SOUTH ASIANS: A POPULATION WITH A DISADVANTAGEOUS METABOLIC PHENOTYPE

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EPIDEMIOLOGY OF CVD IN SOUTH ASIANS

The South Asian population originally descends from the Indian subcontinent (India, Pakistan, Bangladesh, Nepal and Sri Lanka) and comprises approximately 20% of the total world population. Currently, over 200,000 South Asians are living in the Netherlands, especially in The Hague. The burden and mortality of cardiovascular diseases (CVD) are significantly higher among both native and migrant South Asians in comparison to subjects of white Caucasian descent (1-3). The age standardized mortality rate for CVD is around 50% higher in South Asian countries than in Western countries (3). Furthermore, individuals are affected by CVD at a younger age and as a result India suffers the highest loss in potentially productive years of life due to cardiovascular deaths (2;4;5). In 2030, this loss is estimated to be ten times higher than in the United States, whereas the population size is only three times larger (2). CVD risk is also higher in migrant South Asians living in Western countries. Studies consistently show that the risk of CVD among South Asian immigrants is at least two-fold increased compared to native populations as well as other immigrant groups. In Canada, the prevalence of CVD among South Asian immigrants is 10.7%, compared to 5.4% and 2.4% for people from European and Chinese descent, respectively (4). Furthermore, South Asians in the UK show a 40-60% higher mortality rate from coronary heart disease compared to European whites (1;6;7). In addition, in all of these studies South Asian immigrants were affected at a younger age than control groups.

The exceptionally high CVD risk in South Asians poses a major health and socioeconomic burden. Therefore, it is important to gain more insight in the pathogenesis of CVD in this population. It is likely that at least part of the excess risk is explained by genetic factors, since both South Asians in native countries as well as migrated South Asians are at increased risk. In addition, environmental factors, such as changes secondary to urbanization and migration, may play a role. Indeed, the risk of CVD appears to increase as South Asians move from rural India to urban India to immigrant populations (8).

When considering underlying causes for the increased CVD risk in the South Asian population, the pathogenesis of CVD should be discussed first.

PATHOGENESIS OF CARDIOVASCULAR DISEASE

The major cause of CVD is atherosclerosis, which is present many years before any clinical symptoms of CVD become manifest, including ischemic heart disease, cerebrovascular accident and peripheral arterial occlusive disease.

Atherosclerosis development starts with endothelial damage and dysfunction, often promoted by inflammatory mediators or shear stress induced by nonlaminar blood flow. This results in enhanced recruitment of inflammatory leukocytes such as monocytes and T-lymphocytes towards the damaged site, and migration of monocytes into the subendothelial intima followed by transformation into macrophages. At the same time, low-density lipo-

protein (LDL) particles may infiltrate into the vessel wall and become oxidized (e.g. due to release of reactive oxygen species or cigarette smoke). Macrophages within the vessel wall can take up oxidized LDL via receptors such as scavenger receptor A (SRA) and CD36, and become lipid-laden foam cells (9). What follows is an inflammatory status in which leukocytes and local endothelial cells excrete pro-inflammatory cytokines, including interferon γ (IFN-γ), tumor-necrosis factor-α (TNF-α) and growth factors, further stimulating leukocyte recruitment, accumulation of macrophages as well as proliferation of smooth muscle cells in the vascular intima, which produce elastin and collagen (10). This all sequentially leads to plaque formation, plaque expansion and formation of a fibrous cap. High-density lipoprotein (HDL) supposedly has an atheroprotective role, primarily by removing cholesterol from atherosclerotic plaques and transporting it back to the liver for excretion via the bile. Furthermore, it prevents LDL from oxidation and has anti-inflammatory properties (11).

From the above-mentioned pathophysiology it becomes clear that the development of atherosclerosis may be promoted by metabolic as well as inflammatory risk factors. Metabolic or 'classical' risk factors include dyslipidemia (marked by elevated LDL-C and decreased HDL-C levels), hypertension (resulting in nonlaminar blood flow), and smoking (resulting in endothelial dysfunction) (12). Furthermore, insulin resistance and central obesity are metabolic risk factors that are associated with increased CVD risk (13-15). Most of these classical cardiovascular risk factors are highly present in South Asians. In addition, although the precise mechanism is still under debate, also inflammatory or 'nonclassical' risk factors may contribute to development of CVD. Among these are systemic inflammation (marked by elevated C-reactive protein and/or TNF-α levels), as well as HDL dysfunction and endothelial dysfunction which can both give rise to inflammation (16).

Next, we will discuss the classical (metabolic) and nonclassical (inflammatory) risk factors for CVD in South Asian subjects.

CLASSICAL CVD RISK FACTORS IN SOUTH ASIANS

Dyslipidemia

Dyslipidemia, often comprising increased levels of LDL-C and triglycerides and decreased levels of HDL-C, is one of the main risk factors for CVD. South Asians were consistently shown to have higher triglyceride and lower HDL-C levels (7;17;18). Some studies also reported higher LDL-C levels in South Asian subjects compared to white Caucasians (4;19).

Obesity

South Asians have a disadvantageous fat distribution pattern with relatively thin extremities and higher abdominal adiposity (20;21). Furthermore, at a similar level of BMI, body fat percentage is higher in South Asians compared to white Caucasians (20;22). South Asians also have a tendency for higher deposition of fat within cells of non-adipose tissues such as

muscle and liver, so called "ectopic" sites. Petersen *et al.* (23) showed that in healthy lean South Asians hepatic triglyceride content was two-fold higher than in healthy lean white Caucasians. This higher triglyceride content was associated with hepatic insulin resistance and increased levels of pro-inflammatory cytokines. Storage of fat in these ectopic sites has a disruptive effect on glucose metabolism and it is now increasingly recognized that hepatic steatosis may be causally related to hepatic insulin resistance, the metabolic syndrome, systemic inflammation and even CVD (23-26).

Insulin resistance

Insulin resistance and elevated fasting glucose levels are more prevalent in non-diabetic South Asians compared to non-diabetic white Caucasians (7;18). In South Asians, the high rate of type 2 diabetes (T2D) is most striking. In 35-60 year old South Asian males living in the UK, diabetes prevalence was 16% compared with only 4% among European whites (7;18;27). Other studies, amongst which one conducted in the Netherlands, have reported an even higher prevalence of up to 25.4% for both South Asian men and women (28;29). Furthermore, the onset of diabetes is over 10 years earlier in South Asians (28), and diabetes occurs at a lower BMI compared to white Caucasians: the risk of developing T2D of a South Asian with a BMI of 21 kg/m^2 is comparable to the risk of a white Caucasian with a BMI of 30 kg/m^2 (3;19). Finally, South Asians often develop more diabetes-related complications, such as diabetic nephropathy and retinopathy (30).

Thus, in South Asians a disadvantageous metabolic profile consisting of central obesity, insulin resistance, and dyslipidemia, is highly prevalent. It is commonly assumed that an ethnic susceptibility towards a disturbed energy homeostasis *(e.g.* lower oxidation of glucose and fatty acids by mitochondria) might underlie this phenotype (31). In line with this, South Asian subjects have lower energy expenditure (32). As no efficient treatment is available for their disadvantageous phenotype, unravelling its cause is of great importance and may be beneficial in preventing, at least in part, the development of T2D and CVD in the South Asian population.

Excess risk

Studies have shown that after correction for the above-mentioned classical risk factors, ethnicity still remains an independent determinant of cardiovascular events in the South Asian population (1;4;7). Thus, residual risk is present suggesting that additional factors *(i.e.* nonclassical cardiovascular risk factors) may play a role. Aberrancies in several pathways may contribute to these nonclassical risk factors which are shortly summarized as 'inflammatory' factors. These factors will be shortly discussed in the following section; inflammation, HDL dysfunction and endothelial activation.

NON-CLASSICAL CVD RISK FACTORS IN SOUTH ASIANS

C-reactive protein

As mentioned above, inflammation is a well-recognized key player in the pathogenesis of atherosclerosis and may, therefore, be considered a risk factor for CVD. Besides promoting initiation of atherosclerosis development through monocyte attraction, it may lead to instability of the fibrous cap of the atherosclerotic plaque, resulting in rupture of the plaque and a subsequent cardiovascular event. C-reactive protein (CRP), which is synthesized by the liver in response to inflammatory factors released by macrophages and adipocytes (33;34), is a sensitive marker of inflammation (35). In a study of Chambers et al (17), CRP levels were found to be significantly higher in South Asians compared with Europeans even after adjustment for conventional risk factors such as age, smoking and body mass index, suggesting a chronic state of low grade inflammation in this population. However, in their study the difference in CRP levels was predominantly explained by greater central obesity and insulin resistance in South Asians. Visceral adipose tissue has been found to be a major source of cytokine release into the circulation (17;36). Intriguingly, the increased risk of CVD in South Asians was associated with a larger amount of visceral adipose tissue (37). Not only do South Asians have more visceral adipose tissue, their adipocytes appear to be more inflammatory as well. Several studies reported that South Asian adipocytes release higher levels of pro-inflammatory cytokines, such as CRP, interleukin 6 (IL-6) and TNF-α in comparison to white Caucasians (23;38), indicating a chronic inflammatory state.

HDL dysfunction

Multiple studies have consistently shown lower HDL-C levels in South Asians compared to white Caucasians (6;17;21;39-42). The cardiovascular protective effects of HDL have been attributed to several atheroprotective properties. Firstly, HDL stimulates cholesterol efflux from foam cells present in atherosclerotic plaques by acting as cholesterol acceptor and transporting cholesterol back to the liver for excretion into the bile (43). Secondly, HDL prevents LDL from oxidation (44-46). Thirdly, HDL has anti-inflammatory properties; during the early phase of atherosclerosis development, HDL may prevent leukocyte adhesion to endothelial cells by lowering expression of monocyte chemotactic protein 1 (MCP-1) and vascular cell adhesion molecule (VCAM-1) and by counteracting platelet-activating factor (PAF) induced adhesion of leukocytes (44-47). Fourthly, HDL induces vasodilatation through stimulation of nitric oxide (NO) release by endothelial cells (54). This results in lower endothelial shear stress and thereby slows down initiation of atherosclerosis development. Thus, dysfunction of HDL may not only directly aggrevate atherosclerosis development as a consequence of lower cholesterol uptake from the vascular wall, but also indirectly through induction of inflammation as well as endothelial dysfunction.

Recent evidence suggests that HDL functionality may be more importantly linked to CVD than plasma HDL-C levels *per se* (48;49). In trials that aimed at raising HDL-C levels, *e.g.* with dalcetrapib (50) or niacin (51) on top of statin, no decrease in the occurrence of cardio-

vascular endpoints was observed. Furthermore, several studies showed that HDL is dysfunctional in patients with coronary atherosclerosis, in men with cardiovascular risk factors, and in patients with an acute phase response after surgery (52-55).

Remarkably, little is known about HDL functionality in South Asians. To date only one cross-sectional, uncontrolled pilot study assessed the anti-oxidative capacity of HDL in South Asian immigrants living in the USA. They found dysfunctional HDL in 50% of the participants, which was significantly correlated with carotid intima media thickness, a surrogate marker of atherosclerosis (56). However, they lacked a control group of another ethnicity, so no statements could be made on the implication of this percentage for their increased risk of CVD. Future studies in this direction are, therefore, highly warranted.

Endothelial activation

Endothelial activation is regarded an important initiating factor in the pathogenesis of atherosclerosis and CVD (57). Endothelial activation is characterized by a proadhesion, proinflammatory, and procoagulatory milieu that favours all stages of atherogenesis. A hallmark of endothelial activation is a reduction in the bioavailability of endothelium-derived NO. An impaired NO-mediated vasodilatory response has been demonstrated in patients with cardiac risk factors or established atherosclerosis (58;59). Furthermore, the degree of impairment is related to the severity and extent of coronary artery disease (60).

Interestingly, previous studies have demonstrated reduced flow-mediated vasodilatation in South Asians compared to white Caucasians, pointing to endothelial activation and lower NO bioavailability (27;61). Of note, NO is mainly produced by the endothelium as a consequence of an interaction with HDL (62;63). Hence, besides a susceptibility for endothelial activation, dysfunctionality of HDL with respect to its ability to induce endothelial NO production is another possible explanation for the lower NO bioavailability in South Asians.

Circulating endothelial progenitor cells (EPCs), mobilized from the bone marrow, have an important role in the repair and regeneration of the endothelium (64-66). The number of circulating EPCs is lower in patients with established coronary artery disease, is predictive of future cardiovascular events, and is positively correlated with measures of endothelial function (67;68). Intriguingly, South Asians have lower circulating numbers of EPCs compared to white Caucasians, which may lead to a reduced capacity for endothelial repair (61;69). Furthermore, exercise-induced EPC mobilization was reduced in South Asian men (69). Interestingly, NO appears to be critical for EPC mobilization in response to exercise (69). Hence, the reduced exercise mediated EPC mobilization in South Asians may be secondary to their reduced NO bioavailability. Future studies should be directed at further investigating endothelial activation in this population and at developing strategies that enhance EPC mobilization by augmenting NO bioavailability.

CONCLUDING REMARKS

In conclusion, South Asians are more liable to develop CVD at an early age, and classical risk factors associated with CVD, including dyslipidemia, central obesity and insulin resistance, are more prevalent in this ethnicity compared to subjects of white Caucasian origin. The underlying cause for this highly prevalent disadvantageous metabolic phenotype is currently unknown. Of note, these 'metabolic' risk factors seem to account for only part of the increased risk in South Asians as ethnicity remains an independent determinant of cardiovascular events. Nonclassical 'inflammatory' risk factors, i.e. higher levels of inflammation, HDL dysfunction, and endothelial activation may be involved in the residual CVD risk in South Asians (see **FIGURE 1)**, although the presence of these risk factors requires further investigation as it has not always been properly studied. Furthermore, which of these factors plays a dominant role and is therefore the most promising therapeutic target to lower the excess CVD risk in South Asians remains to be investigated.

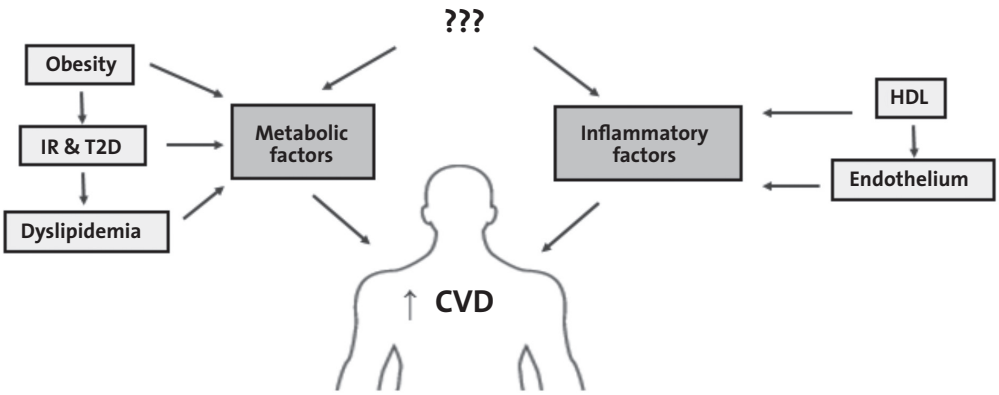

FIGURE 1 - **Proposed underlying mechanisms in the high cardiovascular risk in the South Asian population.** Classical (metabolic) risk factors, i.e. central obesity, insulin resistance and type 2 diabetes, and dyslipidemia are highly prevalent in the South Asian population. Furthermore, dysfunctional HDL and endothelium may enhance inflammation and these nonclassical risk factors may contribute to the 'residual' CVD risk of the South Asian population. Whether the high prevalence of metabolic and inflammatory factors in the South Asian population is due to a common (disadvantageous) factor remains to be determined. *CVD,* cardiovascular disease; *IR,* insulin resistance; *HDL,* high-density lipoprotein; *T2D,* type 2 diabetes.

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OUTLINE OF THESIS

As is evident from **CHAPTER 1** and **2** described in the first part of the thesis, brown adipose tissue (BAT) is a recently identified player in energy metabolism in human adults and a promising new target to treat obesity and related diseases. Interestingly, as described in **CHAPTER 3**, South Asians have lower energy expenditure, which may thus theoretically be caused by a reduction in BAT activity. Therefore, the studies of which the results are described in this thesis were aimed at 1) gaining more insight in the physiology of BAT, 2) identifying novel targets that may activate BAT, and 3) investigating the involvement of BAT in metabolism in humans with a focus on potential differences between South Asians and white Caucasians.

In the second part of this thesis, the role of BAT in metabolism and obesity is investigated using mouse studies. Since fatty acids (FA) are the main fuel for thermogenesis in BAT, and intracellular FA stores rapidly diminish upon BAT activation, BAT is required to take up FA from the plasma. It has been previously suggested that BAT takes up FA by uptake of whole lipoproteins *(i.e.* chylomicrons and VLDL), but this is in contrast to selective TG-derived FA uptake as exerted by white adipose tissue and muscle. Therefore, in **CHAPTER 4**, we aimed to investigate the mechanism by which BAT takes up lipoprotein-TG-derived FA by performing kinetic studies using glycerol tri[^{3}H]oleate and [^{14}C]cholesteryl oleate double-labeled lipoprotein-mimicking particles. Next, we focused on novel tools and targets that may activate BAT, thereby enhancing clearance of plasma TG and increasing energy expenditure. Based on the discovery that BMP7 can activate BAT, in **CHAPTER 5**, we investigated the mechanism by which BMP7 activates BAT, with focus on the role of the sympathetic nervous system, by treating high-fat fed lean mice and diet-induced obese mice with BMP7 under regular room temperature and thermoneutral temperature. In **CHAPTER 6**, the mechanism by which the anti-diabetic drug metformin lowers plasma TG was investigated. To this end, we performed VLDL-TG production and TG clearance experiments in dyslipidemic APOE*3-Leiden.CETP transgenic mice, as well as mechanistic studies *in vitro* using a brown adipocyte line, and put special focus on activation of intracellular AMPK. In **CHAPTER 7**, we investigated whether the TG-lowering effect of systemic cannabinoid 1 receptor blockade that was previously found in patients treated with rimonabant was mediated by activation of BAT in diet-induced obese APOE*3-Leiden.CETP transgenic mice. We further explored the underlying mechanism by performing experiments at thermoneutrality as well as using a strictly peripheral cannabinoid 1 receptor blocker. The brain is an important activator of BAT, especially in case of cold exposure. In **CHAPTER 8** we aimed to gain more insight in mediators that modulate BAT activity via the brain by studying the role of the melanocortin system on BAT function. To this end, we antagonized the central melanocortin 3 and 4 receptor in APOE*3-Leiden.CETP mice and studied BAT function and activity. To investigate whether BAT activation could protect against atherosclerosis development, in **CHAPTER 9**, we treated dyslipidemic APOE*3-Leiden.CETP mice with the ß3-adrenergic agonist CL316243 and studied energy expenditure, lipid metabolism and atherosclerosis development.

In the third part of the thesis, human studies on the role of BAT in metabolism and

obesity are described. A well-known cause of obesity is long term high-fat feeding, which may result in development of insulin resistance and eventually type 2 diabetes. To gain more insight in underlying mechanisms responsible for the development of high-fat diet induced insulin resistance, in **CHAPTER 10** we studied the effects of short-term high fat feeding on macrophage markers in skeletal muscle in healthy male subjects. The South Asian population is especially prone to develop obesity and related disorders, such as type 2 diabetes and cardiovascular disease (CVD). In **CHAPTER 11**, we investigated whether the high CVD risk in the South Asian population may be due to an ethnic susceptibility to develop endothelial activation. To this end, we measured markers for endothelial activation in cord blood of South Asian and white Caucasian neonates. In **CHAPTER 12**, we investigated whether HDL dysfunction may be present in the South Asian population by measuring different measures of HDL functionality in 3 cohorts of South Asian subjects and matched white Caucasian subjects *(i.e.,* neonates, adolescents and adults). The highly prevalent disadvantageous metabolic phenotype consisting of obesity, dyslipidemia and insulin resistance likely also underlies the high CVD risk in the South Asian population. Therefore, in **CHAPTER 13** we investigated whether a lower BAT volume or activity may be present in the South Asian population by performing cold-induced ^{18}F-FDG PET-CT scans in healthy lean Dutch South Asian and matched white Caucasian subjects. Since the ^{18}F-FDG PET-CT scan is currently the 'gold standard' to determine BAT volume and activity, but its use is limited by cost and radiation exposure, in **CHAPTER 14** we investigated whether supraclavicular skin temperature, the location at which most BAT is located, may serve as a quantitative measure of ^{18}F-FDG uptake in human subjects by use of wireless iButtons.

Finally in the fourth part of the thesis, the results from these studies and their implications are discussed in **CHAPTER 15** and summarized in **CHAPTER 16**.