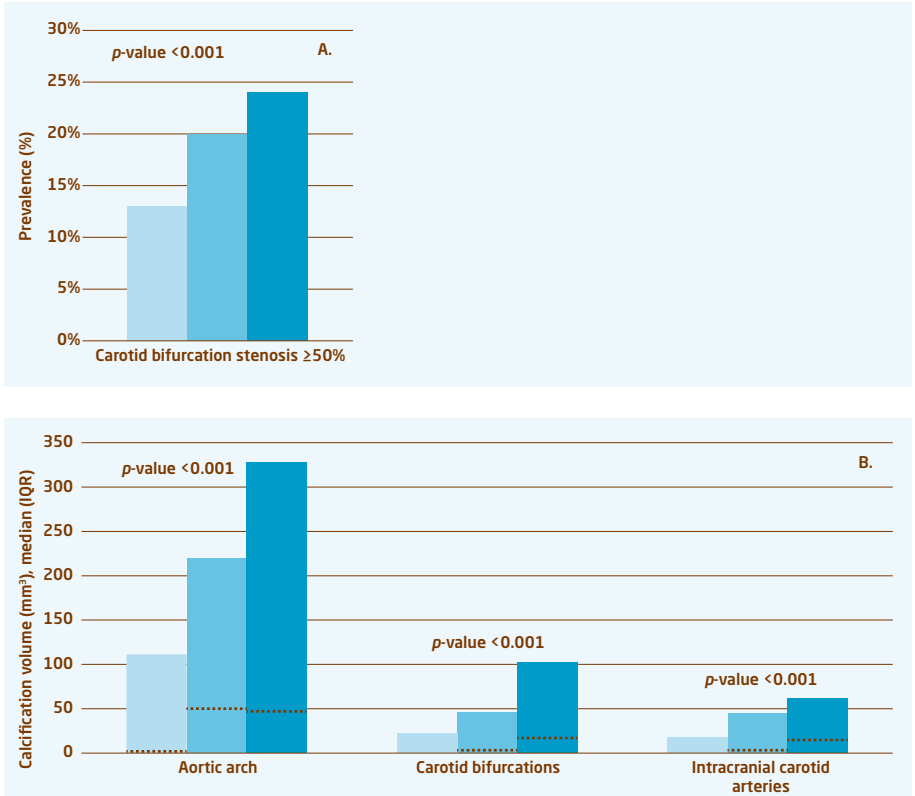

Figure - Atherosclerotic measurements in patients with normal glucose metabolism (light blue), newly-diagnosed disturbed glucose metabolism (medium blue) and pre-existent diabetes mellitus (dark blue): A. prevalence of stenosis ≥50% in carotid bifurcation, B. calcification volume (mm³, median (dashed line) and IQR) in aortic arch, carotid bifurcations, and intracranial carotid arteries.

role of calcification volume in atherosclerosis is less clear. A recent study investigating the relation between carotid bifurcation calcification volume and carotid bifurcation stenosis in symptomatic patients showed no relation [35]. In contrast, another study found that calcified plaques resulted more often in obstructive stenosis in symptomatic patients with diabetes mellitus [31]. Moreover, it has been hypothesized that calcified atherosclerotic plaques are associated with plaque stability and with less neurological symptoms [36, 37].

Conflicting results exist on the role of sex in the association between diabetes mellitus and calcification volume. Two population-based studies have shown an association between diabetes mellitus and calcification volume in the aortic arch, carotid bifurcation and intracranial carotid arteries only in women [16, 17]. Another study on subjects with a family history of coronary artery disease found also an association between fasting plasma glucose levels and coronary artery calcification volume in women only [19]. However, a population-based study found an association between diabetes mellitus and coronary artery calcification volume both in men and women [20] and another population-based study found no association between diabetes mellitus and aortic arch calcification volume at all [18].

TABLE 2 - ATHEROSCLEROSIS MEASUREMENTS IN DIFFERENT GLUCOSE GROUPS

	Normal glucose metabolism (n=623)	Newly-diagnosed disturbed glucose metabolism (n=384)	Pre-existent diabetes mellitus (n=210)	*p*-value
Stenosis ≥50%, n (%)				
All patients	78 (13)	77 (20)	50 (24)	<0.001
Women	35 (11)	23 (13)	14 (17)	0.435
Men	43 (14)	54 (27)	36 (30)	<0.001
Calcification volume in aortic arch (mm³), median (IQR)				
All patients	3 (0-111)	50 (0-219)	48 (1-329)	<0.001
Women	1 (0-160)	75 (0-301)	33 (0-288)	<0.001
Men	3 (0-85)	28 (0-149)	65 (4-337)	<0.001
Calcification volume in carotid bifurcations (mm³), median (IQR)				
All patients	0 (0-22)	5 (0-46)	17 (0-102)	<0.001
Women	0 (0-16)	3 (0-42)	4 (0-59)	0.001
Men	1 (0-27)	6 (0-46)	26 (3-137)	<0.001
Calcification volume intracranial carotid arteries (mm³), median (IQR)				
All patients	0 (0-18)	5 (0-45)	16 (1-63)	<0.001
Women	0 (0-12)	1 (0-21)	10 (0-50)	<0.001
Men	1 (0-23)	10 (0-59)	23 (2-73)	<0.001

In contrast with these previous studies, we found that newly-diagnosed disturbed glucose metabolism and pre-existent diabetes mellitus was associated with atherosclerosis only in men, but not in women. However, we also found no significant interaction between glucose and sex. We therefore conclude that there is no consensus on the role of sex in the association between disturbed glucose metabolism and calcification volume. It is known that women with diabetes mellitus have a three- to seven-fold increased risk for coronary heart disease, compared to a two- to three-fold increased risk in men with diabetes mellitus [38]. Therefore, it would be interesting to investigate the possible different effects of glucose metabolism on atherosclerosis in men and women.

Atherosclerosis is an important vascular complication in patients with diabetes mellitus. The pathogenesis of atherosclerosis in diabetes mellitus is multifactorial. Not only the co-existence of other cardiovascular risk factors in patients with diabetes mellitus like hypertension and dyslipidemia, but also insulin resistance, endothelial dysfunction, dyslipidemia, chronic inflammation, procoagulability, and impaired fibrinolysis promote atherosclerosis [39, 40]. In patients with pre-diabetes these processes may already play a role. This is also reflected in the Oxford Plaque Study, in which histological features of symptomatic atherosclerotic carotid plaques were assessed in patients with impaired glucose tolerance and diabetes mellitus. Not only in patients with diabetes mellitus, but also in patients with impaired glucose tolerance, surface thrombus and plaque macrophages seemed to persist longer in plaques, compared to plaques from those with normal glucose tolerance [41]. A recently developed mouse model could be used to investigate the causal role of impaired glucose tolerance in the pathogenesis of atherosclerosis [42].

TABLE 3 - ASSOCIATION BETWEEN BOTH NEWLY-DIAGNOSED DISTURBED GLUCOSE METABOLISM AND PRE-EXISTENT DIABETES MELLITUS AND ATHEROSCLEROSIS, ASSESSED WITH MULTIVARIABLE LOGISTIC AND LINEAR REGRESSION ANALYSIS

	Stenosis ≥50% aOR† (95%CI)	Calcification volume aortic arch Adjusted beta coeff‡ (95%CI)	Calcification volume carotid bifurcations Adjusted beta coeff‡ (95%CI)	Calcification volume intracranial carotid arteries Adjusted beta coeff‡ (95%CI)
Newly-diagnosed disturbed glucose metabolism vs. normal glucose metabolism				
All patients*	1.51 (1.04-2.20)	0.13 (-0.12-0.39)	0.08 (-0.15-0.31)	0.12 (-0.14-0.38)
Women**	0.76 (0.40-1.43)	0.09 (-0.28-0.46)	0.02 (-0.30-0.33)	-0.27 (-0.62-0.09)
Men**	2.15 (1.32-3.48)	0.15 (-0.20-0.51)	0.13 (-0.20-0.46)	0.49 (0.12-0.86)
Pre-existent diabetes mellitus vs. normal glucose metabolism				
All patients*	1.65 (1.06-2.58)	0.49 (0.18-0.81)	0.50 (0.22-0.79)	0.57 (0.25-0.88)
Women**	1.00 (0.47-2.15)	0.40 (-0.09-0.88)	0.21 (-0.20-0.62)	0.58 (0.12-1.03)
Men**	2.00 (1.13-3.52)	0.56 (0.13-0.99)	0.71 (0.31-1.11)	0.57 (0.13-1.01)

† adjusted odds ratio with corresponding 95% confidence interval
‡ adjusted beta coefficient with corresponding 95% confidence interval
* Adjusted for age, sex, hypertension, dyslipidemia, current smoking, previous ischemic heart disease, previous TIA/ischemic stroke, BMI
** Adjusted for age, hypertension, dyslipidemia, current smoking, previous ischemic heart disease, previous TIA/ischemic stroke, BMI

However, whether pre-diabetes is associated with atherosclerosis remains controversial [13-15, 21, 22, 43, 44]. Two recent meta-analyses found an association between impaired glucose tolerance and a small increase in CIMT [22, 45].

To our knowledge, this is the first study that assessed the association between newly-diagnosed disturbed glucose metabolism and atherosclerosis in patients with ischemic stroke or TIA. Strengths of this study are the large sample size, detailed clinical information, the use of three different glucose tests and two different methods to assess atherosclerosis.

Our study has also some limitations. The glucose tests were not repeated to rule out laboratory error and the acute phase effect. Nonetheless, according to the American Diabetes Association two different simultaneously disturbed glucose tests are sufficient to diagnose pre-diabetes and newly-diagnosed diabetes mellitus and repeating the tests is therefore not obligatory [46]. Second, we excluded patients with missing glucose assessment and missing CTA, which might compromise the generalizability of our findings. Reasons for missing glucose assessments were mainly the poor medical condition of the patient, and these patients might therefore benefit less of secondary stroke prevention.

In conclusion, newly-diagnosed disturbed glucose metabolism, just like pre-existent diabetes mellitus, is independently associated with atherosclerosis in patients after ischemic stroke or TIA. This association may partly explain the increased risk for recurrent stroke in these patients. This study therefore suggests that patients with newly-diagnosed disturbed glucose metabolism after ischemic stroke or TIA should be treated more aggressively to lower glucose levels. Future studies may include the effects of treatment on the progression of atherosclerosis in these patients.

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CHAPTER 4.2

ASSOCIATIONS BETWEEN GENETIC VARIATIONS IN METABOLIC TRAITS AND ARTERIAL STENOSIS IN PATIENTS WITH CEREBRAL ISCHEMIA

AUTHORS

Evita G. van den Herik [1*]
Maksim V. Struchalin [2*]
Susanne Fonville [1]
Lonneke M.L. de Lau [1]
Heleen M. den Hertog [1,3]
Peter J. Koudstaal [1]
Cornelia M. van Duijn [2]

1
DEPARTMENT OF NEUROLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

2
DEPARTMENT OF EPIDEMIOLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

3
DEPARTMENT OF NEUROLOGY
MEDISCH SPECTRUM TWENTE
Enschede, The Netherlands

* AUTHORS CONTRIBUTED EQUALLY

SUBMITTED

Abstract

OBJECTIVE

Recent large genomewide association (GWA) studies have found many new genes to be associated with metabolic traits including hypertension, lipid levels and diabetes. A next step after gene discovery is to investigate associations between these genes and clinically relevant endpoints. Therefore, we studied the association between recently discovered genetic variations in metabolic traits and craniocervical artery stenosis in patients with ischemic stroke or transient ischemic attack (TIA).

METHODS AND RESULTS

We included 700 patients with recent ischemic stroke or TIA. In all patients, CT angiography from the ascending aorta to the intracranial circulation was performed and scored for degree of stenosis in several arteries. Our primary outcome was the presence of a stenosis ≥30% in any of the scored arteries. Genotyping was performed with Metabochip, a targeted gene chip for metabolic traits containing ~200,000 SNPs.
Five loci were found to be strongly associated with presence of stenosis (GLIS3, p=1.6x10-6; AGBL2, p=1.2x10-4; SBF2, p=7.9x10-5; SCAMP5, p=8.7x10-5; and MC4R, p=1.7x10-4). After adjusting for multiple testing using permutation the risk allele in the GLIS3 locus was associated with a 2.2 (95%CI 1.6-3.1)-fold increase of stenosis risk. This gene was previously shown to lead to elevated fasting glucose levels. We found no significant association between loci implicated in hypertension or hypercholesterolemia and presence of stenosis.

CONCLUSIONS

Our study suggests that a gene implicated in increased fasting glucose levels, but not blood pressure- or lipid-related genes, are associated with atherosclerosis in patients with a recent ischemic stroke or TIA.

Introduction

During the past years many large gene discovery studies have been undertaken, uncovering new genes and loci that are associated with various disorders and traits. For metabolic traits such as blood pressure, lipid and glucose levels, very large cohorts of over 100,000 participants have shown many new loci [1-6]. In these studies, genotyping has generally been broad but not dense, rendering the genetic variants found in these large studies likely not to be the true functional variant, but closely linked to it [7].

To find causative genetic variants, one possibility is to perform a dense genotyping and sequencing of the implicated locus. This is time-consuming and may not reveal a truly functional variant. Apart from uncovering functional genetic variants, it could also be useful to study newfound genes and loci with respect to clinical parameters, to solidify their association with the studied traits. This increases knowledge on functional pathways in which the genetic variations could exert their functions, and leads to better comprehension of results of large-scale genetic studies.

Important risk factors for ischemic stroke and transient ischemic attack (TIA) are dyslipidemia, diabetes mellitus and hypertension. Many single nucleotide polymorphisms (SNPs) have been found to be associated with these metabolic parameters. However, studies of these discovered variants with regard to clinically relevant parameters such as carotid artery stenosis have not been performed yet, despite the fact that a stenosis can have a causal relation to ischemic stroke or TIA [8]. It also remains unclear which metabolic traits are important in the pathogenesis of atherosclerosis, of which stenosis is a marker.

A recent designed custom genotyping array, called the Metabochip, is available and assays ~200.000 SNP markers for type 2 diabetes, cardiovascular disease, body mass index (BMI), glucose and insulin levels, lipid levels and blood pressure [9]. We therefore investigated the associations between SNPs on the Metabochip and stenosis in the craniocervical arteries in a large hospital-based cohort of patients with a recent ischemic stroke or TIA.

Methods

STUDY POPULATION

Patients were derived from the Erasmus Stroke Study, an ongoing registry of patients with cerebrovascular diseases treated at the Erasmus MC University Medical Center, from December 2005 [10, 11]. From all patients, detailed clinical and radiological data, blood samples and DNA are collected. The study was approved by the Medical Ethics Committee of our hospital.
All consecutive patients with a clinical diagnosis of ischemic stroke or TIA between December 2005 and April 2010 were included. Ischemic stroke was defined as a focal neurological deficit of sudden onset of presumed vascular origin lasting 24 hours or more, with brain imaging showing no abnormalities or typical signs of infarction. TIA was defined similarly, but with a duration of symptoms of <24 hours and with no signs of recent infarction on CT-scan. All patients gave written informed consent.

CT ACQUISITION AND ANALYSIS

CT angiography (CTA) of the carotid artery was routinely performed with a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Sensation 64, Definition, Definition AS+ or Definition flash, Siemens Medical Solutions, Erlangen, Germany) using a standardized optimized contrast-enhanced CTA protocol (120 kVp, 180-200 mAs, collimation 16 x 0.75 mm; 32 x 2 x 0.6 mm; 64 x 2 x 0.6 mm, pitch < 1). The scan range extended from the ascending aorta to the intracranial circulation (3 cm above the sella turcica). All patients received 80 ml of contrast agent (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, UK), followed by 45 ml saline bolus chaser, both at an injection rate of 4 or 5 ml/s. Image reconstructions were made with field of view of 120 mm, matrix size 512 × 512, slice thickness 0.75 or 1.0 mm, increment 0.4 – 1.0 mm and with an intermediate reconstruction algorithm.

STENOSIS

The CTA was scored for the degree of stenosis, per vessel segment. The stenosis degree was determined for the brachiocephalic trunk, subclavian artery, common carotid artery, carotid bifurcation, internal carotid artery, carotid siphon and vertebral artery, all according to the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [12].
As stenosis degrees are often categorized as 0-29%, 30-49%, 50-69% and over 70% [13], we chose a threshold of 30% stenosis to resemble the presence of clinical relevant atherosclerosis. The presence of stenosis was therefore defined as a stenosis of 30% or more in any of the studied arteries.

INTRACLASS CORRELATION EFFICIENT

An intraclass correlation coefficient for the degree of stenosis was based on the ratings of 2 independent observers (E.v.d.H. & S.F.) on 50 CT examinations: 0.96.

GENOTYPING AND QUALITY CONTROL

Genotyping was performed with the Metabochip, a custom Illumina iSelect genotyping array. This chip was designed to fine-map regions that were discovered in high throughput studies with metabolic traits, including blood pressure and hypertension, lipid levels and hypercholesterolemia, glucose levels and diabetes, and BMI. It contains dense mapping of known loci, as well as single SNPs, with a total of approximately 200.000 SNPs [9]. Most loci contain SNPs in very high linkage disequilibrium.
Quality control was performed, with the following exclusion criteria: significant deviation from Hardy Weinberg equilibrium ($P < 5x10^{-7}$), call rates <0.90, minor allele frequency (MAF) <0.005, discrepancy between recorded and genotype-determined sex and results of identity by state estimations. After quality control 700 individuals and 163,735 SNPs remained.

STATISTICAL ANALYSIS

We performed a principal component analysis to evaluate the genetic structure of the sample. According to the first two principle components, our population was made up of at least three ethnic groups with the largest group made up of white participants (Figure 1).

We fitted an additive model to determine associations between SNPs and presence of stenosis by means of multiple logistic regression analysis, adjusted for the first three principle components to account for genetic stratification. This reduced inflation of test statistics (lambda) to 0.97.

TABLE 1 - CHARACTERISTICS OF THE STUDY POPULATION (N=700)

Characteristics	
Age	61.7±14.0
Female	370 (53)
Cerebral ischemia	
Transient ischemic attack	303 (43)
Ischemic attack	397 (57)
Cardiovascular risk factors	
Hypertension	378 (54)
Hypercholesterolemia	245 (35)
Diabetes mellitus	101 (14)
Current smoking*	209 (31)
Atherosclerosis on CT-angiography	
Stenosis ≥30%†	337 (49)

Numbers resemble N(%) or mean±SD as appropriate.
* smoking status known in 665 patients
† scored for all vessels in 693 patients

All analyses were performed in the GenABEL R package [14]. The threshold corresponding to a = 0.05 with Bonferroni correction for 163,735 independent tests, $3x10^{-7}$, was considered too conservative as the chip was designed for finemapping and contains mostly loci of several hundred SNPs that are highly correlated to each other. Therefore, the number of independent tests is substantially reduced, and this makes Bonferroni correction over conservative [15, 16]. In the results section and tables we report p-values before permutation analysis. To estimate empirical significance, we performed permutation tests (1,000 replications) on the complete set of SNPs [16].

Results

From December 2005 until April 2010, 1299 patients with ischemic stroke or TIA were included in the Erasmus Stroke Study. From 256 patients (20%), DNA samples were unavailable, and CTA was unavailable in 166 patients (13%). Reasons for not performing a CTA were contrast allergy, renal failure (glomerular filtration rate <45 ml/min), severe neurological symptoms with short life expectancy and severe comorbidity with concomitant high surgical risk. From the remaining 877 patients, 149 (11%) were not genotyped for various practical reasons, leaving 728 patients for genotyping. After quality control of genotyping data, 700 patients (54%) were left and included in this study. The mean age of the study population was 61.7±14 years, 370 patients (53%) were female, and cardiovascular risk factors were highly prevalent (Table 1).

In 337 patients (49%), a stenosis ≥30% was present in any of the assessed craniocervical arteries. SNPs in several loci showed an association with the presence of a stenosis (Figures 2 and 3). Figure 2 shows that although findings are not genomewide significant taking all SNPs together, there is evidence for a deviation from the expected null value when adjusting for stratification by principal components analysis. Particularly for chromosome 9, there is evidence for associations with multiple SNPs in the same locus with *p*-values below 10^{-6}. Table 2 shows associations between SNPs with *P*-values $\leq 10^{-3}$ and presence of a stenosis ≥30%, expressed as odds ratios. This table

TABLE 2 - SNPS MOST SIGNIFICANTLY ASSOCIATED WITH PRESENCE OF STENOSIS ($P<10^{-4}$)

SNP	SNP location	Minor allele	MAF	Chromosome position	Odds ratio (95% CI)	*P*-value	Closest gene*		SNP number**	Associated trait
							Name	Distance		
Unnamed	Intronic	A	0.14	9:4251222	2.22 (1.60-3.08)	$1.56x10^{-6}$	GLIS3	Within gene	23	Increased fasting glucose [4]
rs7717939	Upstream	G	0.46	5:2277557	0.62 (0.50-0.78)	$3.50x10^{-5}$	IRX4	343	0	-
rs11865869	Intronic	G	0.22	16:7987404	0.58 (0.45-0.75)	$3.77x10^{-5}$	BCMO1	Within gene	0	-
rs12066353	Intronic	C	0.13	1:87293907	2.02 (1.43-2.84)	$5.66x10^{-5}$	HS2ST1	Within gene	0	-
rs10800152	Intronic	A	0.26	1:163968300	0.60 (0.47-0.77)	$6.10x10^{-5}$	TMCO1	Within gene	0	Glaucoma [38]
rs4251961	Upstream	G	0.38	2:113590938	1.57 (1.26-1.96)	$6.41x10^{-5}$	IL1RN	1	0	Inflammation
rs8024657	Upstream	A	0.36	15:35868908	1.60 (1.27-2.01)	$6.66x10^{-5}$	TMCO5	145	0	-
rs11049257	Upstream	G	0.20	12:28030272	0.56 (0.42-0.75)	$7.03x10^{-5}$	PTHLH	13	0	Atherosclerosis [39]
Unnamed	Intronic	C	0.14	11:10092608	0.53 (0.38-0.73)	$7.93x10^{-5}$	SBF2	Within gene	53	Increased HDL [1]
rs12544899	Upstream	G	0.23	8:38688488	0.59 (0.46-0.77)	$8.16x10^{-5}$	TACC1	75	0	Carcinogenesis [40]
Unnamed	Exonic	G	0.08	15:73099023	0.44 (0.29-0.66)	$8.69x10^{-5}$	SCAMP5	Within gene	23	-
rs13155847	Downstream	G	0.19	5:5374098	0.56 (0.42-0.75)	$8.71x10^{-5}$	ADAMTS16	0.7	0	-
rs10769288	Intronic	A	0.38	11:47685756	0.63 (0.50-0.80)	$1.23x10^{-4}$	AGBL2†	Within gene	54	†Increased BMI [2]
Unnamed	Downstream	G	0.29	18:56090278	1.58 (1.24-2.01)	$1.67x10^{-4}$	MC4R	87	30	Decreased HDL [5]

Stenosis is defined as stenosis >30% in any craniocervical artery.
MAF: Minor allele frequency
* distance in kilobase pairs
** number of SNPs in a given locus with P-value less than 10^{-3}
† locus continues into MTCH2 gene

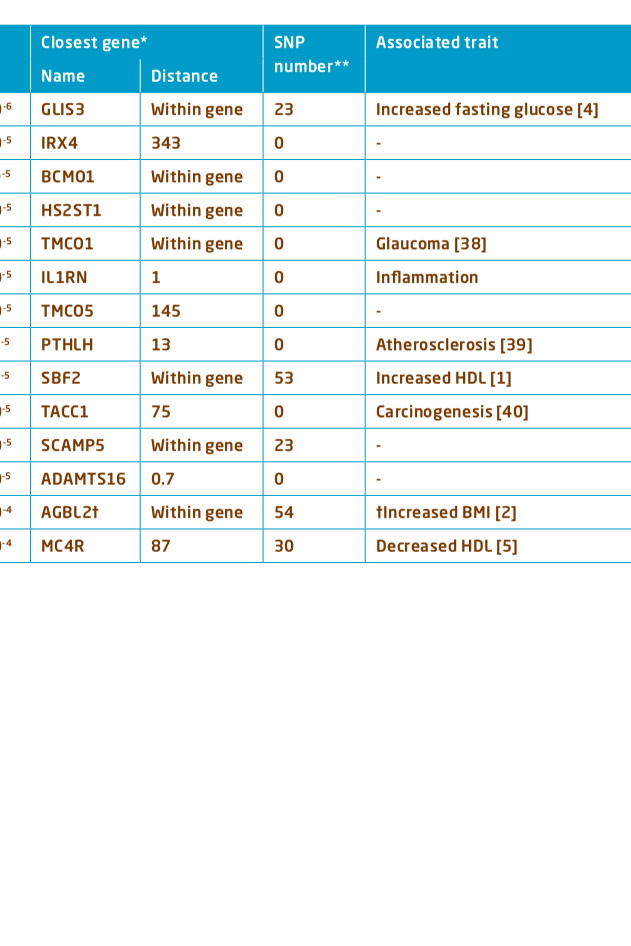

Figure 1 - Multidimensional scaling plot showing all participants, plotted according to the score of the first two principal components. The large cloud in the top left corner are Caucasians, the two spread groups indicate other ethnicities.

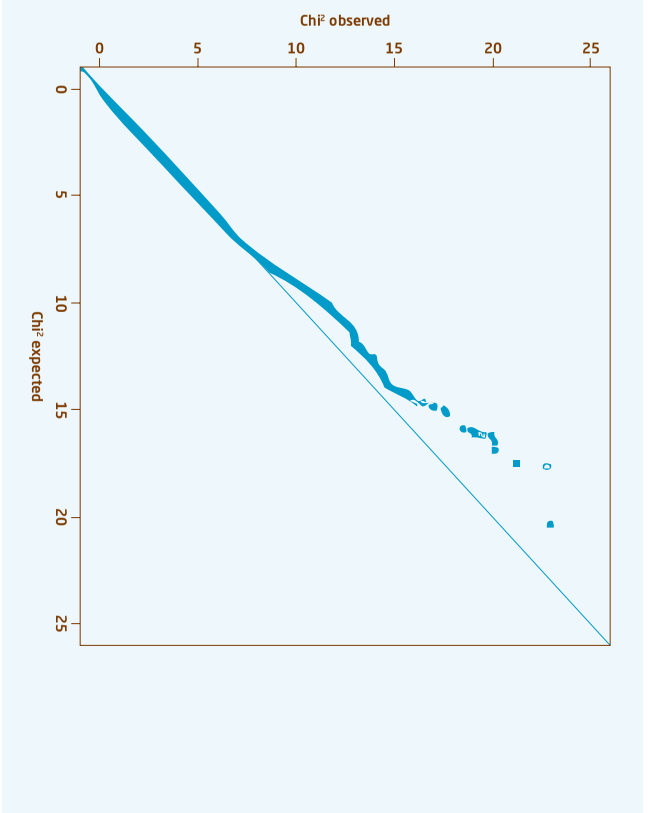

Figure 2 - Quantile-quantile (QQ) plot showing observed versus expected Chi^2 values after analysis for presence of stenosis ≥30% in a craniocervical artery. The red line shows the distribution under the null hypothesis of no association.

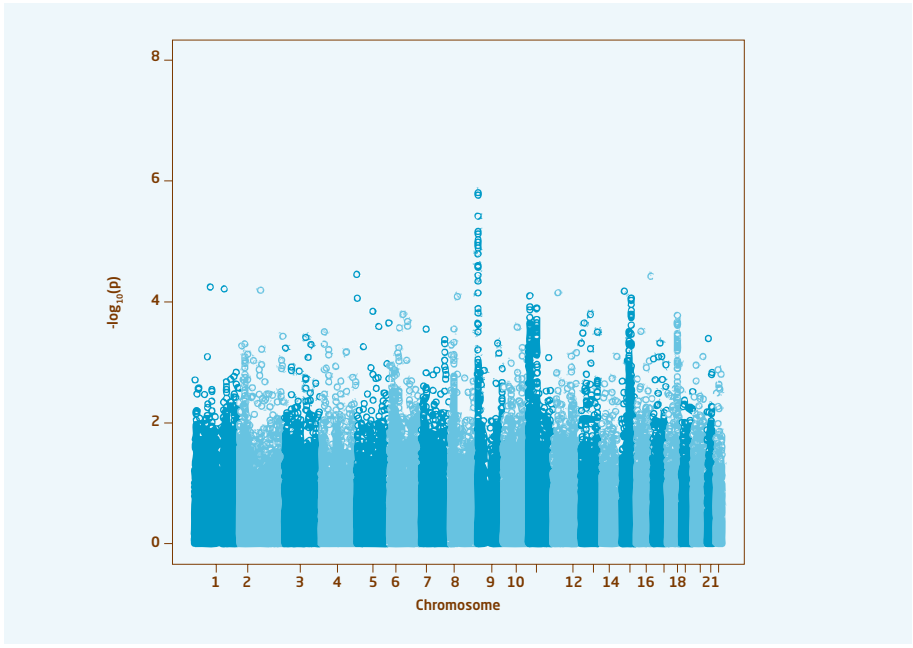

Figure 3 - Results of test for association between each SNP and stenosis in the craniocervical arteries ≥30%. *P* values ($-\log_{10}(P)$) are shown for each SNP according to its genomic position.

includes five loci, where in each locus a large number of SNPs are associated with stenosis (GLIS3, $p=1.6\times10^{-6}$; AGBL2, $p=1.2\times10^{-4}$; SBF2, $p=7.9\times10^{-5}$; SCAMP5, $p=8.7\times10^{-5}$; and MC4R, $p=1.7\times10^{-4}$). We were unable to find an association between SNPs in loci associated with blood pressure and hypertension, HDL or total cholesterol levels, diabetes mellitus, and risk of stenosis.

After permutation testing, the most significant locus was an intronic SNP (unnamed) in GLIS3, which was initially associated with fasting glucose, with a 2.22 (95%CI 1.60-3.08)-fold increase of stenosis risk ($p=0.055$).

Discussion

We found that an intronic SNP in the GLIS 3 gene was borderline significantly associated with the occurrence of craniocervical stenosis in patients with a recent ischemic stroke or TIA. No associations were found between SNPs associated with other cardiovascular risk factors, like lipid levels or blood pressure, and presence of atherosclerosis.

GLIS3 is a gene encoding the transcription factor *GLIS family zinc finger 3 isoform*, a protein participating in the development of β-cells in the pancreas [17]. Previous genetic studies have found SNPs in the GLIS3 locus to be associated with increased fasting plasma glucose, insulin and 2-hour post-load glucose levels [4, 18] and diabetes mellitus type 1 and 2 [4, 19, 20]. Deficiency of GLIS3 in $Glis3^{zf/zf}$ mutant mice led to decreased glucose-stimulated insulin response, resulting in hyperglycemia and hypoinsulinaemia [17].

Our finding that GLIS3 is associated with craniocervical stenosis is in line with previous findings. Several studies assessed the association between glucose levels and/or diabetes mellitus and atherosclerosis. Most studies used carotid intima media thickness (CIMT) measured with duplex ultrasound as a proxy for atherosclerosis. We used craniocervical stenosis, which represents the same atherosclerotic process, but is also an important clinical risk factor for ischemic stroke. Diabetes mellitus increases the risk of (high-grade) carotid artery stenosis [21, 22] . Elevated fasting plasma glucose levels also have been associated with increased CIMT [23-26]. Also, a longitudinal study demonstrated that development of increased CIMT after ten years of follow-up was associated with higher levels of fasting glucose at baseline [27]. Furthermore, insulin resistance and subsequent endothelial dysfunction has also been associated with atherosclerosis in patients with type 2 diabetes [28, 29].
Recently, genetic determinants of fasting glucose levels were shown to be associated with CIMT as well, indicating a possible causal role for elevated fasting glucose in atherosclerosis [30]. Apart from candidate-gene studies there have been some less-hypothesis driven studies on genetic determinants of CIMT, without significant results [31, 32].
Surprisingly we did not find any associations between loci associated with blood pressure and lipid levels and stenosis, although these are important risk factors for ischemic stroke and TIA and associated with carotid artery stenosis as well [33, 34].

Earlier studies on genetic risk of atherosclerosis showed the 9p21 locus to be associated with coronary artery atherosclerosis and cerebrovascular disease [35, 36], but not with CIMT [37]. In our study, we did not find an association with SNPs in the 9p21 locus that are commonly used to construct haplotypes. The locus with the borderline significance in our study, GLIS3, is located on chromosome 9p24.2, which is situated over 17 Mb pairs from the 9p21 locus and is therefore unlikely to co-segregate.

Our study has some limitations. Firstly, our population size is small for a genetic association study. However, the number of SNPs is limited and mostly highly linked to each other, limiting the number of independent tests. Furthermore, no replication study was performed, because we are not aware of a cohort with the same extensive CTA scoring. However, a replication study is necessary to confirm our findings [7].

Second, we had to exclude a considerable number of patients because CT angiography was not performed due to comorbidity or short life expectancy, leading to selection bias. However, this is a consequence of a hospital-based cohort.

Lastly, a well-known issue in genome wide association studies is the problem of multiple testing. The Bonferroni correction method is often used, but has proven to be very conservative [15, 16]. We used permutation-based corrections as an alternative. Using this technique, we found only 1 SNP borderline significantly associated with atherosclerosis. Other methods like SLIDE and simple μ are available [15, 16] and using these methods might have led to significant associations.

A strength of our study is the detailed scoring of the CTAs, enabling us to study stenosis in each vessel segment that can lead to cerebral ischemia. Also, this is one of the first studies to use a chip containing all known genetic variations for metabolic traits with a common clinical parameter. This allowed us to study associations between SNPs for all common metabolic cardiovascular risk factors and a marker of atherosclerosis, craniocervical arterial stenosis.

This study implicates a possible important role of glucose levels in the pathogenesis of atherosclerosis in patients with ischemic stroke or TIA. If this finding will be confirmed in a replication study, patients with increased glucose levels after ischemic stroke or TIA should be treated more aggressively in the context of secondary stroke prevention.

In this targeted genomic association study, we found that a locus associated with fasting glucose levels was borderline significantly associated with the presence of a stenosis in the craniocervical arteries in patients with ischemic stroke or TIA. We found no evidence for an association between occurrence of stenosis and previously suspected traits such as loci for blood pressure or lipid levels.

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CHAPTER 4.3

ABNORMAL DIPPING BLOOD PRESSURE PATTERNS: PREVALENCE AFTER TIA OR ISCHEMIC STROKE AND ASSOCIATION WITH ATHEROSCLEROSIS

AUTHORS

Susanne Fonville [1]
Christine Verboon [1]
Anouk C. van Dijk [1,2]
Aad van der Lugt [2]
Peter J. Koudstaal [1]
Anton H. van der Meiracker [3]
Heleen M. den Hertog [1,4]

1
DEPARTMENT OF NEUROLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

2
DEPARTMENT OF RADIOLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

3
DEPARTMENT OF INTERNAL MEDICINE
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

4
DEPARTMENT OF NEUROLOGY
MEDISCH SPECTRUM TWENTE
Enschede, The Netherlands

SUBMITTED

Abstract

OBJECTIVES

Non- and reverse dipping blood pressure patterns are often present in patients with transient ischemic attack (TIA) or ischemic stroke in the acute phase. We aimed to assess the prevalence of abnormal dipping blood pressure patterns in the post-acute phase of TIA or ischemic stroke and their association with extra- and intracranial atherosclerosis.

METHODS

We prospectively studied patients with a TIA or ischemic stroke in the post-acute phase who underwent 24-hour ambulatory blood pressure measurement. Dipping percentages were calculated as (awake mean arterial pressure (MAP) – asleep MAP) / (awake MAP) * 100. Extreme dipping was defined as ≥20% dipping, normal dipping as ≥10% and <20%, non-dipping as ≥0% and <10% and reverse dipping as <0%. We used CT-angiography to assess the degree of stenosis in the carotid artery bifurcations and semi-automatically scored calcification volume in the aortic arch, carotid bifurcations and intracranial carotid arteries. The relation between blood pressure patterns and atherosclerosis was expressed as odds ratios and beta coefficients. Adjustments were made for potential confounders with multiple logistic and linear regression models.

RESULTS

Of the 192 patients 75 (39%) had normal dipping, 50 (26%) extreme dipping, 48 (25%) non-dipping and 19 (10%) reverse dipping. Reverse dipping tended to be associated with extra- and intracranial calcification volume (adjusted beta coefficient (95%-CI) 0.64 (-0.50-1.79), 0.73 (-0.35-1.82) and 0.64 (-0.28-1.55) for aortic arch, carotid bifurcations and intracranial carotid arteries respectively).

CONCLUSIONS

Non- and reverse dipping nocturnal blood pressure patterns are often present after TIA or ischemic stroke and there is a trend towards an association with extra- and intracranial atherosclerosis.

Introduction

Hypertension is the most important modifiable risk factor for first-ever ischemic stroke and recurrent stroke [1, 2]. Ambulatory blood pressure measurement has proven to be a more accurate predictor for cardiovascular events and mortality than office measurements [3, 4]. Blood pressure follows a circadian pattern with normally a 10-20% nocturnal decline compared with daytime blood pressure, which is called dipping [5, 6]. A nocturnal decline in blood pressure of 20% or over is called extreme dipping. A reduced decline in nocturnal blood pressure of less than 10% has been coined non-dipping and a rise in nocturnal blood pressure reverse dipping. However, it is still unproven that these arbitrary cut-off points are more discriminative than others [6, 7]. Non-dipping and reverse dipping blood pressure patterns are associated with increased risk of cardiovascular disease both in normotensive and hypertensive subjects [8-10]. Non- and reverse dipping blood pressure patterns are not only a risk factor for ischemic stroke [11], but have also been associated with silent lacunar infarctions [12, 13]. A potential underlying mechanism for this increased cardiovascular risk is atherosclerosis [14, 15].

Most previous studies have determined the prevalence of abnormal dipping blood pressure patterns in the acute phase of stroke [16-19]. However, the first few days after stroke hypertension is often present due to an acute phase reaction, followed by a return of blood pressure to pre-stroke values [20-22], and therefore may influence the dipping pattern. We therefore aimed to assess the prevalence of the different dipping blood pressure patterns in patients with a transient ischemic attack (TIA) or ischemic stroke in the post-acute phase. Furthermore, we studied the association between nocturnal blood pressure and dipping patterns and extra- and intracranial atherosclerosis in these patients.

Methods

STUDY SAMPLE

We prospectively studied consecutive patients with minor ischemic stroke (modified Rankin Scale score of ≤3 [23]) who visited the post-stroke care clinic 4-6 weeks after the stroke and patients with TIA who visited our specialized TIA clinic between May 2009 and December 2011 and underwent a 24-hour ambulatory blood pressure measurement. Patients with an ischemic stroke within the previous 4 days or with a TIA within the previous 24 hours were excluded to avoid the acute phase reaction. Written informed consent was obtained from all participants as approved by the Institutional Ethics Committee.

Ischemic stroke was defined as a focal neurological deficit of sudden onset of presumed vascular origin, lasting 24 hours or more, with brain imaging showing typical signs of brain infarction or no abnormalities. The diagnosis of TIA was defined as a focal neurologic deficit of sudden onset lasting less than 24 hours and with no signs of recent infarction on CT-scan.

CLINICAL DATA

Demographic data, vascular history and risk factors, use of antihypertensive drugs, renal function, and data on event characteristics were collected. Because blood pressure is influenced by the acute phase reaction, hypertension was not defined by the blood pressure during admission, but on the use of antihypertensive drugs prior to the event. Dyslipidemia was defined as the use of statins and/or low-density lipoprotein (LDL) level higher than 2.5mmol/L. Diabetes was defined as the use of oral and/or parenteral antidiabetic agents and/or fasting plasma glucose levels of

7.0mmol/L or higher. Stroke subtype was classified with the Trial of Org 10710 in Acute Stroke Treatment (TOAST) classification, a classification system which denotes 5 stroke subtypes: large-artery atherosclerosis, cardio-embolism, small-vessel occlusion, stroke of other determined etiology and stroke of undetermined etiology [24]. Stroke severity was assessed by means of the National Institutes of Health Stroke Scale (NIHSS) score, a 15-item standardized neurologic examination with scores that range from 0 to 42 points, with higher values indicating greater severity [25].

24-HOUR BLOOD PRESSURE MEASUREMENT

The 24-hour ambulatory blood pressure measurement was recorded with the oscillometric SpaceLabs 90207 ambulatory blood pressure monitor (SpaceLabs Healthcare, Issaquah, Washington, United States of America) [26]. The ambulatory blood pressure measurement device was attached to the non-dominant arm. Patients were instructed to relax their arm during the measurement and to pursue their normal daily activities. They were asked to note down these activities, sleep quality and their true sleeping times in a diary. Blood pressure and heart rate were measured with a 20-minute interval between 6:00 AM and 10:00 PM and at a 30-minute interval between 10.00 PM and 6.00 AM. According to recent proposals the awake period was defined as 9.00 AM – 9.00 PM and the asleep period as 1.00 AM – 6.00 AM. Patients with less than 70% of successful recordings and/or less than 20 valid awake measurements and/or less than 7 valid asleep measurements and/or less than 2 valid awake measurements per hour and/or less than 1 valid asleep measurement per hour were excluded [6]. Dipping percentage was calculated by: (awake mean arterial pressure [MAP] – asleep MAP)/awake MAP *100. We chose MAP over systolic and diastolic blood pressure to calculate dipping percentage, because with an oscillometric device MAP, rather than systolic or diastolic blood pressure, is assessed most accurately [7]. Patients were classified according to their dipping percentage in 4 categories, according to the frequently used arbitrary cut-off points: extreme dipping (nocturnal blood pressure fall ≥20%), normal dipping (≥10% and <20%), non-dipping (≥0% and <10%) and reverse dipping (<0%) [7].

CT ACQUISITION AND ANALYSIS

CT Angiography (CTA) of the carotid artery was performed with a 16 slice, 64 slice or 128 slice multi-detector CT system (Sensation 16, Sensation 64, Definition, Definition AS+ or Definition Flash, Siemens Medical Solutions, Erlangen, Germany) using a standardized optimized contrast-enhanced CTA protocol (120 kVp, 180-200 mAs, collimation 16 x 0.75 mm; 32 x 2 x 0.6 mm; 64 x 2 x 0.6 mm, pitch < 1). The scan range extended from the ascending aorta to the intracranial circulation (3 cm above the sella turcica). All patients received 80 ml of contrast agent (320 mg/mL iodixanol, Visipaque, Amersham Health, Little Chalfont, UK), followed by 45 ml saline bolus chaser, both at an injection rate of 4 or 5 ml/s. Image reconstructions were made with field of view of 120 mm, matrix size 512 × 512, slice thickness 0.75 or 1.0 mm, increment 0.4 – 1.0 mm and with an intermediate reconstruction algorithm.

STENOSIS

The severity of extracranial carotid artery stenosis within 3 cm proximal and distal of the bifurcation was assessed with the North American Symptomatic Carotid Endarterectomy Trial (NASCET) criteria [27]. A stenosis of 30% or more was considered clinically relevant. An intraclass correlation coefficient (ICC) for the degree of stenosis was based on the ratings of 3 observers on 50 CT examinations: 0.96-0.99.

VOLUME OF CALCIFICATIONS

Dedicated commercially available software (Syngo CalciumScoring, Siemens) was used to semi-automatically quantify calcifications in the aortic arch and extracranial carotid arteries, expressed as calcification volume in mm^3. The aortic arch was defined as the origin of the aortic arch (the image in which the ascending and descending aorta merge into the inner curvature of the aortic arch) to the first 1 cm of the common carotid arteries, the vertebral arteries and the subclavian arteries beyond the origin of the vertebral arteries. Both carotid arteries were scored within 3 cm proximal and distal of the bifurcation. A threshold of 600 Hounsfield units (HU) was used to differentiate calcifications from contrast material in the lumen. A detailed description of the measurement is provided elsewhere [28].

A custom-made plug-in for the freely available software ImageJ (Rasband, National Institute of Mental Health, Bethesda, MD, available at http://rsb.info.nih.gov/ij) was used for quantification of intracranial internal carotid artery calcifications. The intracranial internal carotid artery comprised the horizontal segment of the petrous internal carotid artery to the top of the internal carotid artery. Due to the close relationship of calcium in the arterial wall to the skull base, the previous mentioned semi-automatic tool could not be used. With the plug-in, it is possible to draw regions of interest in axial multidetector (MD) CTA images and to calculate automatically the total number of pixels above a predefined threshold. The volume of the intracranial calcifications (in mm^3) was calculated as the product of the number of pixels above the threshold (600 HU), the pixel size, and the increment [29].

ICC for extracranial and intracranial calcifications was assessed, based on the ratings of respectively 3 observers on 35 CT examinations (ICC, 1.00) and the ratings of 2 observers on 40 CT examinations (ICC, 0.99).

STATISTICAL ANALYSIS

Statistical analyses were performed with Stata/SE 12.1 for Windows (Statacorp, College Station, Texas). We compared clinical variables, laboratory assessments and radiological data between dipping groups, with normal dipping blood pressure pattern as a reference. Categorical variables were compared with the chi-squared-test and normally distributed continuous variables with one-way analysis of variance (ANOVA). Non-normally distributed continuous variables were compared with the Kruskal-Wallis test. In the analysis with severity of stenosis, we used the highest degree of stenosis in both carotid artery bifurcations. In the analysis with calcification as continuous measure, we used log-transformed values and added 1.0 mm^3 to the non-transformed values to deal with patients with a calcium score of zero. Calcification volumes of the left and right side were summed for the extra- and intracranial carotid arteries separately. The relation between dipping groups on the one hand and atherosclerosis on the other was studied with a logistic and linear regression model. Adjustments were made for age, sex, number of antihypertensive drugs, diabetes, and TIA vs. ischemic stroke with a multivariable logistic and linear regression model. Furthermore, we investigated the association between asleep MAP and asleep hypertension (defined as ≥120/70 mmHg) and atherosclerosis, as these variables are a superior predictor of cardiovascular outcome and mortality compared to awake values [6].

P-values of less than 0.05 were considered statistically significant.

Results

PREVALENCE OF THE DIFFERENT DIPPING PATTERNS

Between May 2009 and December 2011, 261 patients (40%) with a minor ischemic stroke visited the post-stroke care clinic 4-6 weeks after their stroke and 388 patients (60%) with a TIA visited our specialized TIA clinic. Two-hundred fifty-four patients (39%) gave informed consent and underwent a 24-h ambulatory blood pressure measurement. Six patients underwent their measurement in the acute phase and were excluded. Fifty-six patients (23%) were excluded because their ambulatory blood pressure recordings did not meet the quality criteria. This left 192 patients eligible for the analysis, with a median of 91% successful recordings (IQR 86-96). Normal dipping was present in 75 patients (39%), extreme dipping in 50 patients (26%), non-dipping in 48 patients (25%), and reverse dipping in 19 patients (10%).

CLINICAL CHARACTERISTICS OF DIPPING PATTERNS

Demographic variables, stroke risk factors, medical history, laboratory variables and event characteristics for the four dipping groups are shown in Table 1. Non- and reverse dippers are older, had more often hypertension, previous ischemic stroke or TIA, previous non-ischemic heart disease, and renal dysfunction. They also tended to have more often dyslipidemia and diabetes mellitus. Reverse dippers were also less often current smokers.

Blood pressure characteristics in the different dipping groups are shown in Table 2. The median time between the event and 24h ambulatory blood pressure measurement was 66 days (IQR 18-100) and did not differ between the dipping groups. Awake systolic blood pressure, diastolic blood pressure and MAP were not different among dipping group. Asleep, but not awake blood pressure increased in the patients with less dipping. The number of antihypertensive drugs used during the measurement was higher in non- and reverse dippers.

Of the 192 patients, 112 (58%) kept a diary. Sleep quality did not differ among the dipping groups.

ASSOCIATION WITH ATHEROSCLEROSIS

A total of 175 patients (91%) underwent CT-angiography. Main reasons for not performing CT-angiography were renal failure, contrast allergy, or the performance of duplex ultrasound in another hospital. The results are shown in the Figure. The prevalence of stenosis increased with decreasing nocturnal dipping. However, there were no significant differences between the dipping groups (Table 3).

Calcification volumes in the aortic arch, carotid bifurcation and intracranial carotid artery were scored in resp. 175 (91%), 167 (87%), and 171 (89%) patients. Calcification volume could not be assessed in case of technical failure or artifacts (movement, clips, coils, and stents). Calcification volume in the aortic arch and intracranial carotids differed between the dipping groups, with the highest volumes in the reverse dippers (Figure). However, adjustment for potential confounders attenuated these results (Table 3).

There was a trend toward an association between asleep hypertension and stenosis, but no significant associations between asleep MAP and extra- and intracranial atherosclerosis.

Discussion

We found that non- and reverse dipping blood pressure patterns are often present in the post-acute phase after a TIA or ischemic stroke. Also, a trend toward an association between reverse

TABLE 1 - PATIENT AND EVENT CHARACTERISTICS PER DIPPING GROUP

Total number of patients = 192	Extreme dipping* (n=50)	Normal dipping* (n=75)	Non-dipping* (n=48)	Reverse dipping* (n=19)	*p*-value
Male sex, n (%)	24 (48)	49 (65)	35 (73)	13 (68)	0.064
Age (yrs), mean (SD)	56 (11)	60 (15)	63 (13)	66 (15)	0.027
Vascular risk factors					
Current smoking, n (%)	22 (44)	22 (29)	13 (27)	2 (11)	0.043
Hypertension**, n (%)	26 (52)	33 (44)	32 (67)	15 (79)	0.012
Dyslipidemia***, n (%)	41 (82)	64 (85)	44 (92)	19 (100)	0.159
Diabetes mellitus****, n (%)	12 (24)	15 (20)	16 (33)	8 (42)	0.151
Atrial fibrillation, n (%)	4 (8)	3 (4)	4 (8)	1 (5)	0.730
Body mass index (kg/m²)†, mean (SD)	27 (4)	27 (5)	27 (5)	28 (4)	0.908
Medical history					
Ischemic stroke/TIA, n (%)	11 (22)	11 (15)	17 (35)	7 (37)	0.031
Ischemic heart disease, n (%)	3 (6)	13 (17)	9 (19)	5 (16)	0.128
Non-ischemic heart disease, n (%)	3 (6)	8 (11)	13 (27)	3 (16)	0.017
Event characteristics					
TIA, n (%)	24 (48)	36 (48)	29 (60)	11 (58)	0.488
NIHSS‡, median (IQR)	1 (1-4)	2 (1-4)	2 (0-5)	2 (1-4)	0.647
TOAST classification					0.496
Large artery disease, n (%)	4 (8)	12 (16)	8 (17)	6 (32)	
Cardio-embolism, n (%)	7 (14)	7 (9)	5 (10)	3 (16)	
Small vessel disease, n (%)	20 (40)	25 (33)	13 (27)	4 (21)	
Other determined, n (%)	0 (0)	3 (4)	1 (2)	0 (0)	
Undetermined, n (%)	19 (38)	28 (37)	21 (44)	6 (32)	
Physical examination					
Office mean arterial pressure (mmHg), mean (SD)	92 (10)	94 (11)	95 (15)	92 (11)	0.673
Laboratory assessment					
GFR ≤60mL/min, n (%)	6 (12)	12 (16)	13 (28)	8 (42)	0.018

* Definitions dipping groups: extreme dipping: ≥20%, normal dipping: 10-20%, non-dipping: 0-10%, reverse dipping: <0%
** defined as the use of antihypertensive drugs
*** defined as the use of statins or LDL ≥2.5mmol/L
**** defined as the use of oral and/or parenteral antidiabetics or fasting plasma glucose ≥7.0mmol/L
† missing in 21 patients
‡ in patients with ischemic stroke only

dipping blood pressure patterns and extra- and intracranial atherosclerosis could be established. Most previous studies that assessed the prevalence of dipping blood pressure patterns in patients with ischemic stroke or intracerebral hemorrhage have performed 24h ambulatory blood pressure measurement within 5 days after the event, and used systolic blood pressure, diastolic blood pressure or MAP to calculate the dipping percentage [16-19]. These studies found an even higher prevalence of abnormal dipping, with extreme dipping present in 2-5%, normal dipping in 9-23%, non-dipping in

TABLE 2 - 24-HOUR AMBULATORY BLOOD PRESSURE MEASUREMENT PER DIPPING GROUP

	Extreme dipping (n=50)	Normal dipping (n=75)	Non-dipping (n=48)	Reverse dipping (n=19)	*p*-value
Time between event and 24-h ambulatory blood pressure measurement (days), median (IQR)	73 (31-100)	69 (24-100)	57 (22-90)	35 (21-99)	0.676
Awake (9.00-21.00)					
Systolic blood pressure (mmHg), mean (SD)	132 (15)	129 (13)	132 (14)	131 (15)	0.580
Diastolic blood pressure (mmHg), mean (SD)	81 (10)	78 (9)	78 (10)	74 (10)	0.079
Mean arterial pressure (mmHg), mean (SD)	99 (11)	96 (10)	96 (10)	94 (11)	0.372
Daytime hypertension*, n (%)	16 (32)	13 (17)	8 (17)	3 (16)	0.162
Heart rate (beats/minute), mean (SD)	79 (11)	77 (11)	75 (11)	72 (11)	0.081
Asleep (1.00-6.00)					
Systolic blood pressure (mmHg), mean (SD)	103 (11)	113 (13)	127 (15)	139 (18)	<0.001
Diastolic blood pressure (mmHg), mean (SD)	58 (9)	64 (8)	72 (9)	78 (9)	<0.001
Mean arterial pressure (mmHg), mean (SD)	74 (8)	81 (8)	91 (9)	100 (11)	<0.001
Night-time hypertension**, n (%)	2 (4)	11 (15)	26 (54)	15 (79)	<0.001
Heart rate (beats/minute), mean (SD)	69 (9)	67 (8)	67 (10)	71 (12)	0.218
Dipping percentage					
Based on systolic blood pressure, mean (SD)	22 (5)	13 (4)	4 (4)	-6 (5)	<0.001
Based on diastolic blood pressure, mean (SD)	28 (4)	17 (4)	7 (4)	-5 (7)	<0.001
Based on MAP, mean (SD)	25 (4)	15 (3)	5 (3)	-6 (5)	<0.001
Medication					
Number of antihypertensive drugs, median (IQR)	1 (0-2)	1 (0-1)	2 (1-3)	2 (1-2)	0.002
Diary					
Normal sleep quality†, n (%)	12 (46)	28 (57)	8 (31)	4 (36)	0.153
Dipping percentage based on MAP using self-reported sleeping-timest, mean (SD)	23 (3)	14 (4)	7 (4)	-4 (5)	<0.001

* defined by awake average ≥135/85mmHg
** defined by asleep average ≥120/70mmHg
† in 112 patients

37-88% and reverse dipping in 36-40% of the patients. Compared to the present study, the criteria to define the dipping pattern in these studies were less rigorous. Most patients with acute stroke initially have an elevated blood pressure, returning to pre-event values over the first few days [20-22]. This might have resulted in an overestimation of the frequency of non- and reverse dipping patterns since hypertension per se associates with abnormal dipping blood pressure patterns [30].

In another study of 81 patients with a first ever stroke (intracerebral hemorrhage or ischemic stroke), the prevalence of dipping patterns in the post-stroke period was assessed 120 days after the event. Dipping percentage was based on systolic blood pressure. Normal dipping blood pressure pattern was present in 23% of the patients, non-dipping in 56% and reverse dipping in 21% [31]. The prevalence of dipping patterns cannot be directly compared with the results of our study because of differences in stroke subtypes, time between event and 24h ambulatory blood pressure measurement and method of calculating dipping percentage.

TABLE 3 - ASSOCIATION BETWEEN ASLEEP BLOOD PRESSURE OR DIPPING PATTERN AND ATHEROSCLEROSIS

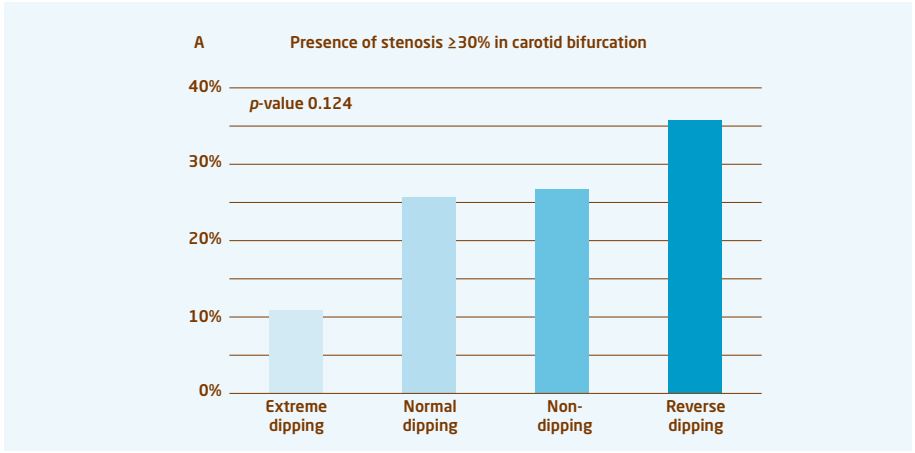
	Presence of stenosis ≥30%	Calcification volume in aortic arch (per mm³)	Calcification volume in carotid bifurcation (per mm³)	Intracranial calcification volume (per mm³)
	aOR (95%-CI)*	Adj. beta coeff (95%-CI)*	Adj. beta coeff (95%-CI)*	Adj. beta coeff (95%-CI)*
Asleep blood pressure				
Asleep MAP (per mmHg)	1.02 (0.99-1.05)	0.01 (-0.01-0.04)	0.02 (-0.01-0.04)	0.01 (-0.01-0.04)
Asleep hypertension‡	1.81 (0.80-4.12)	-0.10 (-0.80-0.59)	0.42 (-0.21-1.05)	0.11 (-0.46-0.69)
Dipping pattern				
Dipping percentage (MAP)	0.98 (0.94-1.02)	-0.01 (-0.04-0.03)	-0.02 (-0.05-0.01)	-0.03 (-0.05-0.00)†
Non-dipping vs. dipping	1.12 (0.50-2.50)	-0.12 (-0.80-0.56)	0.29 (-0.32-0.91)	0.15 (-0.40-0.70)
Vs. normal dipping				
Extreme dipping	0.42 (0.14-1.28)	-0.19 (-0.96-0.57)	-0.12 (-0.81-0.57)	-0.24 (-0.85-0.37)
Non-dipping	0.76 (0.30-1.94)	-0.48 (-1.27-0.31)	0.09 (-0.64-0.82)	-0.15 (-0.80-0.49)
Reverse dipping	1.22 (0.34-4.41)	0.64 (-0.50-1.79)	0.73 (-0.35-1.82)	0.64 (-0.28-1.55)

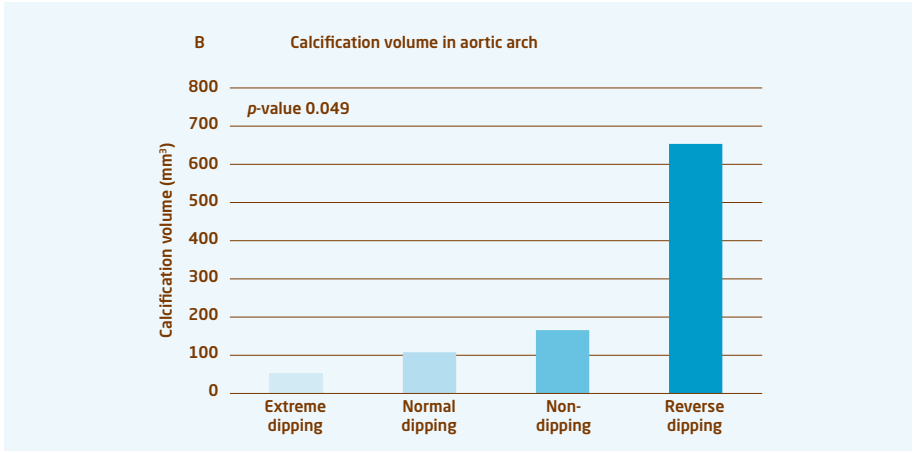
* Adjusted odds ratio (aOR) and adjusted beta coefficient (Adj. beta coeff) with corresponding 95% confidence intervals (95%-CI) based on multivariable logistic and linear regression analysis with adjustments for age, sex, number of antihypertensive drugs, diabetes mellitus, and TIA vs. ischemic stroke.
† p-value 0.043
‡ defined by asleep average ≥120/70mmHg

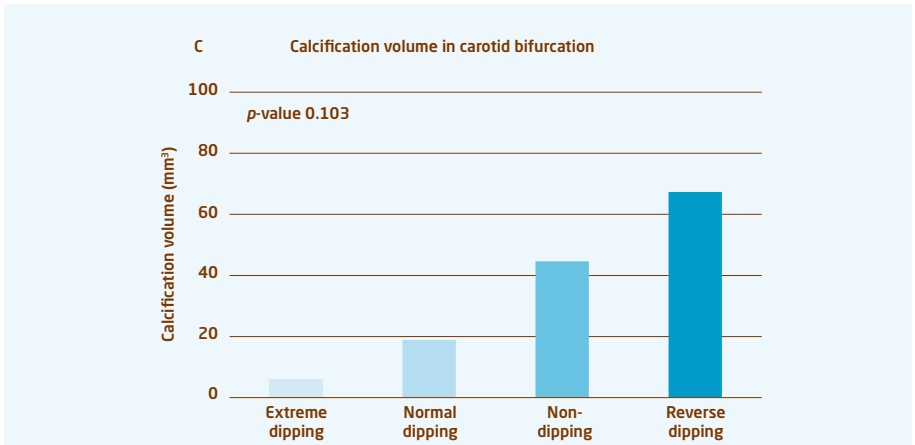
The trend towards an association between reverse-dipping blood pressure patterns and extra-and intracranial atherosclerosis has been demonstrated in hypertensive patients without previous stroke by means of intima-media thickness (IMT). Some studies found an association between non-dipping patterns (non- and reverse dipping combined) and IMT [14, 15, 32, 33], however, in line with our results, others revealed that this association attenuates after adjustment for confounders [34, 35].

After adjusting for confounders, we found no significant associations between abnormal dipping and extra- and intracranial atherosclerosis. Patients with non- and reverse dipping blood pressure patterns more often had vascular risk factors like increasing age, hypertension, dyslipidemia and diabetes mellitus compared to patients with normal dipping blood pressure patterns.
The question whether non- or reverse dipping is the cause or the consequence of atherosclerosis remains unanswered. It is possible that reverse dipping increases the risk of atherosclerosis due to the continuously high blood pressure levels. However, not all patients with an abnormal dipping pattern have hypertension. Possibly, extra- and intracranial atherosclerosis leads to reverse dipping as a defense mechanism to maintain sufficient cerebral perfusion. Population-based longitudinal studies with repeated measurements might give more insight in the direction of the relationship between abnormal dipping patterns and atherosclerosis.

To our knowledge our study is the first that has assessed the prevalence of abnormal blood pressure dipping patterns and their association with both intra- and extracranial atherosclerosis in patients with TIA or ischemic stroke. Another strength is the detailed data on patient and event characteristics.







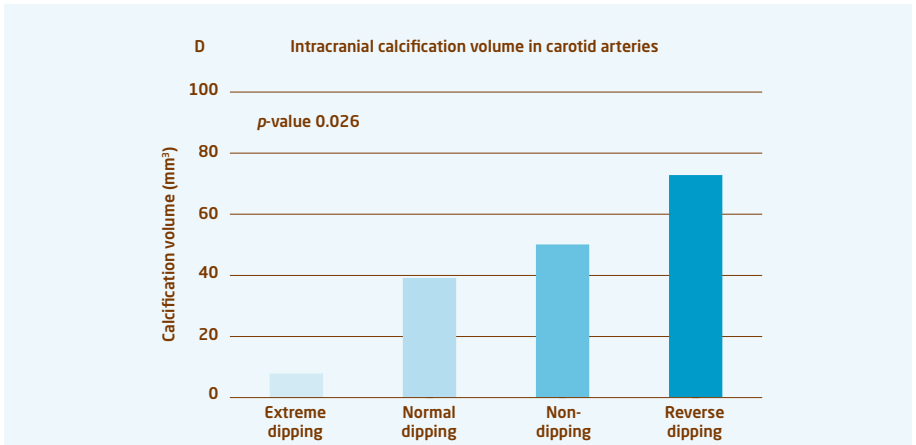


Figure - Presence of stenosis ≥30% in carotid bifurcations (A), calcification volume in aortic arch (median (IQR)) (B), calcification volume in carotid bifurcations (median (IQR)) (C), and intracranial calcification volume in carotid arteries (median (IQR)) (D) per dipping group.

Our study has also limitations. We used fixed narrow time intervals with discarding transition periods between daytime and nighttime [6]. Although this is preferable to fixed wide time intervals (daytime 6.00 AM – 10.00 PM and night-time 10.00 PM – 6.00 AM) as there is considerable variation of blood pressure during these transition periods [6], it would be better to use the true sleeping times based on the diary [36] or even actimeter-derived sleeping times [36, 37]. Second, the ambulatory blood pressure measurement was not repeated due to practical reasons. The reproducibility of the dipping percentage has been subject of several studies and ranges from poor to satisfactory [38-40]. Third, morning intake of antihypertensive drugs might have induced a non- or reverse dipping blood pressure pattern in some of our patients, as the antihypertensive effect may attenuate during the day. Timing of drug intake, drug type, and amount of drugs, however, was not uniform among the dipping groups, making it hard to study its effect on dipping patterns. Finally, the number of patients was relatively small, especially the number of patients with reverse dipping. This might explain that we found only a trend towards an association with atherosclerosis.

In conclusion, our study shows that non- and reverse dipping are often present in the post-acute phase after TIA or ischemic stroke. These dipping patterns are associated with vascular risk factors and a trend towards an association between reverse dipping and extra- and intracranial atherosclerosis could be established. Up to now, unraveling the relationship between dipping patterns and atherosclerosis is mainly interesting from a mechanistic point of view. Abnormal dipping patterns may be an important potential treatment target. Apart from changing the timing of intake of antihypertensive agents treatment options are not available yet. Moreover, as a second step it has to be proven that an induced change in dipping pattern results in an improved cardiovascular outcome.

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CHAPTER 5

TREATMENT OF DISTURBED GLUCOSE METABOLISM AFTER TIA OR ISCHEMIC STROKE

CHAPTER 5.1

METFORMIN AND SITAGLIPTIN IN PATIENTS WITH IMPAIRED GLUCOSE TOLERANCE AND A RECENT TIA OR MINOR ISCHEMIC STROKE (MAAS) – A MULTICENTER, RANDOMIZED, OPEN-LABEL PHASE II TRIAL

AUTHORS

Susanne Fonville [1]
Lisa Osei [2]
Adrienne A.M. Zandbergen [3]
Paul J.A.M. Brouwers [2]
Laus J.M.M. Mulder [4]
Marleen Witteveen [3]
Diederik W.J. Dippel [1]
Peter J. Koudstaal [1]
Heleen M. den Hertog [1,2]

1
DEPARTMENT OF NEUROLOGY
ERASMUS MC UNIVERSITY MEDICAL CENTER
Rotterdam, The Netherlands

2
DEPARTMENT OF NEUROLOGY
MEDISCH SPECTRUM TWENTE
Enschede, The Netherlands

3
DEPARTMENT OF INTERNAL MEDICINE
IKAZIA HOSPITAL
Rotterdam, The Netherlands

4
DEPARTMENT OF NEUROLOGY
IKAZIA HOSPITAL
Rotterdam, The Netherlands

SUBMITTED

Abstract

RATIONALE

Impaired glucose tolerance is present in one third of patients with a transient ischemic attack (TIA) or ischemic stroke, and is associated with a two-fold risk of recurrent stroke. Metformin improves glucose tolerance, but often leads to side effects.

AIMS

To compare the feasibility, safety, and effects on glucose metabolism of both metformin and sitagliptin in patients with TIA or minor ischemic stroke and impaired glucose tolerance. We will also assess whether a slow increase in dose of metformin and better support and information on this treatment will reduce the incidence of side effects in these patients.

DESIGN

The MAAS trial is a phase II, multicenter, randomized, controlled, open-label trial with blinded outcome assessment. Patients (n=100) with a recent (< 6 months) TIA, amaurosis fugax or minor ischemic stroke (modified Rankin scale ≤ 3) and impaired glucose tolerance will be included. The patients will be randomly assigned in a 1:1:2 ratio to metformin, sitagliptin or 'no treatment'. Patients allocated to metformin will start with 500 mg twice daily, which will be slowly increased in 6-weeks time to a daily dose of two times 1000 mg. Patients allocated to sitagliptin will be treated with a daily fixed dose of 100 mg. The study has been registered as NTR 3196 in The Netherlands Trial Register.

STUDY OUTCOMES

Primary outcomes include percentage still on treatment, percentage of (serious) adverse events, and the baseline adjusted difference in 2-hour post-load glucose levels at 6 months.

DISCUSSION

This study will give more information about the feasibility and safety of metformin and sitagliptin as well as the effect on 2-hour post-load glucose levels at 6 months in patients with TIA or ischemic stroke and impaired glucose tolerance.

Introduction and rationale

Impaired glucose tolerance, an intermediate metabolic state between normal glucose tolerance and diabetes mellitus, is present in about a third of patients with transient ischemic attack (TIA) or ischemic stroke [1-4], and is associated with a two-fold risk of recurrent stroke [5]. The mechanisms underlying this association are not fully understood, but include insulin resistance, endothelial dysfunction, dyslipidemia, chronic inflammation, procoagulability, and impaired fibrinolysis [6-8].
Pharmacological interventions reduce the rate of progression to type 2 diabetes by 10-60% in people with impaired glucose tolerance [9-12]. Lifestyle interventions are likely to be at least as effective as drug treatment [9, 12], but often difficult to carry out successfully, and life style advice needs to be reinforced on a regular basis.

There is no clear evidence that tight glycemic control reduces the risk of stroke in patients with diabetes or impaired glucose tolerance. In the UK Prospective Diabetes Study, however, metformin therapy was associated with less cardiovascular events in newly-diagnosed type 2 diabetics [13]. Furthermore, a large randomized placebo-controlled trial found that metformin reduces macrovascular complications when added to insulin treatment in type 2 diabetes [14]. A recent meta-analysis on glucose-lowering pharmacological interventions in patients with impaired glucose tolerance found no beneficial effects on all-cause mortality or death due to major cardiovascular events, with the possible exception of stroke [15].

The widely-used oral glucose-lowering drug metformin is a biguanide that improves insulin sensitivity, enhances peripheral uptake of glucose, and decreases hepatic glucose output. It is recommended as first-line treatment in type 2 diabetes mellitus, and is cheap as compared to the newer antidiabetic drugs. Our recent findings suggest that metformin treatment is safe in patients with TIA or ischemic stroke and impaired glucose tolerance, and probably leads to improved glucose tolerance [16]. However, 50% of the patients experienced gastrointestinal side effects resulting in permanent discontinuation in 25%. Slower increase in dose of metformin and better information and support on the temporarily nature of the side effects might prevent the high incidence of side effects and discontinuation of treatment respectively.

Novel drugs for type 2 diabetes might have fewer side effects than metformin, and might be at least as effective as metformin. Sitagliptin, a selective dipeptidyl peptidase-4 (DPP-4) inhibitor, improves glycemic control and β-cell function and has a safety profile similar to placebo, with low risk of gastrointestinal side effects [6, 7].

The aim of the study is to compare the feasibility, safety, and effect on glucose metabolism of both metformin and sitagliptin in patients with TIA or minor ischemic stroke and impaired glucose tolerance. Also, we will assess whether a slow increase in dose of metformin and better support and information on this treatment will reduce the incidence of side effects in these patients.

Methods

DESIGN

We conduct a phase II, multicenter prospective, randomized, open-label trial, blinded end point (PROBE) trial of standard care plus metformin or sitagliptin, as compared with standard care without antidiabetic treatment.

PATIENT POPULATION – INCLUSION AND EXCLUSION CRITERIA

All adult patients attending the TIA outpatient clinic or admitted to the stroke unit in 3 hospitals in the Netherlands with TIA, amaurosis fugax or minor ischemic stroke (defined as a modified Rankin scale (mRS) [17] score of 3 or less) within the previous 6 months, and impaired glucose tolerance, defined as 2-hour post-load glucose levels between 7.8 and 11.0 mmol/L [18] after standard oral glucose tolerance test (OGTT) [19], will be invited to participate in the trial. The OGTT will be repeated after 2-4 weeks to rule out laboratory error and the acute phase effect. If the second OGTT confirms the diagnosis of impaired glucose tolerance, and all the selection criteria are fulfilled, the patient will be asked for written informed consent. Inclusion and exclusion criteria are shown in Table 1.

RANDOMIZATION, BLINDING AND TREATMENT ALLOCATION

Patients will be randomized to receive either open-label metformin or sitagliptin or "no treatment" in a 1:1:2 ratio for 6 months. The randomization process will be available online by means of a list generated by computer before the start of the trial. Treatment allocation will be only possible after registration in the database. From this moment, it will not be possible to remove a patient from the database. The list with information regarding the treatment allocation will be kept separate from the study database. An independent statistician, who otherwise will not be involved in the study, will provide the list. The statistician will report unblinded data to the Data Safety and Monitoring Board (DSMB) for evaluation and interim analysis. The steering committee will be kept unaware of these results unless necessary (as judged by the DSMB), and the code will not be broken until the last patients have completed the six-months of follow-up.

Irrespective of treatment allocation, patients will receive optimal standard care, including antithrombotic and antihypertensive agents as well as cholesterol lowering drugs, where appropriate [20]. In addition, a stroke nurse specialist will provide general lifestyle advice including healthy diet, stop smoking, and regular physical exercise.

INTERVENTION

Patients will be randomly allocated to open-label metformin or sitagliptin or "no treatment" for a 6 months period. Patients allocated to metformin will start with 500 mg twice daily, which will be slowly increased in a 6-week period to 1000 mg twice daily (week 1: 2 times 500 mg, week 3: 2 times 850 mg, week 7: 2 times 1000 mg). If there are unmanageable side effects at an increased dose, the lower dose will be resumed and the increase will be tried again the next week. Patients allocated to sitagliptin will be treated with a daily fixed dose of 100 mg.

STUDY PROCEDURES

All patients will be assessed at baseline, and at 6 months. At baseline, data on clinical features of TIA or ischemic stroke, demographic data, medical history, vascular risk factors, and medication use will be obtained.

TABLE 1 – INCLUSION AND EXCLUSION CRITERIA

Inclusion criteria
Age ≥18-years
TIA, amaurosis fugax or minor ischemic stroke (mRS≤3)
Symptom onset <6 months
Impaired glucose tolerance (2-hour post-load glucose level between 7.8 and 11.0 mmol/L) in 2 consecutive measurements
Exclusion criteria
Diabetes mellitus
History of diabetic ketoacidosis
Symptoms of type 1 diabetes
Signs of renal impairment (creatinin of 135 µmol/L or higher for men, and 110 µmol/L or higher for women
Known liver disease or disturbed liver function tests (alanine amino transferase, aspartate amino transferas, alkaline phosphastase, or γ glutamyl transferase increased to more than twice the upper limit of typical values)
History of lactic acidosis
Heart failure requiring pharmacological therapy
Pancreatitis
Chronic hypoxic lung disease
Digoxin use
Pregnancy or breast feeding

At the follow-up visit, patients will be asked to complete a single questionnaire to determine compliance and nature of any of the side effects of the study medication. Patients will also be contacted by telephone for recording of possible adverse events and to support continuation of treatment at 2 weeks, 6 weeks, and 3 months after inclusion. If necessary (e.g. in case of unmanageable side effects), the number of telephone contacts and or follow up visits will be intensified. Before randomization and at 6 months, all patients will undergo an OGTT with 75-gram of glucose. Fasting glucose levels, body mass index (BMI), waist circumference, blood pressure and lipid profile will be assessed at baseline, and at 6 months.

PRIMARY OUTCOMES

The primary outcomes are tolerability of metformin and sitagliptin, assessed as the number of patients still on treatment after 6 months, the number of adverse events and serious adverse events, and the baseline adjusted difference in 2-hour post-load glucose levels.

SECONDARY OUTCOMES

Secondary outcomes are the effect of metformin and sitagliptin on fasting plasma glucose levels at 6 months, the percentage of patients with normal glucose tolerance at 6 months and on the BMI and waist circumference at 6 months.

DATA SAFETY MONITORING BOARD

The DSMB is composed of independent experts in the field of statistics, neurology, and vascular internal medicine. It monitors the progress and safety of the trial, and performs an interim analysis. Based on this information, they advise the steering committee on pre-specified grounds, as formulated by the DSMB.

SAMPLE SIZE

A sample size of 100 patients (25 on metformin, 25 on sitagliptin, and 50 in the control group) will provide a power of 80%, to detect a difference of 8% in 2-hour post-load glucose level after 6 months between treatment groups, assuming a significance level of α =0.05 and a mean glucose level of 9.0 mmol/l in the control group, with a standard deviation of 1.0 mmol/l.

STATISTICAL ANALYSES

Analyses will be done by intention-to-treat and all patients who are randomly assigned to treatment will be included in the pre-specified analyses. The effect in each of the two treatment groups will be compared to the control group separately.

We will estimate the baseline adjusted differences in mean 2-hour post-load glucose levels and fasting glucose levels between treatment groups with 95% confidence intervals (CI) with univariable linear regression. Adjustments will be made with a multivariable linear regression model that will include the following factors: age, sex, time to treatment, and baseline waist. Similar analyses will be performed to study the effect of treatment with metformin or sitagliptin on BMI and waist circumference. We will compare the incidence of (serious) adverse events and percentage of patients with a normal glucose tolerance at 6 months between treatment groups with chi-square test.

Discussion

Impaired glucose tolerance is present in up to one third of patients with TIA or stroke and is associated with a two-fold increased risk of recurrent stroke. Intensive glucose control with oral antidiabetic drugs reduces the rate of progression to type 2 diabetes in patients with impaired glucose tolerance [9-12]. Whether pharmacotherapeutical intervention reduces the risk of cardiovascular events in patients with TIA or minor ischemic stroke (who are often older with more co-medication) and impaired glucose tolerance is unknown. Our recent study (LIMIT) has shown that metformin treatment is safe and improves glucose tolerance in these patients, but often leads to gastrointestinal side effects [16].

The rationale for choosing metformin and sitagliptin is as follows: metformin is recommended as first-line treatment in type 2 diabetes, is cheap, and widely used in The Netherlands. However, in the LIMIT trial metformin caused frequent gastrointestinal side effects and consequently discontinuation of drug adherence in a large percentage of the patients [16]. When slow increase in dose of metformin and better support and information on this treatment proves to reduce the incidence of side effects in these patients, metformin will be a cheap, easy and widely used drug to improve glucose tolerance in a large proportion of stroke patients. Sitagliptin has proven to be equally effective and safe as metformin, with a lower risk of gastrointestinal side effects [6, 7]. This combined with the once daily, fixed dose makes it a patient friendly drug and is more prone to good adherence. However, the costs of sitagliptin are more than 16 times higher compared to metformin. To compare the effect in both treatment groups with the control group separately, a randomization ratio of 1:1:2 was chosen.

TABLE 2 - MAAS STUDY GROUP

MAAS Study Group	Name	Affiliations
Principal investigators	Heleen M. den Hertog	Neurologist, PhD, Medisch Spectrum Twente, Enschede
	Adrienne A.M. Zandbergen	Vascular internist, PhD, Ikazia Hospital, Rotterdam
Other study group members	Susanne Fonville	Resident in neurology, Erasmus Medical Center University Hospital, Rotterdam
	Lisa Osei	Resident in neurology, Medisch Spectrum Twente, Enschede
	Marleen Witteveen	Resident in internal medicine, Ikazia Hospital, Rotterdam
	Diederik W.J. Dippel	Neurologist, PhD, Erasmus Medical Center University Hospital, Rotterdam
	Peter J. Koudstaal	Neurologist, PhD, Erasmus Medical Center University Hospital, Rotterdam
	Laus J.M.M. Mulder	Neurologist, Ikazia Hospital, Rotterdam
	Paul J.A.M. Brouwers	Neurologist, PhD, Medisch Spectrum Twente, Enschede
Trial statistician	Hester F. Lingsma	Clinical epidemiologist, PhD, Erasmus Medical Center University Hospital, Rotterdam
Data monitoring and safety committee	Ale Algra	Clinical epidemiologist, PhD, Julius Center, University Medical Center Utrecht, Utrecht (chair)
	Hester F. Lingsma	Clinical epidemiologist, PhD, Erasmus Medical Center University Hospital, Rotterdam
	Paul J. Nederkoorn	Neurologist, PhD, Amsterdam Medical Center, Amsterdam
	Behiye Özcan	Vascular internist, PhD, Erasmus Medical Center University Hospital, Rotterdam
Study coordinators	Susanne Fonville	Resident in neurology, Erasmus Medical Center University Hospital, Rotterdam
	Lisa Osei	Resident in neurology, Medisch Spectrum Twente, Enschede
	Marleen Witteveen	Resident in internal medicine, Ikazia Hospital, Rotterdam
Trial registration	NTR-number: 3196	

Although a double-blind, placebo-controlled designed trial would have been superior, the current trial has a PROBE design. The main reason is that the former comes with greater costs. Potential limitations are therefore the lack of a placebo group and its open design. However, outcome assessment will be blinded for treatment allocation, and the design will resemble the effect in clinical practice after implementation.

Although lifestyle intervention is equally effective in lowering glucose levels as glucose lowering drugs, it is hard to sustain [9, 12]. If we can prove with the MAAS trial that metformin and/or sitagliptin are safe and feasible in lowering glucose levels, a phase III trial is necessary to investigate the effect on the incidence of recurrent stroke and other cardiovascular complications, to improve secondary prevention in these patients.

Summary

In the MAAS trial, we will investigate whether metformin and sitagliptin are feasible and safe in patients with TIA or minor ischemic stroke and impaired glucose tolerance. The design is a multicenter PROBE trial.

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CHAPTER 6

GENERAL DISCUSSION

In this thesis, I have focused on the role of newly-diagnosed disturbed glucose metabolism (pre-diabetes or newly-diagnosed diabetes mellitus) as a potential treatable risk factor in secondary stroke prevention. Pre-diabetes is an intermediate metabolic state between normal glucose metabolism and diabetes mellitus and its diagnosis is based on impaired fasting glucose, impaired glucose tolerance and/or disturbed glycosylated hemoglobin levels. It heralds an increased risk of developing diabetes mellitus and an increased cardiovascular risk. Furthermore, another potential treatable risk factor, nocturnal blood pressure patterns, is also discussed.
In this chapter, I will summarize the main findings of my studies, and discuss methodological issues, and the significance and implications for clinical practice. In addition, I will also present some perspectives for future research.

Newly-diagnosed disturbed glucose metabolism after transient ischemic attack (TIA) or stroke: scope of the problem and predicting the persistence at 3 months

SCOPE OF THE PROBLEM 'NEWLY-DIAGNOSED DISTURBED GLUCOSE METABOLISM' IN PATIENTS WITH TIA OR STROKE

Three different detection methods are known to identify people with (pre-) diabetes: fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels [1]. With these three detection methods, we found that 79% of the patients with a recent TIA or stroke had either pre-diabetes or newly-diagnosed diabetes mellitus. About one third of these patients would not have been identified by fasting plasma glucose levels alone. We found a high prevalence of pre-diabetes after TIA or stroke (52% vs. 23-53%) [2, 3], most likely due to the fact that we were the first to use all three detection methods simultaneously.

To date, assessment of fasting plasma glucose levels still dominates screening of disturbed glucose metabolism in patients with TIA or stroke, as the oral glucose tolerance test is more time-consuming and inconvenient than assessing fasting plasma glucose levels. Furthermore, patients often dislike the glucose load and this causes occasionally nausea and vomiting with possible failure of the test as a result. Assessment of glycosylated hemoglobin has recently been advocated as a diagnostic tool of diabetes mellitus as well [1]. Measurement of glycosylated hemoglobin does not require a fasting sample, has much less intra-individual variation, can be used as a marker of chronic hyperglycemia, and is less influenced by the events related to acute stroke. However, it is more expensive and it may take several days before the results are available.

It is known that the concordance between the three detection methods is not 100% [1]. I showed that without the 2-hour post-load glucose and glycosylated hemoglobin levels the majority of patients with pre-diabetes and newly-diagnosed diabetes mellitus would not have been identified. The three detection methods should therefore be considered as complimentary rather than individual tests, as no gold standard exist. Glucose and glycosylated hemoglobin levels in the pre-diabetic range represent an increased risk for developing diabetes mellitus and an increased cardiovascular risk [4, 5]. In my view, ideally all three detection methods should be used to identify all patients at risk. Moreover, I think it is also possible in the current practice to perform all three tests during admission at the stroke units or a visit to a specialized TIA clinic.

PREDICTING PERSISTENT IMPAIRED GLUCOSE TOLERANCE

In Chapter 3.2, I demonstrated that about half of the patients with impaired glucose tolerance after a TIA or ischemic stroke have persistent impaired glucose tolerance at 3 months. To identify patients at risk for persistent impaired glucose tolerance, I developed a prediction model based on clinical predictors readily available at the time of admission.

The most important predictors for persistent impaired glucose tolerance were statin use, low triglycerides levels and high fasting plasma glucose levels. Impaired glucose tolerance is associated with dyslipidemia, with high triglycerides and/or low HDL levels [6]. Statin use can be used as a proxy of dyslipidemia, which explains its identification as a predictor of persistent impaired glucose metabolism. The fact that lower levels of triglycerides predict persistent impaired glucose tolerance, might be explained by the frequent use of statins in these patients and the associated correlation between lipid levels and statin use.
Although impaired fasting glucose and impaired glucose tolerance have different pathophysiological mechanisms, they often co-exist indicating more advanced disease. Also patients with both impaired fasting glucose and impaired glucose tolerance have an even higher risk of developing diabetes mellitus compared with patients with impaired fasting glucose or impaired glucose tolerance alone. [7, 8] Fasting plasma glucose levels were therefore not an unexpected predictor of persistent impaired glucose tolerance.
However, 2-hour post-load glucose and glycosylated hemoglobin surprisingly did not have an added value in our prediction model. Our hypothesis was that higher 2-hour post-load glucose levels after a TIA or stroke were associated with an increased risk of persistent impaired glucose tolerance, particularly as higher levels of 2-hour post-load glucose in impaired glucose tolerance are known to increase the risk of developing diabetes mellitus.
As glycosylated hemoglobin levels reflect the glucose levels of the previous 2-3 months, a simple hypothesis would be that increased levels of glycosylated hemoglobin in patients with impaired glucose levels after a stroke reflect a pre-existent problem in the glucose metabolism. Transient impaired glucose metabolism due to an acute stress reaction would therefore be less likely in these patients. However, both 2-hour post-load glucose and glycosylated hemoglobin levels were no predictors of persistent impaired glucose tolerance.

To assess the internal validity of our prediction model, I performed bootstrapping techniques. This is an elegant technique to perform in small samples. In the bootstrap procedure 500 random samples of patients were drawn from the original sample, and in each sample the modeling steps were repeated. The 500 resulting models were subsequently evaluated on the original sample. The mean area under the receiver operating characteristics curve (AUROC) was compared with the original AUROC. The bootstrapped AUROC of our prediction model was 0.777.
The AUROC gives an indication of the discriminative ability. An AUROC equal to 1 indicates a perfect discriminative model and an AUROC equal to 0.5 means that the model does not perform better than chance. This indicates that our model has a good performance, but is not perfect yet.

I used potential predictors readily available on admission, so the model would be easy to use in daily practice. However, this may also have attenuated the accuracy of our model as the role of unknown potential factors was discarded. An interesting example might be pancreatic beta cell dysfunction, as this seemed to be correlated to transient impaired glucose tolerance in patients with an acute subarachnoid hemorrhage as well [9]. The pancreatic beta cell function can be assessed with homeostatic model assessment (HOMA) and newer techniques are being developed.

Also, because lifestyle modification can prevent the conversion of impaired glucose tolerance to diabetes mellitus [10], weight reduction and increasing physical activity are important factors to take into account. However, these factors are not available on admission yet and therefore not used in our prediction model.

Of course, the model should be externally validated in an independent comparable population before it can be recommended for daily practice. If patients with increased risk for persistent impaired glucose tolerance are identified, lifestyle modification and/or treatment with glucose lowering agents should be considered to prevent progression to diabetes and reduce the risk of recurrent cardiovascular events. However, it remains unknown whether glucose-lowering drugs reduces the risk of cardiovascular events in these patients. A phase III trial is needed to confirm this effect. In preparation of this phase III trial, we therefore initiated the MAAS trial (**M**etformin and sit**A**gliptin in patients with imp**A**ired glucose tolerance and a recent TIA or minor ischemic **S**troke), a phase II trial to investigate the feasibility, safety, and effects on glucose metabolism of glucose-lowering drugs in patients with impaired glucose tolerance after a TIA or ischemic stroke.

MAAS trial: rationale

In **Chapter 5**, I have presented the protocol of the ongoing MAAS trial. We compare the feasibility, safety and effects on glucose metabolism of both metformin and sitagliptin in patients with TIA or minor ischemic stroke and impaired glucose tolerance. Patients are randomized to receive either open-label metformin or sitagliptin or "no treatment" in a 1:1:2 ratio for 6 months. Our recent LIMIT trial investigated the effects of metformin on glucose metabolism in patients with a recent TIA or ischemic stroke and impaired glucose tolerance. We found that metformin was safe and showed a trend towards improving glucose tolerance. However, 50% of the patients experienced gastrointestinal side effects, resulting in permanent discontinuation in 25% [11]. In the MAAS trial, we have therefore made 3 major protocol changes, compared with the LIMIT trial protocol.

First, the dose increase of metformin will be slowed. Instead of increasing the dose from 500mg once daily to two times 1000mg in 1 month, we start with 500mg twice daily and increase the dose in 6 weeks time to two times 1000mg. Patients also receive more information and support about the possible side effects. In case of unmanageable side effects at an increased dose, the increase is slowed down. I expect that the slower increase and better information and support on the temporarily nature of the side effects will prevent high incidence of side effects and discontinuation of treatment respectively.

Second, we added sitagliptin as a separate treatment arm. Sitagliptin is a selective dipeptidyl peptidase-4 inhibitor, and has therefore a different pharmacotherapeutical effect compared to metformin. It is at least as effective as metformin, and is supposed to have fewer side effects [12, 13]. Also, sitagliptin has a fixed, once daily dose, which makes it a patient friendly drug and more prone to good adherence. However, it comes with greater costs.

Lastly, we repeated the OGTT before randomization. Patients with impaired glucose tolerance after a TIA or minor ischemic stroke are asked to participate in the trial. After 2-4 weeks the OGTT is repeated to rule out laboratory error and the acute phase effect. Patients with transient impaired glucose tolerance are excluded. We therefore prevent that patients are unnecessarily treated with glucose-lowering drugs with potential side effects.

The MAAS trial has a multicenter PROBE design: prospective, randomized, open-label trial with blinded end point assessment. A double-blind, placebo-controlled designed trial is obviously superior, but comes with greater costs. As the outcome assessment is blinded and the design resembles the effect in clinical practice after implementation, I think the use of this design is justifiable.

Finally, lifestyle intervention has proven to be equally effective in lowering glucose levels as glucose-lowering drugs, but is hard to sustain [14, 15]. The MAAS trial is therefore an important step to prove that metformin and/or sitagliptin are safe and feasible in lowering glucose levels in patients with a TIA or ischemic stroke and impaired glucose tolerance. Subsequently, a phase III trial is necessary to investigate the effect on incidence of recurrent stroke and other cardiovascular complications, to improve secondary stroke prevention.

Atherosclerosis: association with newly-diagnosed disturbed glucose metabolism and abnormal nocturnal dipping blood pressure patterns

NEWLY-DIAGNOSED DISTURBED GLUCOSE METABOLISM AND ATHEROSCLEROSIS

Patients with diabetes mellitus are highly susceptible for atherosclerosis. The co-existence of cardiovascular risk factors like hypertension and dyslipidemia promote atherosclerosis. Also insulin resistance, endothelial dysfunction, chronic inflammation, procoagulability, and impaired fibrinolysis play an important role [16, 17].

The factors that increment the susceptibility for atherosclerosis in diabetics may already play a role in patients with pre-diabetes. We hypothesized that this might be the cause of the increased cardiovascular risk in these patients [4, 5, 18] and therefore investigated the association between newly-diagnosed disturbed glucose metabolism (both pre-diabetes and newly-diagnosed diabetes) after a TIA or ischemic stroke and atherosclerosis in **Chapter 4.1**.

We found that newly-diagnosed disturbed glucose metabolism in patients with a recent TIA or ischemic stroke is associated with more severe extra- and intracranial atherosclerosis, similar to pre-existent diabetes mellitus.

Because of the conflicting results on the role of sex in the association between diabetes mellitus and atherosclerosis, and our finding that the association seemed more prominent in men, we performed a test for interaction and found no significant interaction. Prior studies have shown that women with diabetes mellitus have a three- to seven-fold increased risk for coronary heart disease, compared to a two- to three-fold increased risk in men with diabetes mellitus [19]. Hence, I still consider it worthwhile to further investigate the possible different effects of glucose metabolism on atherosclerosis in men and women.

Patients with newly-diagnosed disturbed glucose metabolism after a TIA or ischemic stroke have an increased cardiovascular risk, which might be explained by its association with atherosclerosis. This underlines the importance of the detection of disturbed glucose metabolism after a TIA

or ischemic stroke and therefore the use of all three detection methods. Furthermore, as these patients have manifest atherosclerosis, this offers a rationale for more aggressive treatment of newly-diagnosed disturbed glucose metabolism after a TIA or ischemic stroke. However, the effect of this treatment on the progression of atherosclerosis and (recurrent) stroke should be assessed first.

Interestingly, in Chapter 4.2 we found also a genetic association between glucose metabolism and atherosclerosis. Contrary to our expectation, genes related to dyslipidemia and hypertension were not significantly associated with atherosclerosis in this study population of patients with recent TIA or stroke. A SNP in the GLIS 3 gene, however, emerged as being strongly associated with carotid bifurcation stenosis. The GLIS 3 gene is associated with increased fasting plasma glucose, insulin and 2-hour post-load glucose levels and diabetes mellitus [20]. A replication study is necessary to confirm our finding, but this study supports our previous results that glucose metabolism plays an important role in the pathogenesis of atherosclerosis in patients with a TIA or stroke.

ABNORMAL NOCTURNAL DIPPING BLOOD PRESSURE PATTERNS AND ATHEROSCLEROSIS

In my quest to improve secondary stroke prevention, I came across another interesting potential new risk factor for (recurrent) stroke: abnormal nocturnal blood pressure patterns. In Chapter 4.3, I assessed the prevalence of abnormal dipping patterns in the post-acute phase after a TIA or ischemic stroke. I found that only 39% of our patients had a normal dipping pattern. Extreme dipping was present in 26%, non-dipping in 25%, and reverse dipping in 10%. It is difficult to compare our results with previous studies, because only a single study assessed the dipping patterns in the post-acute phase and across all studies different blood pressure measurements were used. Also, we used the new definitions for awake and asleep periods, which have only recently been proposed [21].

Non- and reverse dipping are associated with an increased risk of cardiovascular disease [22-24]. A potential underlying mechanism for this increased risk is atherosclerosis. I therefore investigated the association between abnormal dipping patterns and atherosclerosis. We found no significant associations between non- and reverse dipping blood pressure patterns and extra- and intracranial atherosclerosis. This is most likely due to the small sample size, because we found a trend toward an association between reverse dipping and with extra- and intracranial atherosclerosis. Our findings were in line with previous studies demonstrating an association with non-dipping and intima-media thickness (IMT), attenuated after adjustments for confounders.

It remains unknown whether non- and reverse dipping are the cause or the consequence of atherosclerosis. Continuously elevated blood pressure might increase the risk for atherosclerosis. However, not all patients with non- or reverse dipping have hypertension. Otherwise, atherosclerosis might induce non- or reverse dipping as a defense mechanism to maintain sufficient cerebral blood flow. A population-based study with repeated measurements will give more insight in the direction of the relationship between abnormal dipping patterns and atherosclerosis.

In my view, 24-hour ambulatory blood pressure measurements should not be part of standard stroke care yet. At present, no evidence based treatment for abnormal nocturnal dipping blood pressure patterns is available, apart from adjusting the timing of intake of antihypertensive drugs.

Also, a trial is necessary to prove that treatment of abnormal nocturnal dipping blood pressure patterns reduces the increased cardiovascular risk. Nevertheless, I think that an abnormal dipping blood pressure pattern should be considered as an interesting potential treatable risk factor.

Conclusion

Secondary stroke prevention is not perfect yet. Stroke incidence is increasing and the search for new treatable risk factors is warranted. Diabetes mellitus is an important known risk factor. But pre-diabetes appears to be an interesting risk factor as well.

Newly-diagnosed disturbed glucose metabolism (both pre-diabetes and newly-diagnosed diabetes mellitus) is more often present after TIA or stroke than previously thought and is associated with an increased risk for recurrent stroke. Good and simple diagnostic tests that complement each other are readily available and I therefore recommend assessing 2-hour post-load glucose and glycosylated hemoglobin levels next to fasting plasma glucose levels to diagnose (pre-) diabetes mellitus after TIA or stroke. However, these tests are still rarely performed routinely in common practice, due to unfamiliarity and practical objections. With this thesis I hope to give a rationale to use all 3 glucose tests to diagnose (pre-) diabetes mellitus after a TIA or stroke. It is also important to differentiate between transient and persistent disturbed glucose metabolism, to identify the patients with the highest risk.

Furthermore, I show that not only diabetes mellitus, but also newly-diagnosed disturbed glucose metabolism (including pre-diabetes), is associated with atherosclerosis in patients with a TIA or ischemic stroke. Treatment of pre-diabetes can prevent conversion to diabetes mellitus, but it is unknown whether treatment also decreases the risk of recurrent stroke. The MAAS trial is the first step, before future studies can focus on the effect of treatment on the incidence of recurrent stroke and the progression of atherosclerosis in these patients.

A small side step to nocturnal dipping blood pressure patterns led to another interesting potential treatable risk factor for recurrent ischemic stroke. Future studies should concentrate on the treatment of abnormal dipping patterns, before the effect of treatment on the increased cardiovascular risk in these patients can be studied.

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CHAPTER 7

SUMMARY / NEDERLANDSE SAMENVATTING

Summary

Stroke is the third leading cause of death and the first cause of disability in developed countries. After the initial acute stroke treatment, prevention of recurrent stroke is important. Pharmacological treatment, lifestyle modification and surgical intervention are the standard treatment. However, with the current treatment, secondary stroke prevention is not perfect, and the identification of new treatable risk factors is needed to optimize secondary stroke prevention.

Diabetes mellitus is an important risk factor for first stroke and stroke recurrence. Pre-diabetes is an intermediate metabolic state between normal glucose metabolism and diabetes mellitus, and is associated with a nearly two-fold risk of stroke in patients with transient ischemic attack (TIA) or stroke. The prevalence of pre-diabetes is increasing and hence a potential treatable risk factor in secondary stroke prevention, similar to overt diabetes mellitus.

This thesis focuses on the role of newly-diagnosed disturbed glucose metabolism (pre-diabetes and newly-diagnosed diabetes mellitus) as a potential treatable risk factor in the secondary stroke prevention after TIA or ischemic stroke.

Chapter 1, the general introduction, describes the background and the rationale for the research described in this thesis.

In **Chapter 2**, I reviewed the literature concerning pre-diabetes in patients with stroke or TIA. I discuss the different detection methods to identify pre-diabetes, the prevalence in patients with stroke or TIA, pathophysiology, pre-diabetes as potential risk factor and prognostic factor of outcome after stroke, and treatment strategies. Up to 53% of the non-diabetic patients with a recent ischemic stroke or TIA have pre-diabetes, which is clearly more than in the community. To detect all patients with an increased risk for diabetes mellitus and recurrent stroke, it has been advised to assess 2-hour post-load glucose and glycosylated hemoglobin levels besides fasting plasma glucose levels. Pre-diabetes increases the risk of cardiovascular disease and recurrent ischemic stroke, making this an important target for both primary and secondary prevention. Lifestyle interventions are at least equally effective as pharmacologic interventions in preventing progression to diabetes mellitus, but more difficult to carry out. The effect of these interventions on the risk of (recurrent) cardiovascular disease is still unclear.

The Erasmus Stroke Study is an ongoing registry of all neurovascular patients in the Erasmus MC University Medical Center. It provided the basis of the clinical studies described in this thesis. We have included patients with a recent TIA, ischemic or hemorrhagic stroke to answer several questions concerning the role of newly-diagnosed disturbed glucose metabolism.

In **Chapter 3.1**, I assessed the prevalence of pre-diabetes and newly-diagnosed diabetes mellitus with different screening methods, including fasting plasma glucose, 2-hour post-load glucose and glycosylated hemoglobin levels, in patients with recent TIA, ischemic or hemorrhagic stroke without known diabetes mellitus. With the 3 detection methods combined, 52% of the patients were diagnosed with pre-diabetes and 27% with newly-diagnosed diabetes mellitus. The majority of these patients would not have been identified by fasting plasma glucose levels alone. Hence, our study provides a rationale for the use of the 2-hour post-load glucose and glycosylated hemoglobin levels, besides fasting plasma glucose, as a part of standard care for patients with a TIA or stroke.

It is important to differentiate between transient and persistent impaired glucose tolerance after TIA or stroke, to identify those at risk for recurrent stroke. In **Chapter 3.2**, we therefore developed a prediction model, by means of a multivariable logistic regression model. Almost half of the patients had persistent impaired glucose tolerance. Our prediction model including age, current smoking, statin use, triglycerides, hypertension, previous ischemic cardiovascular disease, body mass index (BMI) and fasting plasma glucose accurately predicted persistent impaired glucose tolerance, with statin use, triglycerides and fasting plasma glucose as the most important predictors.

Atherosclerosis is the most common cause of ischemic stroke. Patients with diabetes mellitus are highly susceptible for atherosclerosis and that might be one of the underlying mechanisms of their increased risk of recurrent stroke. In **Chapter 4.1**, I assessed whether newly-diagnosed disturbed glucose metabolism is associated with atherosclerosis in patients with TIA or ischemic stroke compared with patients with normal glucose metabolism and with known diabetes mellitus. The prevalence of stenosis ≥50% and calcification volume in aortic arch, carotid bifurcation and intracranial carotids increased with more disturbed glucose metabolism. Newly-diagnosed disturbed glucose metabolism in patients with a recent TIA or ischemic stroke was associated with more severe extra- and intracranial atherosclerosis, similar to pre-existent diabetes mellitus.

Chapter 4.2 describes a candidate gene study on the association between genetic variations in metabolic traits and craniocervical artery stenosis in patients with ischemic stroke or TIA. A gene associated with diabetes mellitus, fasting plasma glucose, insulin and 2-hour post-load glucose levels, GLIS3, was associated with a 2.2 (95%CI 1.6-3.1)-fold increase of stenosis risk.

Another interesting potential risk factor for (recurrent) stroke is abnormal nocturnal blood pressure patterns. Blood pressure follows a circadian pattern with normally a 10-20% nocturnal decline compared with daytime blood pressure, which is called dipping. Non-dipping (a 0-10% nocturnal decline) and reverse dipping (a rise in blood pressure during the night) blood pressure patterns are associated with increased risk of cardiovascular disease. In **Chapter 4.3,** I studied the association between nocturnal blood pressure patterns and extra- and intracranial atherosclerosis in patients with a recent TIA or minor ischemic stroke. Seventy-five of the 192 patients (39%) had a normal dipping pattern, 50 (26%) extreme dipping, 48 (25%) non-dipping and 19 (10%) reverse dipping. We found that there was a trend towards an association between reverse dipping and extra- and intracranial atherosclerosis.

Chapter 5 focuses on treatment of disturbed glucose metabolism after stroke. I describe the rationale and protocol for a multicenter, randomized, open-label phase II trial: **M**etformin and sit**A**gliptin in patients with imp**A**ired glucose tolerance and a recent TIA or minor ischemic **S**troke (MAAS) trial. This study will give more information about the feasibility and safety of metformin and sitagliptin as well as the effect on 2-hour post-load glucose levels at 6 months in patients with TIA or ischemic stroke and impaired glucose tolerance. If we can prove with the MAAS trial that metformin and/or sitagliptin are safe and feasible in lowering glucose levels, a phase III trial is the next step to investigate the effect on the incidence of recurrent stroke and other cardiovascular complications.

I discuss clinical and scientific implications of the studies described in this thesis in **Chapter 6**. Newly-diagnosed disturbed glucose metabolism is often present after a TIA or stroke and is associated with atherosclerosis. It is therefore important to differentiate between transient and persistent newly-diagnosed disturbed glucose metabolism. Future research should focus on the treatment of newly-diagnosed disturbed glucose metabolism and the effects on the incidence of recurrent stroke. Furthermore, abnormal nocturnal dipping blood pressure patterns are another interesting potential treatable risk factor for recurrent stroke. There is a trend to an association between abnormal nocturnal dipping blood pressure patterns and atherosclerosis and future research has to prove that treatment leads to a lower risk of stroke as well.

Nederlands samenvatting

Een beroerte (herseninfarct of hersenbloeding) is de derde doodsoorzaak en de belangrijkste oorzaak van invaliditeit in de westerse wereld. Naast de acute behandeling van een beroerte, is het verlagen van het risico op opnieuw een beroerte en andere hart-en vaatziekten van belang. Medicamenteuze en chirurgische behandeling van risicofactoren verlaagt de kans op vasculaire complicaties na een transient ischemic attack (TIA) of herseninfarct. Echter, met de huidige behandeling is de secundaire preventie niet perfect en het identificeren van nieuwe behandelbare risicofactoren is van groot belang om dit te optimaliseren.

Diabetes mellitus is een belangrijke risicofactor voor een beroerte en een recidief beroerte. Pre-diabetes is een voorstadium van diabetes mellitus, met een verhoogd risico op het ontwikkelen van diabetes mellitus in de toekomst en een verhoogd risico op hart- en vaatziekten en een recidief beroerte bij patiënten met een recente TIA of beroerte. Pre-diabetes komt steeds vaker voor en is daarom een mogelijke behandelbare risicofactor voor het voorkomen van een beroerte.

In dit proefschrift heb ik mij gericht op de rol van nieuw-gediagnosticeerde gestoord glucose metabolisme (pre-diabetes en nieuw-gediagnosticieerde diabetes mellitus) als mogelijke behandelbare risicofactor in de secundaire preventie na een TIA of herseninfarct.

In **Hoofdstuk 1**, de algemene introductie, heb ik de achtergrond en motivatie van het onderzoek van dit proefschrift beschreven.

In **Hoofdstuk 2** heb ik een overzicht gegeven van publicaties over pre-diabetes bij patiënten met een beroerte. Daarin heb ik aandacht geschonken aan de verschillende methodes om pre-diabetes te diagnosticeren, het vóórkomen van pre-diabetes bij patiënten met een beroerte of TIA, de pathofysiologie, pre-diabetes als mogelijke risicofactor en voorspeller van de uitkomst na een TIA of herseninfarct en behandelstrategieën. Tot 53% van de patiënten met een TIA of een beroerte en zonder diabetes mellitus in de voorgeschiedenis heeft pre-diabetes, hetgeen duidelijk meer is dan in de algemene populatie. Om alle patiënten met een verhoogd risico op het ontwikkelen van diabetes mellitus en een recidief beroerte te detecteren wordt wel aangeraden om niet alleen het nuchter glucose te bepalen, maar dit aan te vullen met glucosewaarden na glucosebelasting en geglycosyleerde hemoglobine. Pre-diabetes verhoogt het risico op andere hart-en vaatziekten en een recidief herseninfarct, dat pre-diabetes een belangrijk aangrijpingspunt maakt voor zowel primaire als secundaire preventie. Leefstijlveranderingen zijn even effectief als farmacologische interventies als het gaat om het ontwikkelen van diabetes mellitus, maar zijn vaak moeilijker vol te houden. De effecten van deze behandelingen op het risico op een (recidief) beroerte zijn nog onbekend.

De Erasmus Stroke Study is een lopende registratie database van alle neurovasculaire patiënten in het Erasmus MC. Deze database vormde de basis voor de studies in dit proefschrift. We hebben de gegevens van patiënten met een TIA of een beroerte gebruikt om verschillende vragen te beantwoorden over de rol van nieuw-gediagnosticeerde gestoord glucose metabolisme in deze patiënten.

In **Hoofdstuk 3.1** hebben we gekeken naar het vóórkomen van pre-diabetes en nieuw-gediagnosticeerde diabetes mellitus gebruikmakend van verschillende methodes (nuchter glucose,

glucose na belasting en geglycosyleerde hemoglobine) bij patiënten met een recente TIA of beroerte zonder diabetes mellitus in de voorgeschiedenis. Tweeënvijftig procent van de patiënten had pre-diabetes en 27% nieuw-gediagnosticeerde diabetes mellitus, indien gelijktijdig gebruik gemaakt werd van alle drie de methoden. Het overgrote deel van deze patiënten zouden niet geïdentificeerd zijn als alleen nuchter glucose was gebruikt. Dit benadrukt het belang van het gelijktijdig uitvoeren van alledrie de testen en niet alleen te varen op het nuchter glucose, bij de diagnostiek naar (pre-) diabetes mellitus na een TIA of beroerte.

Het is van groot belang om onderscheid te maken tussen voorbijgaande en blijvende gestoorde glucosetolerantie na een TIA of beroerte, omdat de patiënten met een blijvende gestoorde glucosetolerantie een verhoogd risico hebben op een recidief beroerte. In **Hoofdstuk 3.2** hebben we daarom een voorspellingsmodel gemaakt met behulp van een multivariabele logistische regressiemodel. Ongeveer de helft van de patiënten had een blijvend gestoorde glucose tolerantie. Ons voorspellingsmodel omvatte leeftijd, roken, gebruik van cholesterolverlagende medicijnen, triglycerides, hypertensie, eerdere ischemische hartaandoeningen, body mass index (BMI) en het nuchter glucose. Dit model voorspelt blijvend gestoorde glucosetolerantie vrij goed , waarbij het gebruik van cholesterolverlagende medicijnen, triglycerides en het nuchter glucose de belangrijkste voorspellers waren.

Atherosclerose (aderverkalking) is de meest voorkomende oorzaak van een herseninfarct. Patiënten met diabetes mellitus hebben een zeer hoge kans op het ontwikkelen van atherosclerose en dat zou een onderliggend mechanisme kunnen zijn van het verhoogde risico op een recidief beroerte bij deze patiënten. In **Hoofdstuk 4.1** hebben we daarom onderzocht of nieuw-gediagnosticeerde gestoord glucose metabolisme is geassocieerd met atherosclerose bij patiënten met een TIA of beroerte, en vergeleken met patiënten met normaal glucose metabolisme en met pre-existente diabetes mellitus. Het vóórkomen van een significante stenose in de carotisbifurcatie en kalkvolume in de aortaboog, carotisbifurcatie en intracraniële carotiden neemt toe bij een meer gestoord glucose metabolisme. Nieuw-gediagnosticeerde gestoord glucose metabolisme was geassocieerd met ernstiger extra- en intracraniële atherosclerose bij patiënten met een recente TIA of beroerte, zoals bij pre-existente diabetes mellitus.

Hoofdstuk 4.2 beschrijft een kandidaatgen studie, waarin we hebben gekeken naar de associatie tussen genen die betrokken zijn bij metabole risicofactoren, en de mate van atherosclerose in patiënten met een TIA of herseninfarct. Een gen geassocieerd met diabetes mellitus, nuchter glucose, insuline en glucose na belasting, GLIS3, bleek geassocieerd te zijn met een 2.2 (95%BI 1.6-3.1) verhoogd risico op een stenose.

Een andere nieuwe mogelijke risicofactor voor een (recidief) beroerte is een abnormaal nachtelijke dipping bloeddrukpatroon. Bloeddruk volgt een circadiaan ritme met een nachtelijke daling van 10-20%, een fenomeen dat dipping wordt genoemd. Non-dipping (een daling van 0-10%) en omgekeerde dipping (een stijging van bloeddruk 's nachts) zijn geassocieerd met een verhoogd risico op hart- en vaatziekten. In **Hoofdstuk 4.3** heb ik de associatie tussen deze bloeddrukpatronen en atherosclerose onderzocht. Vijf-en-zeventig van de 192 patiënten (39%) had een normaal dipping patroon, 50 (26%) extreme dipping, 48 (25%) non-dipping en 19 (10%) omgekeerde dipping. Er was een trend naar een associatie tussen omgekeerde dipping en extra- en intracraniele atherosclerose.

Hoofdstuk 5 beschrijft de achtergrond en opzet van een lopend gerandomiseerd, open-label fase 2 onderzoek in meerdere centra in Nederland: **M**etformin and sit**A**gliptin in patients with imp**A**ired glucose tolerance and a recent TIA or minor ischemic **S**troke (MAAS) trial. Dit onderzoek zal inzicht geven in de haalbaarheid en de veiligheid van metformine en sitagliptine en het effect op de glucose na belasting na 6 maanden bij patiënten met een TIA of herseninfarct en een gestoorde glucosetolerantie. Als we met de MAAS trial kunnen bewijzen dat de behandeling met metformine en/of sitagliptine veilig en haalbaar is in het verlagen van glucose in het bloed, dan is een fase III onderzoek naar het effect van metformine en/of sitagliptine op vasculaire complicaties na een TIA of beroerte de volgende stap.

In **Hoofdstuk 6** heb ik de klinische en wetenschappelijke implicaties van de verschillende studies in dit proefschrift besproken. Nieuw-gediagnosticeerde gestoord glucose metabolisme na een TIA of beroerte komt vaak voor en is geassocieerd met atherosclerose. Het is daarom van belang om onderscheid te maken tussen voorbijgaande en blijvend gestoord glucose metabolisme. Toekomstig onderzoek moet gericht zijn op de behandeling van nieuw-gediagnosticeerde gestoord glucose metabolisme en de effecten van deze behandeling op het krijgen van opnieuw een beroerte. Daarnaast is een abnormaal nachtelijk dipping bloeddrukpatroon een interessante potentiële behandelbare risicofactor voor opnieuw een beroerte. Er is een trend naar een associatie tussen abnormale nachtelijke dipping bloeddrukpatronen en atherosclerose en ook hier moet onderzoek aantonen dat behandeling hiervan leidt tot een lager risico op beroerte.

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About the author

Susanne Fonville was born on March 13th, 1982 in Utrecht, The Netherlands. After finishing secondary school at the Sint Bonifatius College in Utrecht in 1999, she started Medical School at the Utrecht University. She obtained her medical degree in 2006.
From December 2006 until November 2007 she worked as a medical doctor at the Neurology department at the Onze Lieve Vrouwe Gasthuis in Amsterdam and in 2008 she worked at the Neurosurgery department in the University Medical Center Utrecht. Hereafter, she started in 2009 her residency in Neurology at the Department of Neurology at the Erasmus MC University Medical Center in Rotterdam (head prof. dr. P.A.E. Sillevis Smitt).
In October 2010 she started the research underlying this thesis at the Department of Neurology of the Erasmus MC University Medical Center under supervision of prof. dr. P.J. Koudstaal and dr. H.M. den Hertog. As part of her research training, she attended summer school in neurovascular diseases at the University of Debrecen (Hungary). From February 2013 she is continuing her training as a neurologist at the Erasmus MC University Medical Center in Rotterdam.
She currently lives both in Rotterdam and in Utrecht, together with her boyfriend Jan Martens.

List of publications

1. **Fonville S**, Zandbergen AAM, Koudstaal PJ, den Hertog HM. Pre-diabetes in patients with stroke or TIA: prevalence, risk and clinical management. *Accepted in Cerebrovascular Disease 2014.*

2. **Fonville S**, Zandbergen AAM, Vermeer SE, Dippel DWJ, Koudstaal PJ, den Hertog HM. Prevalence of pre-diabetes and newly-diagnosed diabetes in patients with a TIA or stroke. *Cerebrovascular Disease 2013; 36: 283-289.*

3. **Fonville S**, den Hertog HM, Zandbergen AAM, Koudstaal PJ, Lingsma HF. Occurrence and predictors of persistent impaired glucose tolerance after acute ischemic stroke or TIA. *Accepted in J Stroke Cerebrovascular Diseases 2014.*

4. **Fonville S**, van Dijk AC, Zadi T, van den Herik EG, Lingsma HF, Koudstaal PJ, van der Lugt A, den Hertog HM. Newly-diagnosed disturbed glucose metabolism and pre-existent diabetes are associated with atherosclerosis in patients with TIA or ischemic stroke. *Submitted.*

5. Van den Herik EG*, Struchalin MV*, **Fonville S**, de Lau LML, den Hertog HM, Koudstaal PJ, van Duijn CM. Associations between recently discovered genetic variations in metabolic traits and arterial stenosis in patients with recent cerebral ischemia. *Submitted.*

6. **Fonville S**, Verboon JC, van Dijk AC, van der Lugt A, Koudstaal PJ, van der Meiracker AH, den Hertog HM. Abnormal dipping blood pressure patterns: prevalence after TIA or ischemic stroke and association with atherosclerosis. *Submitted.*

7. **Fonville S**, Zandbergen AAM, Osei L, Brouwers PJAM, Mulder LJMM, Witteveen M, Dippel DWJ, Koudstaal PJ, den Hertog HM. Metformin and sitAgliptin in patients with impAired glucose tolerance and a recent TIA or minor ischemic Stroke (MAAS): rationale and protocol for a multicenter, randomized, open-label phase II trial. *Submitted.*

8. Van Dijk AC, **Fonville S**, Zadi T, van Hattem AMG, Saiedie G, Koudstaal PJ, van der Lugt A. Association between arterial calcifications and nonlacunar and lacunar ischemic strokes. *Stroke 2014; 45(3): 728-733.*

9. **Fonville S**, van der Worp HB, Maat P, Aldenhoven M, Algra A, van Gijn J. Accuracy and inter-observer variation in the classification of dysarthria from speech recordings. *J Neurol 2008 255(10):1545-8.*

10. *Van der Worp HB,* **Fonville S**, Ramos LM, Rinkel GJ. Recurrent perimesencephalic subarachnoid hemorrhage during antithrombotic therapy. *Neurocrit Care 2009;10(2):209-12.*

PHD PORTFOLIO

SUMMARY OF PHD TRAINING AND TEACHING ACTIVITIES

Name PhD student
SUSANNE FONVILLE

Department
NEUROLOGY, ERASMUS MC UNIVERSITY MEDICAL CENTER ROTTERDAM

Research School
COEUR

PhD period
OCTOBER 2009 – JANUARY 2013

Promotor
PROF.DR. P.J. KOUDSTAAL

Copromotor
DR. H.M. DEN HERTOG

	Year	Workload (ECTS)
General Courses		
Course on SNP's and human diseases	2010	1.5
Introduction to clinical research (Nihes, Rotterdam, The Netherlands)	2011	0.9
Biostatistics for clinicians (Nihes, Rotterdam, The Netherlands)	2011	1.0
Regression analysis for clinicians (Nihes, Rotterdam, The Netherlands)	2011	1.9
Principles of epidemiologic data-analysis (Nihes, Rotterdam, The Netherlands)	2011	0.7
Course Good Clinical Practice (BROK, Rotterdam, The Netherlands)	2011	1.0
In depth Courses		
Neurovascular seminar (Amsterdam, The Netherlands)	2011	0.5
Summer school neurovascular diseases (Debrecen, Hungary)	2012	2.0
(Inter)national conferences		
European Stroke Conference (Hamburg, Germany); oral presentation "Prevalence of non-dipping blood pressure profile and its relationship with impaired glucose metabolism in patients with TIA or ischemic stroke" and 1 poster presentation	2011	2.0
European Stroke Conference (Lisbon, Portugal); 3 poster presentations and 1 e-poster presentation	2012	2.0
European Stroke Conference (London, United Kingdom); 1 poster presentation	2013	0.5
Wetenschappelijke vergadering Nederlandse Vereniging voor Neurologie (Nunspeet, The Netherlands); oral presentation "Afwijkende nachtelijke bloeddrukpatronen: prevalentie na TIA of herseninfarct en de associatie met atherosclerose"	2013	1.0
Teaching activities		
Supervising research projects of 4 medical students at Erasmus MC	2011-2012	6.0