8 High prevalence of cardiovascular disease in South Asians: central role for brown adipose tissue?

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ABSTRACT

Cardiovascular disease is the leading cause of death in modern society. Interestingly, the risk of developing cardiovascular disease varies between different ethnical groups. A particularly high risk is faced by South Asians, representing over one fifth of the world's population. Here, we review potential factors contributing to the increased cardiovascular risk of the South Asian population and discuss novel therapeutic strategies based on recent insights. In South Asians, classical ('metabolic') risk factors associated with cardiovascular disease are highly prevalent and include central obesity, insulin resistance, type 2 diabetes and dyslipidemia. A contributing factor that may underlie the development of this disadvantageous metabolic phenotype is the presence of a lower amount of brown adipose tissue in South Asian subjects, resulting in lower energy expenditure and lower lipid oxidation and glucose uptake. As it has been established that the increased prevalence of classical risk factors in South Asians cannot fully explain their increased risk for cardiovascular disease, other non-classical risk factors must underlie this residual risk. In South Asians, the prevalence of 'inflammatory' risk factors including visceral adipose tissue inflammation, endothelial dysfunction and HDL dysfunction is higher compared to white Caucasians. We conclude that a potential novel therapy to lower cardiovascular disease risk in the South Asian population is to enhance brown adipose tissue volume or its activity in order to diminish classical risk factors. Furthermore, anti-inflammatory therapy may lower non-classical risk factors in this population and the combination of both strategies may be especially effective.

INTRODUCTION

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. The burden and mortality of cardiovascular disease are significantly higher among both native and migrant South Asians in comparison to subjects of white Caucasian descent.[1-3] In addition, South Asian individuals are affected 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] The exceptionally high cardiovascular disease risk in South Asians poses a major health and socioeconomic burden and gaining more insight in the pathogenesis of cardiovascular disease in this population is of great importance. Several studies show that in South Asians classical cardiovascular risk factors, including dyslipidemia, obesity, insulin resistance and type 2 diabetes are more prevalent. However, after correction for these classical risk factors, ethnicity remains an independent determinant of cardiovascular events.[1;4;6] Thus, residual risk is present suggesting that additional, non-classical, cardiovascular risk factors may play a role.

In this review, we discuss classical and non-classical risk factors for cardiovascular disease in subjects of South Asian origin and focus on potential pathophysiological pathways that might clarify the unexplained 'excess' risk for cardiovascular disease in this population. Furthermore, we discuss novel therapeutic strategies based on recent insights.

Epidemiology of cardiovascular disease in South Asians

The epidemiology of cardiovascular disease in South Asians has been studied extensively in countries with large South Asian immigrant populations. These studies consistently show that the risk of cardiovascular disease among South Asian immigrants is at least two-fold increased compared to native populations as well as to other immigrant groups. In Canada, the prevalence of cardiovascular disease among South Asian immigrants is 10.7%, compared to 5.4% and 2.4% for people from European and Chinese descent, respectively.[4] In the UK, South Asians show a 40-60% higher mortality rate from coronary heart disease compared to white Europeans.[1;6;7] Furthermore, in all of these studies South Asian immigrants were affected at a younger age than control groups. Importantly, cardiovascular disease risk is not solely increased in South Asian immigrants but also in native South Asian subjects as the age standardized mortality rate for cardiovascular disease is around 50% higher in South Asian countries as compared to Western countries.[3]

The higher risk of cardiovascular disease in South Asians most likely reflects interactions between genetic susceptibility and environmental factors, such as changes secondary to urbanization and migration. Indeed, the risk of cardiovascular disease

appears to increase as South Asians move from rural India to urban India to immigrant populations.[8] With urbanization and migration to Western environments the consumption of energy-rich diets markedly increases. In addition, energy expenditure decreases due to less physical activity, and exposure to stress increases. Acculturation is positively associated with coronary artery disease and type 2 diabetes in South Asian immigrants in the US.[9] Thus, migration itself could be an aggravating factor in the high cardiovascular disease risk of migrant South Asians. Most studies however, have not examined such variables, nor did they differentiate between first and second generation migration. We propose that future studies should include a more detailed migration history to examine what factors associated with migration may contribute to the increased cardiovascular disease risk of South Asians.

PATHOGENESIS OF CARDIOVASCULAR DISEASE

Initiation of atherosclerosis development

The major cause of cardiovascular disease is atherosclerosis, which is present many years before any clinical symptoms of cardiovascular disease 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.[10] Endothelial activation is characterized by a proadhesion, proinflammatory, and procoagulatory milieu that favours all stages of atherogenesis. 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 lipoprotein (LDL) particles may become oxidized (e.g. due to release of reactive oxygen species or cigarette smoke), resulting in accumulation of oxidized LDL within the vessel wall. Macrophages within the vessel wall engulf this oxidized LDL, and become lipid-laden foam cells.[11] What follows is an inflammatory status in which leukocytes and local endothelial cells excrete pro-inflammatory cytokines, including interferon γ (IFN-γ), tumour 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.[12] This all sequentially leads to plaque formation, plaque expansion and formation of a fibrous cap. High-density lipoprotein (HDL) has been attributed 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.[13] Secondly, HDL prevents LDL from oxidation.[14-16] Thirdly, HDL has anti-inflammatory properties; during

the early phase of atherosclerosis development, HDL prevents 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.[13-15;17] Fourthly, HDL induces vasodilation through stimulation of nitric oxide (NO) release by endothelial cells.[18] This results in lower endothelial shear stress (*i.e.* improved endothelial function) and thereby lower initiation of atherosclerosis development. The vasodilating effect also increases delivery of insulin to tissues that take up glucose.

Classical and non-classical cardiovascular disease risk factors

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-cholesterol and triglycerides, and decreased HDL-cholesterol levels), hypertension (resulting in nonlaminar oscillatory blood flow), and smoking (resulting in endothelial dysfunction).[19] Furthermore, central obesity and insulin resistance are metabolic risk factors that are associated with increased cardiovascular disease risk.[20-22] In addition, although the precise mechanism is still under debate, inflammatory or 'non-classical' risk factors may contribute to development of cardiovascular disease. 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.[23]

Role of brown adipose tissue in whole-body metabolism

Interestingly, brown adipose tissue (BAT) recently emerged as a novel player in energy metabolism in humans. In contrast to white adipose tissue that stores excess triglycerides as fat, BAT takes up glucose and triglyceride-derived fatty acids (FA) from the plasma and subsequently burns FA to generate heat by means of mitochondrial uncoupling, a process called thermogenesis.[24] BAT is physiologically distinct from white adipose tissue as it contains high numbers of mitochondria in order to provide high oxidative capacity and is densely innervated by the sympathetic nervous system. The latter makes sure that BAT is rapidly activated in case of a cold environment, resulting in generation of heat. BAT was long thought to be present only in neonates, as they have minimal shiver capacity due to their underdeveloped muscles, and that with increasing age BAT would gradually disappear. Only in 2009 it has been discovered by means of cold-induced ^{18}F-fluorodeoxyglucose (FDG) PET-CT scans that BAT is still present and functional in adults,[25-27] and that it is mainly located in the supraclavicular and paravertebral regions.[28]

The major involvement of BAT in whole-body metabolism appeared from pre-clinical studies in rodents. Decreasing BAT activity or removal of BAT in mice markedly increased plasma glucose and triglyceride levels as well as the development of obesity and insulin

resistance.[29;30] Furthermore, in humans, BAT activity was found to be inversely related with BMI and fat mass.[26] Hence, a low BAT volume or activity may aggravate development of dyslipidemia, obesity and type 2 diabetes, i.e. classical metabolic risk factors.

Next, we will discuss the classical (metabolic) and non-classical (inflammatory) risk factors for cardiovascular disease in South Asian subjects, and speculate on underlying mechanisms.

Classical cardiovascular disease risk factors in South Asians

Dyslipidemia

Dyslipidemia often is one of the main risk factors of cardiovascular disease. South Asians have consistently been shown to have higher triglyceride levels.[7;31;32] Some studies also reported higher LDL-cholesterol levels in South Asian subjects compared to Caucasians.[4;33] Furthermore, multiple studies have consistently shown lower HDL-cholesterol levels in South Asians compared to Caucasians, even in South Asian neonates,[7;32;34-39] and the low levels of HDL-cholesterol are inversely related to cardiovascular risk.[40-45]

Obesity

South Asians have a disadvantageous fat distribution pattern with relatively thin extremities and increased abdominal adiposity.[46;47] Furthermore, at a similar level of BMI, body fat percentage is higher in South Asians compared to Caucasians.[46;48] South Asians also have a tendency for deposition of fat within cells of non-adipose tissues such as muscle and liver, so called "ectopic" sites. Petersen *et al*[49] showed that in young healthy lean South Asians hepatic triglyceride content was two-fold higher than in healthy lean Caucasians. This higher triglyceride content was associated with insulin resistance and increased levels of pro-inflammatory cytokines. We and others also reported higher fat infiltration in the liver in adult South Asians compared to white Caucasians (Bakker *et al*, submitted data, and others[50]). Storage of fat in these ectopic sites has a disruptive effect on glucose metabolism and it is now increasingly recognized that hepatic steatosis, besides abdominal obesity, may be causally related with hepatic insulin resistance, the metabolic syndrome, systemic inflammation and even cardiovascular disease.[49;51-53]

Insulin resistance

Insulin resistance and elevated fasting glucose levels are more prevalent in non-diabetic South Asians compared to non-diabetic white Caucasians,[6;31] possibly as a consequence of increased obesity and ectopic fat deposition. Most striking is the high rate of type 2 diabetes in South Asians. In 35-60 year old South Asian males living in the UK, diabetes prevalence was 16% compared with only 4% among European whites.[32] Other studies have reported an even higher prevalence of up to 25.4% for both South Asian men and

women.[54] Furthermore, the onset of diabetes is over 10 years earlier in South Asians,[54] and diabetes occurs at a lower BMI compared to Caucasians: the risk of developing type 2 diabetes for a South Asian with a BMI of 21 kg/m2 is comparable to the risk of a white Caucasian with a BMI of 30 kg/m2.[3;33] Finally, South Asians develop diabetes-related complications, such as diabetic nephropathy and retinopathy, more often.[55]

Thus, in South Asians a disadvantageous metabolic profile, including dyslipidemia, obesity insulin resistance, and type 2 diabetes is highly prevalent. Up to recently, little was known about the pathophysiological mechanism that underlies this metabolic phenotype, but it was hypothesized that this may be explained by a disturbed energy homeostasis (i.e. lower oxidation of glucose and fatty acids by mitochondria) in South Asians as they have recently been shown to exhibit reduced fat oxidation during sub-maximal exercise as compared to Caucasians.[56]

Brown adipose tissue: central role in classical cardiovascular disease risk factors?

Recently, we demonstrated that Dutch South Asians adolescents have a markedly lower resting energy expenditure (-32%) than BMI-matched Dutch white Caucasians as well as less cold-induced non-shivering thermogenesis, both of which are consistent with lower BAT function. Indeed, cold-induced ^{18}F-FDG PET-CT scans revealed that South Asian adolescents have a markedly lower BAT volume (-34%) in comparison to white Caucasians.[57] Interestingly, the BAT volume correlated positively with thermoneutral resting energy expenditure, indicating that BAT contributes to energy expenditure even under non-cold-induced conditions. Therefore, it is likely that the reduced BAT activity causally contributes to the adverse metabolic phenotype of South Asians including dyslipidemia, obesity, insulin resistance and type 2 diabetes.

It is interesting to speculate on possible mechanisms that underlie the decreased BAT volume in South Asians. The underlying cause may be genetic, i.e. blunted expression of signalling molecules involved in BAT differentiation, environmental, i.e. clothing behaviour, central heating setting or eating pattern, or a combination of the two. Several key molecules have been shown to be importantly involved in BAT differentiation in rodents, including bone morphogenetic protein 7 (BMP7)[58] and NO[59]. We have previously measured BMP7 levels in several cohorts of South Asian subjects including neonates, and we consistently found increased rather than decreased BMP7 levels compared to Caucasians (unpublished data). Thus, decreased BAT differentiation in South Asians does not seem to be caused by impaired BMP7 availability. NO has been recently linked to BAT, as mice that lack the enzyme NO synthase, crucial for catalysing the conversion of L-arginine to NO, have fewer and smaller mitochondria in BAT and lower energy expenditure leading to obesity.[59] Interestingly, South Asians have reduced bioavailability of NO in comparison to white Caucasians.[60] Thus, an inborn reduction in NO bioavailability

might underlie the lower BAT volume in South Asians and is an interesting subject for future studies.

Taken together, as BAT is an important player in triglyceride and glucose clearance as well as in total energy expenditure, a low BAT volume may well contribute to the high prevalence of classical cardiovascular disease risk factors in South Asians, including dyslipidemia, obesity, insulin resistance and type 2 diabetes.

Non-classical cardiovascular disease risk factors in South Asians

Visceral adipose tissue inflammation

As mentioned above, inflammation is a well-recognized key player in the pathogenesis of atherosclerosis and may, therefore, be considered a risk factor for cardiovascular disease.[61] Besides promoting initiation of atherosclerosis development through monocyte attraction, inflammation 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,[62;63] is a sensitive marker of inflammation.[64] In a study of Chambers *et al*,[32] CRP levels were found to be significantly increased in South Asians compared with Caucasians even after adjustment for factors such as age, smoking and body mass index, suggesting a chronic state of low grade inflammation in this population. 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.[32;65] 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 visceral adipocytes release higher levels of pro-inflammatory cytokines, such as TNF-α and interleukin 6 (IL-6) in comparison to Caucasians,[49;66] which may contribute to increased cardiovascular disease risk. Indeed, a larger amount of visceral adipose tissue associates with the increased risk of cardiovascular disease in South Asians.[67] Of note, enhanced visceral adipose tissue inflammation may also be linked to the lower BAT volume in South Asian subjects, as TNF-α has been shown to induce brown adipocyte apoptosis and hamper BAT differentiation in pre-clinical models.[68]

HDL dysfunction

As described above, HDL has several anti-atherogenic properties and dysfunction of HDL may not only directly aggravate 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 cardiovascular disease than plasma HDL-cholesterol levels per se.[69;70] In trials that aimed at raising HDL-cholesterol levels with dalcetrapib or niacin on top of statin, no decrease in the occurrence of cardiovascular endpoints was observed compared to treatment with statins only.[71;72] In line with this, several studies showed that HDL is dysfunctional in patients with coronary atherosclerosis, in patients with an acute phase response after surgery, and in men with cardiovascular risk factors.[73-76]

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. Dysfunctional HDL was found in 50% of the participants, which was significantly correlated with carotid intima media thickness , a surrogate marker of atherosclerosis.[77] However, another ethnic control group was lacking in the study, so no statements could be made on the implication of this percentage for the South Asian population specifically. Future studies are needed to investigate whether the prevalence of HDL dysfunction is higher in South Asian compared to white Caucasian subjects, as this may contribute to the excess risk of cardiovascular disease in people of South Asian origin.

Endothelial activation

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.[78;79] Furthermore, the degree of impairment is related to the severity and extent of coronary artery disease.[80] NO not only has vasodilating properties, but also anti-platelet, anti-proliferative, and anti-inflammatory properties.[81;82] Thus, there is a close link between inflammation, HDL function, and endothelial function; HDL dysfunction may result in inflammation and endothelial activation and furthermore, endothelial activation could induce inflammation, resulting in a negative feedback loop. In addition, the vasodilating effect of NO is related to insulin resistance and type 2 diabetes: vasodilatation increases delivery of insulin to tissues that take up glucose, and, vice versa, insulin stimulates the release of NO from the endothelium.[83;84] Moreover, as NO is important for BAT development, a link between diminished NO availability and BAT function and thus energy metabolism may also exist.

Interestingly, signs of endothelial activation in South Asians are already present upon birth. We previously found that levels of E-selectin, a marker of endothelial activation, are elevated in South Asian neonates compared with Caucasian neonates.[35] In line with this, several studies demonstrated reduced endothelium-dependent vasodilatation in South Asians compared to white Caucasians, pointing to lower NO bioavailability.[85;86] Of note, NO is mainly produced by the endothelium as a consequence of an interaction with HDL.[81;87] The reduced NO bioavailability in South Asians may thus be a consequence

of endothelial activation *per se* or of dysfunctional HDL. Since release of NO from endothelium is also stimulated by insulin, the highly prevalent insulin resistance in South Asians may also contribute to lower NO availability.[83;84]

Circulating endothelial progenitor cells (EPCs), mobilized from the bone marrow, have an important role in the repair and regeneration of the endothelium.[88-90] 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.[91;92] Intriguingly, South Asians have lower circulating numbers of EPCs compared to Caucasians, which may lead to a reduced capacity for endothelial repair.[60;85] Furthermore, exercise-induced EPC mobilization was reduced in South Asian men.[60] Interestingly, NO appears to be critical for EPC mobilization in response to exercise.[60] Hence, the reduced exercise mediated EPC mobilization in South Asians is likely secondary to their reduced NO bioavailability. Future studies should be directed at developing strategies that enhance EPC mobilization by augmenting NO bioavailability.

CONCLUSIONS AND FUTURE DIRECTIONS

South Asians are more liable to develop cardiovascular disease at an early age, and classical risk factors associated with cardiovascular disease, including dyslipidemia, central obesity and insulin resistance, are more prevalent in this population. However, these 'metabolic' risk factors seem to account for only part of the increased risk in South Asians. We propose that non-classical 'inflammatory' risk factors, i.e. higher levels of (visceral adipose tissue) inflammation, HDL dysfunction, and endothelial activation are involved in the residual cardiovascular disease risk in South Asians (see **Figure 1**).

The pathophysiological mechanism for the high prevalence of classical cardiovascular disease risk factors has not been fully established, but may be due to a lower BAT volume in South Asians. This offers a promising new target for preventive and therapeutic interventions as increasing BAT volume or activity will enhance energy expenditure and subsequent clearance of lipids and glucose from the circulation, resulting in improvement of the disadvantageous metabolic phenotype of South Asians and possibly reducing cardiovascular disease risk. Indeed, we recently showed that activation of BAT by ß3-adrenergic stimulation, in a human-like mouse model of lipoprotein metabolism and atherosclerosis, markedly diminished atherosclerosis development (Berbée *et al*, unpublished). Possible options for increasing BAT volume or activity are cold exposure or medication. BAT can be recruited in human subjects following cold exposure resulting in lowering of fat mass.[93;94] Furthermore, we recently showed in a pre-clinical setting that the commonly used anti-diabetic drug metformin activates BAT, which is responsible for its lipid-lowering effect.[95] Moreover, since South Asians have been shown to exhibit

lower NO bioavailability, BAT function may be improved by targeting the NO pathway. As NO also has anti-inflammatory properties and is involved in EPC mobilization, this strategy may also lower inflammation and improve endothelial function and thereby lower the 'residual' cardiovascular disease risk in South Asians.

Thus, future studies should investigate the efficacy of treatment strategies that target both classical as well as non-classical risk factors in the South Asian population to lower cardiovascular disease risk. A combination of therapies that increases BAT activity (i.e. via cold exposure or medical strategies) and lower inflammation may be especially effective.

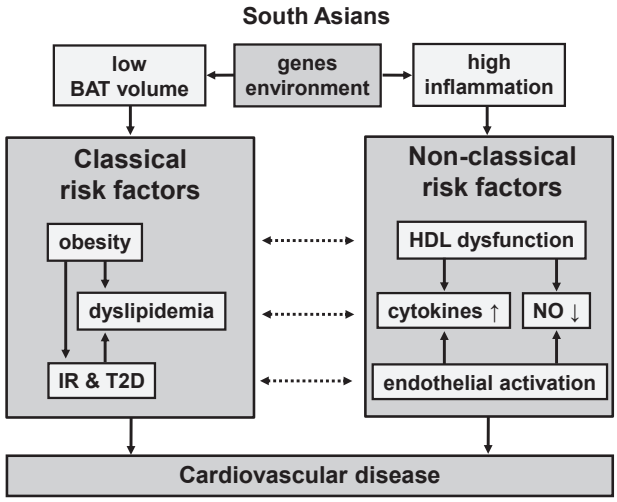

Figure 1. Proposed underlying mechanisms in the high cardiovascular risk in the South Asian population. Classical (metabolic) risk factors, i.e. dyslipidemia, obesity, insulin resistance (IR) and type 2 diabetes (T2D), are highly prevalent in the South Asian population. A low brown adipose tissue (BAT) volume might underlie this disadvantageous metabolic phenotype. In addition, non-classical (inflammatory) risk factors may contribute to the high cardiovascular disease risk in South Asians, such as HDL dysfunction, enhanced pro-inflammatory cytokine release and low nitric oxide (NO) availability, as well as endothelial cell activation. Visceral adipose tissue inflammation may link obesity and inflammation. The classical and non-classical risk factors are likely influenced by both genetic and environmental factors.

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