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General introduction, study objectives and thesis-outline
Diabetes and cardiovascular disease
Type 2 diabetes is becoming a pandemic [1]. The prevalence of diabetes not only increases in western societies but also in developing countries [2]. Central in the pathogenesis of type 2 diabetes is reduced insulin-sensitivity of glucose-utilizing tissues (insulin resistance). Insulin resistance in its turn is strongly associated with mainly visceral-obesity. Both genetic and environmental factors are involved in diabetogenesis.
Diabetic subjects have an increased incidence of both macrovascular (coronary artery events, stroke, peripheral artery disease) and microvascular (nephropathy, retinopathy) disease. Multiple factors contribute to vascular damage, including direct toxic effects of high glucose levels to the vasculature, dyslipidemia and hypertension. Several large-scale intervention trials targeting these factors have shown that the incidence of cardiovascular disease in type 2 diabetic subjects can be significantly reduced [3,4,5].

South Asians, central obesity and type 2 diabetes
In western societies, subjects of South Asian descent have an increased prevalence of insulin resistance and type 2 diabetes [6,7]. Besides exogenous factors like excessive energy ingestion [8] and diminished physical activity [9], genetic factors clearly play a role. Compared to Caucasians, body composition is different in South Asians. South Asians have a preferential truncal (deep adipose tissue) fat distribution [10], and even at the same body mass index the amount of mainly visceral-fat is increased in South Asians [11]. Central obesity is associated with insulin resistance [7]. Compared to the relatively inactive superficial subcutaneous fat (primary compartment), deep subcutaneous and visceral fat (secondary compartment) is an active regulator of body metabolism [12].
Not only fat distribution is different in South Asians, adipose tissue function is also different [13]. The relationship between insulin sensitivity and adiposity is much stronger in South Asians than in Caucasians [10]. Even when central obesity has not (yet) developed, South Asian children have decreased insulin sensitivity compared to Caucasian children [14]. In South Asians, insulin resistance is already present at birth [15].
Several hypotheses try to explain the increased tendency to central obesity and the apparent adipose tissue dysfunction in South Asians. Chronic energy depletion and climatic influences may have favoured energy storage in the quickly mobilizable deep adipose tissue compartment, making fat metabolism
in South Asians more sensitive to fluctuations in energy supply (el-Nino hypothesis) [16]. Alternatively, differences in mitochondrial gene structure and function (favouring energy storage at the cost of heat production) might explain the tendency to central obesity in South Asians (the “mitochondrial efficiency hypothesis”) [17].

Besides its function as an energy storage pool, adipose tissue—especially the deep compartment—plays an important role in immune system maintenance [18-20]. A high burden of mainly gastrointestinal infectious diseases may have resulted in the tendency to prioritize deep visceral adipose tissue depots to meet the immediate demands of the immune system in the defeat of gastrointestinal pathogens, as postulated by the “variable disease selection hypothesis” [21].

**South Asians and cardiovascular disease**

Besides an increased prevalence of central obesity and type 2 diabetes, South Asians also have an increased incidence of cardiovascular disease, especially coronary artery disease and stroke. This is especially true for South Asian immigrants in western societies [22-23], but also for South Asians living in the Indian subcontinent [24]. Although classical risk factors like smoking, hypertension and hypercholesterolemia do contribute to cardiovascular morbidity in South Asians [25], they are not excessive or more prevalent in comparison with Caucasians and hence do not account for the higher rate of cardiovascular disease. Moreover, the higher prevalence of type 2 diabetes in South Asians also fails to fully explain the excessive cardiovascular morbidity [26]. Several other potential cardiovascular risk factors have been studied, including hemostatic factors, inflammatory parameters and metabolic factors. South Asians have higher levels of plasminogen activator inhibitor 1 (PAI-1), fibrinogen and homocysteine, indicating an increased thrombotic tendency [27-29]. South Asians also have higher levels of high-sensitivity C-reactive protein [30], lipoprotein(a) [31] and decreased levels of adiponectin [32]. However, all the above-mentioned factors were examined in cross-sectional studies, and prospective studies addressing their use in predicting cardiovascular disease are lacking.

Not only macrovascular complications are increased, South Asians with type 2 diabetes also have an increased rate of microvascular (renal and retinal) complications [33-35]. Type 2 diabetic South Asians have a 40-fold increased risk for end-stage diabetic nephropathy [36]. In non-diabetic South Asians, central obesity is an independent risk factor for albuminuria [37].
Central obesity, cardiovascular disease and the complement system

As recognized by the “variable disease selection hypothesis” (see above), adipose tissue function and immunity are closely related [18-20]. South Asian ancestors faced a high pressure of mainly gastrointestinal infectious diseases. This might have set the immune system at a higher level of activity, with adipose tissue as a potential mediator. Although protective when faced with infections, enhanced immune activity might turn out to be harmful with respect to cardiovascular disease in the context of excessive energy supply and diminished infectious pressure. Central obesity itself is associated with low grade inflammation, reflected by increased levels of C-reactive protein [27]. In South Asians however, complement factor C3 is even more closely related to the metabolic syndrome (a clustering of cardiovascular risk factors with central obesity as a central feature) than CRP [38], and gene expression and production of several complement factors has been described in mainly visceral adipose tissue [39,40].

The complement system is a key player in the innate immune response, and its involvement in cardiovascular disease is increasingly being recognized [41,42]. The complement system refers to a cascade of proteins which are involved in opsonisation of harmful particles such as bacteria and facilitate the inflammatory response by attraction of inflammatory cells and enhancing vascular permeability.

The complement system can be activated via three pathways: the classical pathway, the alternative pathway and the lectin pathway. These three pathways converge into a C3 convertase, and from this point the cascade follows a final common pathway leading to the formation of C5b-9, also known as the Membrane Attack Complex (MAC). The Membrane Attack Complex induces cell damage by creating pores in the cellular membrane leading to osmotic cell swelling and subsequent cell death, but sublytic effects have also been described [43].

The classical pathway is activated by immune complexes and involves factor 1, 2 and 4. The alternative pathway is dependent on the stabilisation of spontaneously hydrolysed C3. In addition, recent evidence suggests that properdin might act as the primary recognition molecule in the alternative pathway. The lectin pathway, which also involves factor 2 and 4, is activated when Mannan Binding Lectin (MBL) binds to carbohydrate residues present on various pathogenic surfaces. Activation products of the complement system have been detected in atherosclerotic plaques and in kidneys and urine of
diabetic subjects [44-46], and MBL has recently evolved as a cardiovascular and renal risk marker [47-49].

**Figure 1** the complement system

Complement activation occurs via the classical pathway (initiated by the binding of immunoglobulins to antigens), the lectin pathway (initiated by the binding of Mannose Binding Lectin (MBL) or ficolins to sugar residues), or the alternative pathway. The latter pathway is spontaneously activated by slow hydrolysis of C3. The alternative pathway also serves as an amplification loop for the classical and lectin pathway. Recent evidence suggests that binding of properdin to its ligand initiates the alternative pathway. All three pathways converge into a C3 convertase, and from this point the cascade follows a final common pathway leading to the formation of C5b-9, also known as Terminal Complement Complex (TCC) or Membrane Attack Complex (MAC), which induces damage to the cell membrane leading to cell death. Cleavage products of C3 and C5 facilitate the inflammatory response. (prof. T.E.Mollnes, reprinted with permission)
Scope of this thesis
The high rate of cardiovascular complications in type 2 diabetic South Asians, the above-mentioned relationship between central obesity and immune function and the participation of the complement system in vascular damage led us to explore the association of the complement system and vascular complications in type 2 diabetic South Asians. We hypothesized that the activity of the complement system is enhanced in South Asians compared to Caucasians and that this contributes to the increased susceptibility to vascular and renal complications in type 2 diabetic South Asians.

Study population
The studies were accomplished in a population of immigrants of South Asian descent living in The Hague and surroundings. The ancestors of these South Asians originally came from the Indian Subcontinent, in a circumscriptive area of North India called Uttar Pradesh, Uttarakhand and West-Bihar [50]. In the late 19th century, some 30.000 inhabitants of this region migrated to Suriname to work on the plantations. The independence of Suriname in 1975 and the unstable political climate afterwards led many South Asians to migrate to the Netherlands where they took residence mainly in The Hague, Rotterdam and Amsterdam. In the South Asian population in the Hague, type 2 diabetes was shown to be very common, with a prevalence of 40% in those over the age of 60 [51].

The studies described in this thesis are an extension of the previously conducted HINDINEF study [52]. The study population comprised 169 first-degree relatives of South Asian subjects with end-stage renal disease due to diabetic nephropathy, 161 first-degree relatives of type 2 diabetic South Asians without diabetic nephropathy, 43 subjects already known with diabetes but without nephropathy, and 92 “otherwise interested” South Asians (second-degree relatives and spouses). The total population consisted of 465 South Asians. As stated above [51], in this population having a first-degree relative with type 2 diabetes is common rather than exceptional. Out of these 465 subjects, 168 subjects had type 2 diabetes at baseline according to the ADA 2003 criteria. Between 2007 and 2009, follow-up data of these 168 type 2 diabetic subjects were collected. Out of these 168 type 2 diabetic subjects, 54 subjects also underwent vascular function testing. The results of this follow-up study are described in this thesis.
Figure 2  study population

Original HINDINEF study population (n=465)
- 169 first-degree relatives of type 2 diabetic South Asians with end-stage renal disease due to diabetes
- 43 subjects known with diabetes but without albuminuria
- 161 first-degree relatives of type 2 diabetic South Asians without albuminuria
- 92 "otherwise interested" South Asians (second-degree relatives, spouses)

168 South Asians with type 2 diabetes at baseline according to the ADA 2003 criteria
Cross-sectional studies in Chapter 2 and 3

100 randomly selected South Asians without diabetes
Chapter 2 and 3

134 subjects with available follow-up data
Prospective studies in chapter 2,3 and 6

34 subjects lost to follow-up

54 South Asians with type 2 diabetes undergoing vascular function testing
Chapter 7

Not related to the South Asian study population:
70 patients with various proteinuric renal disease
Chapter 5
Thesis outline

As the primary focus of this thesis is on the complement system, chapter 2 - 5 focus on the complement system as a determinant of cardiovascular and renal disease.

Chapter 2 consists of a cross-sectional study comparing levels of complement factor 3 and SC5b-9 –the soluble end product of complement activation- in South Asians and Caucasians and assessing the relation between C3, SC5b-9, Mannose-binding lectin (MBL) and renal damage as reflected by urinary albumin/creatinin ratio. In addition, in a prospective observational part the predictive value of complement factors for the occurrence of cardiovascular events (C3, SC5b-9) and progressive renal failure (C3, SC5b-9, MBL) is studied.

Chapter 3 is a prospective observational study assessing the relationship between Mannose-binding lectin, serum MBL levels and the occurrence of coronary artery events.

In chapter 4 and 5 we study the role of the complement system in the pathogenesis of progressive renal failure. The focus is on properdin, a promotor of the alternative pathway complement activation.

Chapter 4 describes experimental studies on the role of properdin in complement activation on cultured proximal tubular epithelial cells.

Chapter 5 assesses the association between urinary properdin excretion, intrarenal complement activation and renal function. This study was done in seventy patients with proteinuria, mainly Caucasians.

Chapter 6 is a prospective observational study assessing the predictive value of waist-to-hip ratio, a measure of central obesity, for cardiovascular events in type 2 diabetic South Asians.

Chapter 7 is a cross-sectional study addressing the relationship between plasma Connective Tissue Growth Factor (CTGF) – a pro-fibrotic cytokine – and vascular damage as assessed by vascular function testing. This study was performed in 54 type 2 diabetic South Asians.
References:

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