Insulin resistance and adipose tissue in the development of vascular diseases in high-risk patients

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Utrecht, Universiteit Utrecht, Faculteit Geneeskunde
Thesis University Utrecht - with a summary in Dutch
Proefschrift Universiteit Utrecht - met een samenvatting in het Nederlands

ISBN	978-90-9022775-7
Cover	Roy Sanders. Adapted from a photo 'Schuim aan zee' (Martijn Sikking). *Schuim staat hier symbool voor vetweefsel (adipose tissue)*.
Lay-out	Roy Sanders
Print	Gildeprint Drukkerijen B.V., Enschede, The Netherlands

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Insulineresistentie en vetweefsel bij het ontstaan van vaatziekten in hoog risicopatiënten

(met een samenvatting in het Nederlands)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht
op gezag van de rector magnificus, prof. dr. J.C. Stoof,
ingevolge het besluit van het college voor promoties
in het openbaar te verdedigen op

donderdag 6 maart 2008 des ochtends te 10.30 uur

door

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geboren op 10 mei 1979 te `s-Gravenhage

Promotor : Prof. dr. Y. van der Graaf

Co-promotor : Dr. F.L.J. Visseren

Financial support by the Netherlands Heart Foundation and the Dutch Diabetes Research Foundation for the publication of this thesis is gratefully acknowledged.

Financial support was also kindly provided by the Julius Centre for Health Sciences and Primary Care, Astellas Pharma B.V., AstraZeneca B.V., Boehringer Ingelheim B.V., Bristol-Myers Squibb B.V., Eli Lilly Nederland B.V., Genzyme Nederland, GlaxoSmithKline, LEO Pharma B.V., Merck Sharp & Dohme B.V., Novartis Pharma B.V., Novo Nordisk Farma B.V., Pfizer B.V., Philips Medical Systems, Sanofi-Aventis, Servier Nederland Farma B.V. and Schering-Plough B.V.

Manuscripts based on the studies presented in this thesis

Chapter 2

Gorter PM, Olijhoek JK, van der Graaf Y, Algra A, Rabelink TJ, Visseren FLJ, for the SMART Study Group. Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm.
Atherosclerosis. 2004;173:363-369.

Chapter 3

Gorter PM, van der Graaf Y, Visseren FLJ, on behalf of the SMART Study Group. Advanced vascular damage due to insulin resistance or components of metabolic syndrome in patients with manifest arterial disease without known diabetes? The SMART study.
Submitted.

Chapter 4

Gorter PM, Visseren FLJ, Algra A, van der Graaf Y, on behalf of the SMART Study Group. The impact of site and extent of clinically evident cardiovascular disease and atherosclerotic burden on new cardiovascular events in patients with type 2 diabetes. The SMART study.
Diabet Med. 2007;24:1352-1360.

Chapter 5

Gorter PM, Visseren FLJ, Moll FL, van der Graaf Y, on behalf of the SMART Study Group. Intra-abdominal fat and metabolic syndrome are associated with larger infrarenal aortic diameters in patients with manifest arterial disease. The SMART study.
In revision J Vasc Surg.

Chapter 6

Gorter PM, van Lindert ASR, de Vos AM, Meijs MFL, van der Graaf Y, Doevendans PAFM, Prokop M, Visseren FLJ. Quantification of epicardial and peri-coronary fat using cardiac computed tomography; reproducibility and relation with obesity and metabolic syndrome in patients suspected of coronary artery disease.
Atherosclerosis. 2007 Sep 18;[Epub ahead of print].

Chapter 7

Gorter PM, de Vos AM, van der Graaf Y, Stella PR, Doevendans PAFM, Meijs MFL, Prokop M, Visseren FLJ. Epicardial and peri-coronary fat in relation to coronary atherosclerosis and coronary artery calcification in patients undergoing coronary angiography.
Submitted.

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1

General introduction

Cardiovascular disease continues to impose a heavy burden on the Western world because of its associated morbidity and mortality[1]; the incidence and prevalence of cardiovascular diseases are expected to increase over the next 20 years.[2] The underlying pathology of cardiovascular disease is usually atherosclerosis, which has different stages starting with endothelial dysfunction, endothelial injury and vessel wall inflammation.[3,4] Atherosclerotic vascular diseases can become clinically manifest at several sites in the arterial tree leading to cerebrovascular disease, coronary heart disease, peripheral arterial disease or abdominal aortic aneurysm. Well-established risk factors are dyslipidemia, hypertension, hyperglycemia, smoking, and abdominal obesity.[5] Patients with cardiovascular disease or type 2 diabetes mellitus are particularly prone to develop (new) cardiovascular events.[6,7] Worldwide, the number of patients with type 2 diabetes is expected to at least double between now and 2030.[8] The increased burden of cardiovascular disease[1] and type 2 diabetes[8] can partly be attributed to the significant increase in the prevalence of abdominal obesity and associated insulin resistance.[9-11] In the Netherlands, as in most developed countries, the prevalence of overweight (BMI ≥25 kg/m^2) and obesity (BMI ≥30 kg/m^2) has clearly risen in adult males from respectively 37% and 4% in 1981 to 51% and 10% in 2004[12] and is now considered to be an important medical health problem.[13]

Insulin resistance and atherosclerotic vascular disease

Insulin resistance

Insulin is the key hormone for regulation of plasma glucose by activating the glucose uptake in various tissues including skeletal muscle and adipose tissue, and inhibiting the hepatic glucose production. In addition, insulin has stimulating effects on the lipogenesis and glycogen- and protein synthesis and inhibitory effects on glycogenolysis, lipolysis and protein breakdown. Insulin resistance is the result of impaired signalling in the intra-cellular transduction pathway downstream of the insulin receptor.[14,15] Consequently, this leads to an insufficient response of target organs resulting in decreased glucose uptake in adipocytes and skeletal muscle cells and an increased hepatic glucose production (*Figure 1*). Although factors such as genetic susceptibility, physical inactivity, and ageing appear to be involved, adipose tissue is recognized as the main driving force behind insulin resistance.[16] In case of adiposity, the release of free fatty acids and inflammatory cytokines (such as tumor necrosis factor-alpha (TNF-α) and interleukine-6 (IL-6)) from adipocytes and macrophages is increased, whereas the release of adiponectin, an anti-inflammatory adipocytokine solely derived from adipocytes, is reduced. This altered production of adipokines induces insulin resistance in adipose tissue, skeletal muscle cells, and the liver, particularly by inhibiting insulin-receptor mediated phosphorylation of intra-cellular proteins thereby impairing the insulin signalling cascade.[16-18]

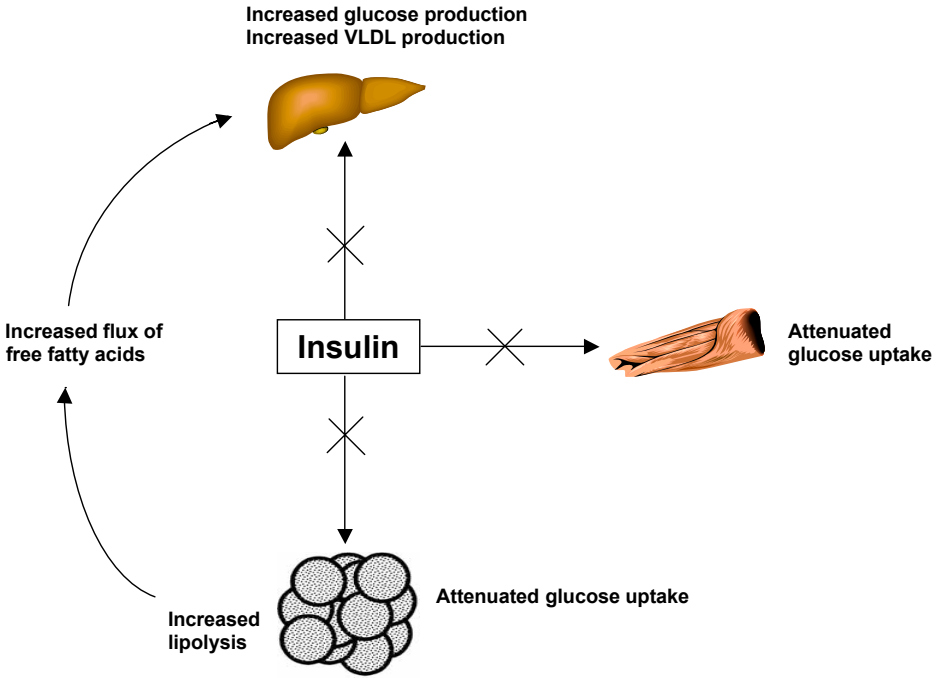

Figure 1. Insulin resistance

Insulin resistance and vascular consequences

Patients with high insulin resistance have an increased cardiovascular risk.[19,20] Although insulin resistance is an important factor in the development of endothelial dysfunction and progressive atherosclerotic vascular disease[21], the underlying mechanisms have not fully been determined. Insulin resistance is considered to be the major driver of interrelated vascular risk factors (e.g. hypertension, dyslipidemia, and hyperglycemia), and may thereby enhance the cardiovascular risk.[22-25] The clustering of several of these risk factors is often referred to as metabolic syndrome and is typically the result of obesity-induced insulin resistance (*Table 1*).[26] The metabolic syndrome is highly prevalent, approximately 24% in healthy adults[27], and is expected to become more prevalent due to the increasing prevalence of obesity.[12] Patients with metabolic syndrome have a two- to threefold higher risk of developing atherosclerotic vascular disease compared to those without metabolic syndrome.[28,29] This increased cardiovascular risk is largely attributed to the clustering of individual risk factors. However, metabolic syndrome was found to be associated with greater vascular damage than what was to be expected from the separate vascular risk factors[30], suggesting that other mechanisms might be involved. Several other mechanisms associated with insulin resistance (metabolic syndrome), such as low-grade inflammation, oxidative stress, hypercoagulability, and oxidized small-dense LDL-cholesterol, might contribute to the atherosclerotic process as well.[31-35]

Table 1. Most used definition of metabolic syndrome according to revised NCEP (ATP III) criteria

Combination of 3 or more of the following metabolic abnormalities:	
Abdominal obesity:	waist circumference >102 cm (men) or >88 cm (women)
High blood pressure:	≥130 mmHg systolic or ≥85 mmHg diastolic and/or medication
High fasting glucose:	fasting serum glucose ≥5.6 mmol/l and/or medication
Hypertriglyceridemia:	serum triglycerides ≥1.70 mmol/l
Low HDL-cholesterol:	serum HDL-cholesterol <1.04 mmol/l for men and <1.29 mmol/l for women

NCEP: National Cholesterol Education Program.
ATP III: Adult Treatment Panel III.

Moreover, in case of insulin resistance, elevated levels of pro-inflammatory adipocytokines and free fatty acids and decreased levels of adiponectin may directly cause endothelial dysfunction, by impairing vasodilatation, and stimulate smooth muscle cell proliferation thereby accelerating atherosclerosis.[34,36-38] Also, hyperinsulinemia, as a result of insulin resistance, has direct effects on the vascular endothelium by stimulating endothelin-1-mediated vasoconstriction.[34] A recent meta-analysis found hyperinsulinemia to be associated with increased cardiovascular mortality, independent of other risk factors.[39] Nevertheless, it remains unclear whether insulin itself accelerates atherosclerosis independently of insulin resistance. In most studies, insulin resistance was found to be associated with atherosclerotic vascular disease.[19,20,40] However, findings were inconsistent as to the independent role of insulin resistance in the atherosclerotic process.

In an insulin resistant pre-diabetic state, during which glucose concentrations are maintained near normal levels by compensatory hyperinsulinemia, the cardiovascular risk is already increased.[41] Type 2 diabetes can be seen as an end-stage of insulin resistance in which there is an inadequate compensatory production of insulin[42,43], meaning that pancreatic beta-cells fail to adapt to an increasing insulin resistance. Patients with type 2 diabetes have an even higher risk of cardiovascular events[44-46], which is almost as high as that of patients with vascular disease.[47,48] Moreover, cardiovascular disease is the most common cause of morbidity and mortality in diabetic patients.[49] Conceptually, several mechanisms underlie the increased cardiovascular risk in diabetic patients. Hyperglycemia does not appear to be the major determinant for the development of macrovascular disease in diabetic patients, as has been established in the UK Prospective Diabetes Study.[50] The high prevalence of cardiovascular risk factors[29] together with other risk factors associated with insulin resistance contribute to the accelerated atherosclerosis in patients with type 2 diabetes. Recent clinical trials showed that treatment directed at multiple risk factors (insulin resistance and/or vascular risk factors) reduces the cardiovascular risk in these patients.[51-53]

Adipose tissue and atherosclerotic vascular disease

Adipose tissue

Adipose tissue can be found in different parts of the body and can be classified into *subcutaneous* and *visceral adipose tissue*. Adipose tissue comprises many cells including adipocytes, preadipocytes, endothelial cells, macrophages, lymphocytes, mast cells and fibroblasts. Although different studies use different terminologies, the following terms are used most often to refer to various depots of adipose tissue. The term visceral adipose tissue refers to intra-abdominal fat. Fat accumulation within organs (e.g. heart, liver, and skeletal muscle) is typically called *ectopic fat*. *Perivascular fat*, also known as *periadventitial fat*, refers to the adipose tissue directly surrounding the arteries and the heart. Recently, two types of perivascular fat have gained more attention: fat along the major branches of the aorta (*periaortic fat*) and fat surrounding the heart (*pericardial fat*). Pericardial fat includes *epicardial adipose tissue* (*EAT*) and *paracardial fat* separated by the fibrous pericardium. The term *EAT* refers to the adipose tissue between the surface of the heart and the fibrous pericardium, and is mainly concentrated in the atrio- and interventricular grooves, along the coronary arteries, and over the right ventricle free wall and apex. *Peri-coronary fat* is defined as the adipose tissue between the surface of the heart and the fibrous pericardium that directly surrounds the coronary arteries.

Function of adipose tissue

The primary physiological role of adipose tissue is to take up free fatty acids from the blood after hydrolysis of triglycerides from triglyceride-rich lipoproteins by lipoprotein lipase and to release free fatty acids during the fasting state (adipocyte lipolysis). Intra-abdominal adipose tissue is currently recognized as an active endocrine and paracrine organ affecting many organs and tissues such as muscle and liver, rather than being solely a simple storage depot for free fatty acids.[54,55] Abdominal adipose tissue is capable of secreting large quantities of adipokines into the systemic circulation (hepatic portal circulation), which are involved in lipid metabolism (e.g. free fatty acids, cholesteryl ester transfer protein), glucose metabolism (e.g. adiponectin), blood pressure regulation and energy homeostasis. In addition, adipose tissue influences inflammation and hemostasis by producing pro-inflammatory cytokines (e.g. TNF-α, leptin, IL-6) and hemostatic factors (e.g. plasminogen activator inhibitor-1 (PAI-1)).
Perivascular fat is widely assumed to serve as a structural support of the vasculature. Only recently interest is going out to the physiological role of perivascular fat.[56,57] It has been proposed that perivascular fat itself might locally regulate vascular tone or might do so by modulating the systemic vasodilating effects of insulin.[57,58] Several studies in rats demonstrated that both periaortic and perimesenteric artery fat have inhibitory effects on vascular contraction, independent of endothelial-derived nitric oxide, by releasing vasoactive substances.[58-61] In addition, EAT may function as a local energy source for cardiomyocytes due to the fact that it has a greater capacity of free fatty acid release and uptake than other fat depots in guinea pigs.[62] Moreover, perivascular fat has the potential to locally secrete chemokines and cytokines and may therefore act as an endocrine and paracrine organ like abdominal fat.[63-65]

Abdominal fat and vascular diseases

Abdominal obesity contributes to chronic inflammation and is associated with the development of both insulin resistance (metabolic syndrome, type 2 diabetes) and atherosclerotic vascular disease.[9] Intra-abdominal fat accumulation causes altered adipocyte function, leading to high systemic plasma levels of TNF-α, IL-6, and free fatty acids, as well as low plasma concentrations of adiponectin, all involved in accelerating atherosclerosis.[37,54,66] Abdominal fat may lead to progressive atherosclerosis through the direct effect of adipocytokines (mainly decreased plasma levels of adiponectin and increased plasma levels of TNF-α) on the vascular endothelium and smooth muscle cells. Furthermore, in case of obesity, TNF-α and free fatty acids may induce insulin resistance in adipocytes, skeletal muscle cells and the liver through various mechanisms such as attenuating the insulin signalling cascade, stimulating adipocyte lipolysis, suppressing the inhibitory effect of insulin on adipocyte lipolysis and hepatic glucose production.[17,18,25] Adiponectin, an anti-inflammatory cytokine, decreases insulin resistance.[67] In insulin resistant obese patients, an increased flux of free fatty acids to the liver leads to the development of atherogenic dyslipidemia (elevated plasma concentration of triglycerides and secondary to that low HDL-cholesterol).[23] Furthermore, an imbalanced production of adipocytokines may promote systemic low-grade inflammation (elevated plasma concentrations of C-reactive protein, IL-6 and TNF-α) and decreased fibrinolytic capacities (elevated PAI-1 plasma concentrations) thereby accelerating the atherothrombotic process.[54,66] In the causal relation between abdominal fat and atherosclerosis it is not known what the relative contributions are of indirect effects through insulin resistance and the direct effects of adipocytokines on the vessel wall.

Perivascular fat and the development of vascular diseases

Atherosclerosis is considered to be a process driven primarily by systemic levels of risk factors, such as plasma lipids, blood pressure, and inflammatory cytokines. However, there is growing evidence that perivascular fat may also affect atherogenesis by the local release of a large number of pro-inflammatory factors that potentially impair vascular function and stimulate local inflammation.[56,57,68,69] Although nearly all arteries are surrounded by perivascular fat, particularly at sites predisposed to atherosclerosis, the role of perivascular fat in affecting vascular function has only very recently gained attention. It has been shown that periaortic fat is a source of cytokines and chemokines with potential chemotactic activity reflected by the accumulation of leukocytes at the interface between periaortic fat and the adventitia of atherosclerotic aortas.[65] Therefore, periaortic fat might contribute to chronic vascular inflammation and atherosclerosis thereby affecting the aortic wall.[65,69] Fat surrounding coronary arteries has the potential for local production of adiponectin and inflammatory cytokines (IL-6, TNF-α)[63,64] and was found to be associated with abdominal obesity.[70] Due to its proximity to coronary arteries[71] and its local production of pro-atherogenic adipocytokines[63,64], fat surrounding coronary arteries may contribute to an increased atherosclerosis and plaque instability from 'outside to inside'. In porcine coronary arteries *in vivo*, adventitial treatment with inflammatory mediators resulted in the migration of macrophages into the vascular wall and intima thickening.[72,73]

So far, the direct contribution of fat surrounding coronary arteries to the development of coronary atherosclerosis is subject of current research.[74,75]

Objectives

The objectives of the studies in this thesis are (1) to investigate the relation between presence of insulin resistance and advanced vascular damage in patients with manifest arterial disease, and the occurrence of (new) vascular events in insulin resistant patients with and without evident vascular disease (**chapters 2, 3 and 4**), and (2) to investigate the relationship between adipose tissue (abdominal fat and fat surrounding coronary arteries) and atherosclerotic vascular damage (**chapters 5, 6 and 7**).

Outline of this thesis

The prevalence of metabolic syndrome has been investigated in many populations, although not in patients with already manifest arterial disease. In **chapter 2** we describe the overall and gender-specific prevalence of metabolic syndrome and its components in patients with manifest arterial disease. The underlying pathophysiology of metabolic syndrome is not fully understood, however insulin resistance is considered to be an important determinant. Although both insulin resistance and metabolic syndrome are important factors in the development of cardiovascular diseases, there is uncertainty regarding the independent role of insulin resistance. In **chapter 3** we examined the relation between insulin resistance and metabolic disturbances and investigated whether advanced vascular damage in patients with manifest arterial disease without known diabetes can be explained by insulin resistance *per se*, or is mediated by the components constituting metabolic syndrome. Patients with type 2 diabetes and evident coronary heart disease have an excess risk of atherosclerotic vascular events. The aim of **chapter 4** was to compare the risk of new cardiovascular events between type 2 diabetic patients with cerebrovascular disease, coronary heart disease or peripheral arterial disease and between diabetic patients with a different number of sites clinically affected by vascular disease. Also, we investigated the additional influence of atherosclerotic burden, assessed as carotid intima-media thickness and albuminuria, on the relationship between clinically evident cardiovascular disease and new cardiovascular events in diabetic patients.

Intra-abdominal fat induces insulin resistance thereby stimulating the development of metabolic syndrome and cardiovascular disease. Intra-abdominal fat may also affect atherogenesis directly by an imbalanced production of pro- and anti-inflammatory adipocytokines. Inflammatory processes appear to be involved in the degeneration of the aortic wall. Therefore, intra-abdominal fat and metabolic syndrome may contribute to the process of aortic dilatation. In **chapter 5** we examined the association between intra-abdominal fat, as well as metabolic syndrome, and infrarenal aortic diameter in patients with manifest arterial disease. Perivascular fat directly surrounding the coronary arteries might aggravate coronary artery disease. To further clarify the possible relation between fat surrounding coronary arteries and coronary atherosclerosis, it is important to reliably quantify this adipose tissue. In **chapter 6** the

reliability of various measurements of EAT and peri-coronary fat using cardiac CT was investigated. Additionally, we examined whether EAT and peri-coronary fat measurements are associated with obesity and metabolic syndrome in patients undergoing coronary angiography. Aim of **chapter 7** was to determine the relation between EAT volume, as well as peri-coronary fat thickness, and the severity of coronary atherosclerosis and the extent of coronary artery calcification in patients undergoing coronary angiography. In **chapter 8** the main findings of the above studies are discussed. Lastly, a summary of the results presented in this thesis is given in **chapter 9**.

References

1. Braunwald E. Shattuck lecture--cardiovascular medicine at the turn of the millennium: triumphs, concerns, and opportunities. *N Engl J Med*. 1997;337:1360-1369.
2. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet*. 1997;349:1498-1504.
3. Ross R. The pathogenesis of atherosclerosis: a perspective for the 1990s. *Nature*. 1993;362:801-809.
4. Ross R. Atherosclerosis--an inflammatory disease. *N Engl J Med*. 1999;340:115-126.
5. Yusuf S, Hawken S, Ounpuu S et al. Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the INTERHEART study): case-control study. *Lancet*. 2004;364:937-952.
6. Drouet L. Atherothrombosis as a systemic disease. *Cerebrovasc Dis*. 2002;13 Suppl 1:1-6.
7. Diabetes mellitus: a major risk factor for cardiovascular disease. A joint editorial statement by the American Diabetes Association; The National Heart, Lung, and Blood Institute; The Juvenile Diabetes Foundation International; The National Institute of Diabetes and Digestive and Kidney Diseases; and The American Heart Association. *Circulation*. 1999;100:1132-1133.
8. Wild S, Roglic G, Green A et al. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care*. 2004;27:1047-1053.
9. Van Gaal LF, Mertens IL, De Block CE. Mechanisms linking obesity with cardiovascular disease. *Nature*. 2006;444:875-880.
10. Yusuf S, Hawken S, Ounpuu S et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet*. 2005;366:1640-1649.
11. Bogers RP, Bemelmans WJ, Hoogenveen RT et al. Association of Overweight With Increased Risk of Coronary Heart Disease Partly Independent of Blood Pressure and Cholesterol Levels: A Meta-analysis of 21 Cohort Studies Including More Than 300 000 Persons. *Arch Intern Med*. 2007;167:1720-1728.
12. Schokker DF, Visscher TL, Nooyens AC et al. Prevalence of overweight and obesity in the Netherlands. *Obes Rev*. 2007;8:101-108.
13. Kopelman PG. Obesity as a medical problem. *Nature*. 2000;404:635-643.
14. White MF. The insulin signalling system and the IRS proteins. *Diabetologia*. 1997;40 Suppl 2:S2-17.
15. Rhodes CJ, White MF. Molecular insights into insulin action and secretion. *Eur J Clin Invest*. 2002;32 Suppl 3:3-13.
16. Jazet IM, Pijl H, Meinders AE. Adipose tissue as an endocrine organ: impact on insulin resistance. *Neth J Med*. 2003;61:194-212.
17. Pittas AG, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. *J Clin Endocrinol Metab*. 2004;89:447-452.
18. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science*. 1993;259:87-91.
19. Bonora E, Kiechl S, Willeit J et al. Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in caucasian subjects from the general population: the Bruneck study. *Diabetes Care*. 2007;30:318-324.
20. Hanley AJ, Williams K, Stern MP et al. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio Heart Study. *Diabetes Care*. 2002;25:1177-1184.
21. Laakso M. Insulin resistance and coronary heart disease. *Curr Opin Lipidol*. 1996;7:217-226.
22. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system. *N Engl J Med*. 1996;334:374-381.
23. Ginsberg HN. Diabetic dyslipidemia: basic mechanisms underlying the common hypertriglyceridemia and low HDL cholesterol levels. *Diabetes*. 1996;45 Suppl 3:S27-30.
24. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet*. 2005;365:1415-1428.

25. Wassink AM, Olijhoek JK, Visseren FL. The metabolic syndrome: metabolic changes with vascular consequences. *Eur J Clin Invest*. 2007;37:8-17.
26. Grundy SM, Cleeman JI, Daniels SR et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;112:2735-2752.
27. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *JAMA*. 2002;287:356-359.
28. Lakka HM, Laaksonen DE, Lakka TA et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA*. 2002;288:2709-2716.
29. Isomaa B, Almgren P, Tuomi T et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001;24:683-689.
30. Scuteri A, Najjar SS, Muller DC et al. Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. *J Am Coll Cardiol*. 2004;43:1388-1395.
31. Sakkinen PA, Wahl P, Cushman M et al. Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. *Am J Epidemiol*. 2000;152:897-907.
32. Hulthe J, Bokemark L, Wikstrand J et al. The metabolic syndrome, LDL particle size, and atherosclerosis: the Atherosclerosis and Insulin Resistance (AIR) study. *Arterioscler Thromb Vasc Biol*. 2000;20:2140-2147.
33. Steinberg HO, Baron AD. Vascular function, insulin resistance and fatty acids. *Diabetologia*. 2002;45:623-634.
34. Wheatcroft SB, Williams IL, Shah AM et al. Pathophysiological implications of insulin resistance on vascular endothelial function. *Diabet Med*. 2003;20:255-268.
35. Reaven G. The metabolic syndrome or the insulin resistance syndrome? Different names, different concepts, and different goals. *Endocrinol Metab Clin North Am*. 2004;33:283-303.
36. Yudkin JS, Kumari M, Humphries SE et al. Inflammation, obesity, stress and coronary heart disease: is interleukin-6 the link? *Atherosclerosis*. 2000;148:209-214.
37. Goldstein BJ, Scalia R. Adipokines and vascular disease in diabetes. *Curr Diab Rep*. 2007;7:25-33.
38. Shioji K, Moriguchi A, Moriwaki S et al. Hypoadiponectinemia implies the development of atherosclerosis in carotid and coronary arteries. *J Cardiol*. 2005;46:105-112.
39. Hu G, Qiao Q, Tuomilehto J et al. Plasma insulin and cardiovascular mortality in non-diabetic European men and women: a meta-analysis of data from eleven prospective studies. *Diabetologia*. 2004;47:1245-1256.
40. Hedblad B, Nilsson P, Engstrom G et al. Insulin resistance in non-diabetic subjects is associated with increased incidence of myocardial infarction and death. *Diabet Med*. 2002;19:470-475.
41. Haffner SM, Mykkanen L, Festa A et al. Insulin-resistant prediabetic subjects have more atherogenic risk factors than insulin-sensitive prediabetic subjects: implications for preventing coronary heart disease during the prediabetic state. *Circulation*. 2000;101:975-980.
42. Kahn BB. Type 2 diabetes: when insulin secretion fails to compensate for insulin resistance. *Cell*. 1998;92:593-596.
43. Stumvoll M, Goldstein BJ, van Haeften TW. Type 2 diabetes: principles of pathogenesis and therapy. *Lancet*. 2005;365:1333-1346.
44. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care*. 1979;2:120-126.
45. Stamler J, Vaccaro O, Neaton JD et al. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care*. 1993;16:434-444.
46. Folsom AR, Rasmussen ML, Chambless LE et al. Prospective associations of fasting insulin, body fat distribution, and diabetes with risk of ischemic stroke. The Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Diabetes Care*. 1999;22:1077-1083.

47. Becker A, Bos G, de Vegt F et al. Cardiovascular events in type 2 diabetes: comparison with nondiabetic individuals without and with prior cardiovascular disease. 10-year follow-up of the Hoorn Study. *Eur Heart J*. 2003;24:1406-1413.
48. Haffner SM, Lehto S, Rönnemaa T et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med*. 1998;339:229-234.
49. Morrish NJ, Wang SL, Stevens LK et al. Mortality and causes of death in the WHO Multinational Study of Vascular Disease in Diabetes. *Diabetologia*. 2001;44 Suppl 2:S14-21.
50. Stratton IM, Adler AI, Neil HA et al. Association of glycaemia with macrovascular and microvascular complications of type 2 diabetes (UKPDS 35): prospective observational study. *BMJ*. 2000;321:405-412.
51. Gaede P, Vedel P, Larsen N et al. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med*. 2003;348:383-393.
52. Efficacy of atenolol and captopril in reducing risk of macrovascular and microvascular complications in type 2 diabetes: UKPDS 39. UK Prospective Diabetes Study Group. *BMJ*. 1998;317:713-720.
53. Dormandy JA, Charbonnel B, Eckland DJ et al. Secondary prevention of macrovascular events in patients with type 2 diabetes in the PROactive Study (PROspective pioglitAzone Clinical Trial In macroVascular Events): a randomised controlled trial. *Lancet*. 2005;366:1279-1289.
54. Sharma AM. Adipose tissue: a mediator of cardiovascular risk. *Int J Obes Relat Metab Disord*. 2002;26 Suppl 4:S5-7.
55. Ahima RS, Flier JS. Adipose tissue as an endocrine organ. *Trends Endocrinol Metab*. 2000;11:327-332.
56. Engeli S. Is there a pathophysiological role for perivascular adipocytes? *Am J Physiol Heart Circ Physiol*. 2005;289:H1794-1795.
57. Yudkin JS, Eringa E, Stehouwer CD. "Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet*. 2005;365:1817-1820.
58. Gollasch M, Dubrovska G. Paracrine role for periadventitial adipose tissue in the regulation of arterial tone. *Trends Pharmacol Sci*. 2004;25:647-653.
59. Lohn M, Dubrovska G, Lauterbach B et al. Periadventitial fat releases a vascular relaxing factor. *FASEB J*. 2002;16:1057-1063.
60. Soltis EE, Cassis LA. Influence of perivascular adipose tissue on rat aortic smooth muscle responsiveness. *Clin Exp Hypertens A*. 1991;13:277-296.
61. Verlohren S, Dubrovska G, Tsang SY et al. Visceral periadventitial adipose tissue regulates arterial tone of mesenteric arteries. *Hypertension*. 2004;44:271-276.
62. Marchington JM, Pond CM. Site-specific properties of pericardial and epicardial adipose tissue: the effects of insulin and high-fat feeding on lipogenesis and the incorporation of fatty acids in vitro. *Int J Obes*. 1990;14:1013-1022.
63. Mazurek T, Zhang L, Zalewski A et al. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation*. 2003;108:2460-2406.
64. Baker AR, Silva NF, Quinn DW et al. Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol*. 2006;5:1.
65. Henrichot E, Juge-Aubry CE, Pernin A et al. Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis? *Arterioscler Thromb Vasc Biol*. 2005;25:2594-2599.
66. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res*. 2005;96:939-949.
67. Weyer C, Funahashi T, Tanaka S et al. Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab*. 2001;86:1930-1935.
68. Eringa EC, Bakker W, Smulders YM et al. Regulation of vascular function and insulin sensitivity by adipose tissue: focus on perivascular adipose tissue. *Microcirculation*. 2007;14:389-402.

69. Stern N, Marcus Y. Perivascular fat: innocent bystander or active player in vascular disease? *J Cardiometab Syndr*. 2006;1:115-120.
70. Iacobellis G, Ribaudo MC, Assael F et al. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. *J Clin Endocrinol Metab*. 2003;88:5163-5168.
71. Prati F, Arbustini E, Labellarte A et al. Eccentric atherosclerotic plaques with positive remodelling have a pericardial distribution: a permissive role of epicardial fat? A three-dimensional intravascular ultrasound study of left anterior descending artery lesions. *Eur Heart J*. 2003;24:329-336.
72. Miyata K, Shimokawa H, Kandabashi T et al. Rho-kinase is involved in macrophage-mediated formation of coronary vascular lesions in pigs in vivo. *Arterioscler Thromb Vasc Biol*. 2000;20:2351-2358.
73. Shimokawa H, Ito A, Fukumoto Y et al. Chronic treatment with interleukin-1 beta induces coronary intimal lesions and vasospastic responses in pigs in vivo. The role of platelet-derived growth factor. *J Clin Invest*. 1996;97:769-776.
74. Chaowalit N, Somers VK, Pellikka PA et al. Subepicardial adipose tissue and the presence and severity of coronary artery disease. *Atherosclerosis*. 2006;186:354-359.
75. Jeong JW, Jeong MH, Yun KH et al. Echocardiographic epicardial fat thickness and coronary artery disease. *Circ J*. 2007;71:536-539.

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Atherosclerosis. 2004;173:363-369.

Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm

Abstract

Metabolic syndrome patients are at increased risk for developing cardiovascular morbidity and mortality. The increasing prevalence of the metabolic syndrome in various asymptomatic populations has been well documented, however, limited information is available about the prevalence in manifest atherosclerotic vascular disease patients. The aim of this study is to determine the overall and gender-specific prevalence of the metabolic syndrome and its components in these patients. This cross-sectional survey of 1117 patients, aged 18-80 years, mean age 60 ± 10 years, comprised patients with coronary heart disease (n = 527), cerebrovascular disease (n = 258), peripheral arterial disease (n = 232) or abdominal aortic aneurysm (n = 100). Metabolic syndrome was defined by Adult Treatment Panel III. The prevalence of the metabolic syndrome in the study population was 46%: 58% in PAD patients, 41% in CHD patients, 43% in CVD patients and 47% in AAA patients. Overall, women had a higher prevalence than men (56% versus 43%). Age did not influence the metabolic syndrome prevalence; crude odds ratios (crude OR) 1.00 (95% CI: 0.99-1.02). Our results demonstrate a high prevalence of the metabolic syndrome in patients with manifest atherosclerotic vascular disease. Screening for metabolic syndrome in patients with high risk for new vascular incidents may identify patients with even higher vascular risk and may direct anti-atherosclerotic treatment in order to prevent new vascular incidents in the same or another vascular bed.

Introduction

Patients with metabolic syndrome have a high risk of developing cardiovascular morbidity and mortality.[1-4] The combination of risk factors comprising the metabolic syndrome interacts synergistically causing or accelerating the progression of atherosclerosis.[1] In patients with metabolic syndrome, a two- to three-fold increased risk of coronary heart disease (CHD) and stroke and cardiovascular mortality has been reported.[1,2,4,5] Several studies evaluated the presence of the metabolic syndrome in different populations. Reported prevalences of the metabolic syndrome in healthy subjects vary between 9 and 22% (24% after age adjustment).[2,6,7] A study in drug-treated hypertensive patients revealed a prevalence varying from 0.8 to 35.3% depending on the definition used.[8] In nearly 80% of the patients with type 2 diabetes mellitus, the metabolic syndrome was present.[1] Variability in the study populations and the use of different diagnostic criteria for the metabolic syndrome are, at least in part, reasons for differences in prevalence rate.[2,6-9]

Although several studies evaluated the prevalence of the metabolic syndrome in different populations without a history of cardiovascular diseases, limited information is available about the prevalence in patients with different clinical manifestations of atherosclerotic vascular disease. Screening for metabolic syndrome in an already high-risk population may help to identify patients with even higher risks for vascular complications and may direct therapy. It is therefore the aim of the current cross-sectional study to assess the overall and gender-specific prevalence of the metabolic syndrome and its components in a cohort of patients with different clinical manifestations of atherosclerotic vascular disease.

Methods

Study settings, participants and design

The Second Manifestations of ARTerial disease (SMART) study, initiated in 1996, is an ongoing, single-centre, prospective cohort study. Patients who are newly referred to the University Medical Centre Utrecht with clinical manifest atherosclerotic vascular disease (coronary heart disease, cerebrovascular disease (CVD), peripheral arterial disease (PAD) or abdominal aortic aneurysm (AAA)) or with marked risk factors for atherosclerosis (hyperlipidemia, type 1 diabetes, type 2 diabetes or hypertension) are included. Patients are non-invasively screened for (asymptomatic) atherosclerosis and for vascular risk factors. Not enrolled in the study are patients above 80 years or those with terminal malignant disease. The Medical Ethics Committee has approved the study and all subjects gave their written informed consent before participating in the study. The main objectives of the SMART study are to determine the prevalence of additional vascular disease and of risk factors for atherosclerosis and to study the incidence of future cardiovascular events. A detailed description of the study design and the criteria used to define the different manifest atherosclerotic vascular diseases was published previously.[10]

For the present study, a cross-sectional evaluation, analyses were based on the screening period of 1 January 1999 to 1 July 2002 and were limited to patients for whom complete data for assessment of the metabolic syndrome were available. A total of 1117 patients, aged 18-80 years, were included. They were newly referred to our hospital and diagnosed with coronary heart disease (n = 527), cerebrovascular disease (n = 258), peripheral arterial disease (n = 232) or abdominal aortic aneurysm (n = 100). CHD includes angina pectoris and myocardial infarction; CVD includes transient ischemic attack, cerebral infarction, amaurosis fugax, and retinal infarction; PAD includes symptomatic and documented obstruction of distal arteries of the leg (Fontaine II and III); AAA includes abdominal aortic aneurysm.

Data collection

At inclusion, patients were asked to complete a standardised health questionnaire covering medical history, symptoms of cardiovascular disease and risk factors, current and former smoking habits, presence of vascular diseases in first degree relatives and information about the use of current medical treatment. All patients entering the SMART study had to undergo a standardised diagnostic protocol including physical examination (weight, length, waist circumference, systolic and diastolic blood pressure) and laboratory tests to determine the lipid profile (serum triglycerides, serum total cholesterol, serum HDL-cholesterol and fasting serum glucose). The laboratory techniques used have been published previously.[10] BMI was calculated as weight in kilograms divided by the square of height in meters.

Definitions

The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III or ATP III) published criteria for the diagnosis of the metabolic syndrome.[11]

Participants having three or more of the following abnormalities were defined as having the metabolic syndrome:

1. *Abdominal obesity*: waist circumference >102 cm in men or >88 cm in women.
2. *High blood pressure*: ≥130 mmHg systolic or ≥85 mmHg diastolic.
3. *Hypertriglyceridemia*: serum triglycerides ≥1.70 mmol/l (150 mg/dl).
4. *Low HDL-cholesterol*: serum HDL-cholesterol <1.04 mmol/l (40 mg/dl) in men or <1.29 mmol/l (50 mg/dl) in women.
5. *High fasting glucose*: fasting serum glucose ≥6.1 mmol/l (110 mg/dl).

Subjects who did not meet the ATP III criteria for high blood pressure or high fasting glucose, but were treated with anti-hypertensive drugs or (self-)reported diabetes mellitus, were also considered to fulfil the criteria for high blood pressure or high fasting glucose, respectively.
Diabetes mellitus was defined as having self-reported diabetes or newly detected diabetes (fasting serum glucose concentrations ≥7.0 mmol/l).[12] History of vascular diseases included atherosclerotic vascular diseases in the medical history other than the vascular diagnosis at inclusion. Current or past smoking included currently smoking patients, those who recently stopped or smoked in the past.

Statistical analysis

Values are given as percentages with number of patients in parenthesis, as mean ± S.D. for normally distributed variables and as median with the interquartile range in parenthesis for non-normally distributed variables. Multiple logistic regression analysis was performed to investigate the independent association of variables with the metabolic syndrome; results were expressed as crude odds ratios (crude OR) with 95% CI or as adjusted odds ratios (adjusted OR) with 95% CI. Presence of the metabolic syndrome was taken as the dependent variable and age, gender and localisation of vascular disease were the independent variables. Variables were included if they significantly changed the crude odds ratio. The statistical analyses were performed with SPSS for Windows 10.1.

Results

Baseline characteristics of the patients are presented in *Table 1* according to the presenting disease: CHD, CVD, PAD or AAA. The majority of the patients were male (79%), and the mean age was 60 years. AAA patients had the highest frequency of a history of vascular diseases (41%). A history of CHD was most common. Diabetes mellitus was present in 19% (n = 213) of all patients and most prevalent in patients with PAD (*Table 1*); in 90% it was diagnosed as type 2 diabetes.
Table 2 displays the prevalence of the metabolic syndrome and its components in relation to gender and vascular disease. The overall prevalence of the metabolic syndrome was 46% and it was most prevalent in the PAD patients (58%). Women had a higher prevalence of the metabolic syndrome than men (56% versus 43%). The prevalence was particularly high in patients with diabetes mellitus: 84% in those who presented with CHD, 76% with CVD, 82% with PAD and 88% with AAA.

Table 1. Baseline characteristics in 18-80-year-old patients with CHD, CVD, PAD or AAA*

	CHD (n = 527)		CVD (n = 258)		PAD (n = 232)		AAA (n = 100)	
	Men	Women	Men	Women	Men	Women	Men	Women
Patients, % (n)	82 (434)	18 (93)	74 (192)	26 (66)	70 (163)	30 (69)	94 (94)	6 (6)
Age (years)	57 ± 9	61 ± 9	61 ± 11	60 ± 11	58 ± 10	59 ± 11	69 ± 6	66 ± 10
Smoking, current or past†, % (n)	81 (350)	52 (48)	84 (162)	80 (53)	94 (153)	87 (60)	87 (82)	100 (6)
History of vascular diseases‡, % (n)	6 (27)	8 (7)	26 (50)	6 (4)	30 (49)	32 (22)	40 (38)	50 (3)
Diabetes mellitus§, % (n)	15 (65)	22 (20)	21 (41)	14 (9)	28 (46)	23 (16)	14 (13)	50 (3)
Anti-hypertensive treatment, % (n)	29 (125)	39 (36)	46 (88)	39 (26)	33 (53)	36 (25)	46 (43)	17 (1)
Lipid-lowering treatment, % (n)	54 (233)	40 (37)	32 (62)	39 (26)	30 (49)	38 (26)	26 (24)	17 (1)
Body weight (kg)	84 ± 11	73 ± 11	82 ± 12	66 ± 11	82 ± 13	71 ± 16	81 ± 12	69 ± 18
BMI (kg/m^2)	27 ± 3	28 ± 4	26 ± 4	25 ± 4	26 ± 4	27 ± 5	26 ± 3	26 ± 7
Waist circumference (cm)	98 ± 9	91 ± 11	97 ± 9	84 ± 9	97 ± 11	89 ± 12	98 ± 11	88 ± 14
Systolic blood pressure (mmHg)	131 ± 18	137 ± 21	146 ± 21	144 ± 27	141 ± 21	149 ± 25	142 ± 20	143 ± 16
Diastolic blood pressure (mmHg)	77 ± 9	74 ± 9	84 ± 11	80 ± 13	81 ± 9	81 ± 10	85 ± 12	83 ± 14
Serum triglycerides (mmol/l)	1.63 (1.21-2.31)	1.55 (1.15-2.07)	1.55 (1.17-2.09)	1.48 (1.07-2.11)	1.78 (1.34-2.73)	1.89 (1.41-2.26)	1.56 (1.16-1.98)	1.67 (1.36-2.64)
Serum HDL-cholesterol (mmol/l)	1.03 (0.89-1.22)	1.22 (1.08-1.53)	1.10 (0.92-1.30)	1.41 (1.16-1.71)	1.00 (0.85-1.25)	1.14 (0.96-1.41)	1.13 (0.93-1.36)	1.09 (0.92-1.32)
Fasting serum glucose (mmol/l)	5.8 (5.4-6.3)	5.5 (5.1-6.3)	5.9 (5.4-6.5)	5.5 (5.2-6.1)	5.9 (5.4-6.8)	5.7 (5.2-6.6)	5.8 (5.4-6.4)	6.9 (5.8-9.2)

All data in percentages (number of patients), mean ± S.D. or median (interquartile range).
* CHD: coronary heart disease; CVD: cerebrovascular disease; PAD: peripheral arterial disease; AAA: abdominal aortic aneurysm.
† Still smoking, recently stopped smoking or previously smoking.
‡ History of vascular disease other than the inclusion diagnosis.
§ Fasting serum glucose ≥7.0 mmol/l or self-reported diabetes mellitus.

Table 2. Prevalence of the metabolic syndrome and its different components in 18-80-year-old patients with CHD, CVD, PAD or AAA*

	Patients (n)	Prevalence of metabolic syndrome		Abdominal obesity		High blood pressure		Hypertriglyceridemia		Low HDL-cholesterol		High fasting glucose	
		Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Total	1117	510	46	377	34	804	72	519	46	555	50	421	38
Localisation of vascular disease													
CHD	527	218	41	185	35	321	61	240	46	269	51	180	34
Men	434	162	37	125	29	255	59	195	45	218	50	148	34
Women	93	56	60	60	65	66	71	45	48	51	55	32	34
CVD	258	111	43	75	29	211	82	104	40	107	41	98	38
Men	192	85	44	52	27	159	83	78	41	80	42	79	41
Women	66	26	39	23	35	52	79	26	39	27	41	19	29
PAD	232	134	58	80	34	192	83	131	56	135	58	105	45
Men	163	89	55	43	26	132	81	91	56	92	56	78	48
Women	69	45	65	37	54	60	87	40	58	43	62	27	39
AAA	100	47	47	37	37	80	80	44	44	44	44	38	38
Men	94	43	46	35	37	75	80	41	44	40	43	34	36
Women	6	4	67	2	33	5	83	3	50	4	67	4	67

* CHD: coronary heart disease; CVD: cerebrovascular disease; PAD: peripheral arterial disease; AAA: abdominal aortic aneurysm. Metabolic syndrome was based on the ATP III criteria (Adult Treatment Panel III). See Section 'Methods' for a description of the criteria used for each component of the metabolic syndrome.

High blood pressure was the component of the metabolic syndrome most often observed, especially in women (78%). High blood pressure, hypertriglyceridemia and low HDL-cholesterol was the most common combination of metabolic abnormalities (n = 246, 48%) in the 510 metabolic syndrome patients. Presence of one or more components of the metabolic syndrome was common, in both sexes: 20% had one component, 27% had two components, 25% had three components, 14% had four components and 7% had five components (*Table 3*). Only a limited number of patients displayed the full cluster of metabolic abnormalities. Metabolic syndrome patients and non-metabolic syndrome patients had the same mean age (60 ± 10 years). Smoking habits were also comparable (82%).

In the diabetic population of our cohort (n = 213), 2% had one component of the metabolic syndrome, 16% had two components, 31% had three components, 28% had four components and 23% had five components. The mean age in this diabetic population was 61 ± 9 years in patients with the metabolic syndrome compared to 63 ± 10 years in patients without the metabolic syndrome.

In the whole cohort, patients with the metabolic syndrome had more frequently a medical history of vascular diseases (21% versus 15%), particularly CVD patients (24% versus 18%) and PAD patients (36% versus 23%). Thirty-four percent of the metabolic syndrome patients were diagnosed with diabetes mellitus compared to 6% in patients without the metabolic syndrome. There was no difference between the number of patients treated with lipid-lowering agents (41%), but patients with the metabolic syndrome were more often treated with anti-hypertensive medication than patients without this syndrome (46% versus 27%).

Multiple logistic regression showed that the metabolic syndrome was 1.7 times more common in women compared to men (reference); OR 1.7 (95% CI: 1.3-2.3). The prevalence of the metabolic syndrome depended not on age (yearly); OR 1.00 (95% CI: 0.99-1.02) and was highest among PAD patients (OR 1.9; 95% CI: 1.4-2.7). Localisation of vascular disease with CHD as reference, showed for CVD and AAA the following ORs (95% CI): 1.1 (0.8-1.4); 1.3 (0.8-2.0). The ORs remained essentially the same after adjustment for gender, age or type of presenting disease, whichever was relevant.

Table 3. Prevalence of the number of components of the metabolic syndrome in 18-80-year-old patients with CHD, CVD, PAD or AAA*

	No. of components											
	0		1		2		3		4		5	
	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage	Number	Percentage
Total	73	7	227	20	307	27	282	25	151	14	77	7
Men	60	7	188	21	256	29	217	25	111	13	51	6
Women	13	6	39	17	51	22	65	28	40	17	26	11
Localisation of vascular disease												
CHD	43	8	114	22	152	29	126	24	61	12	31	6
CVD	18	7	54	21	75	29	65	25	34	13	12	5
PAD	7	3	37	16	54	23	66	28	40	17	28	12
AAA	5	5	22	22	26	26	25	25	16	16	6	6

* CHD: coronary heart disease; CVD: cerebrovascular disease; PAD: peripheral arterial disease; AAA: abdominal aortic aneurysm. Metabolic syndrome was based on the ATP III criteria (Adult Treatment Panel III). See Section 'Methods' for a description of the criteria used for each component of the metabolic syndrome.

Discussion

Evaluation

In the present study, we describe that the metabolic syndrome, according to the ATP III criteria, is highly prevalent in patients with a recent diagnosis of a clinical manifestation of atherosclerosis (46%), especially in PAD patients (58%). Particularly in patients with CHD, the metabolic syndrome was more often present in women than in men (60% versus 37%). The prevalence of the metabolic syndrome was not influenced by age.
Patients with a recently established atherosclerotic vascular disease are at high risk for developing another vascular complication in the same or another part of the vascular system. Patients with the metabolic syndrome are at increased risk for developing cardiovascular morbidity and mortality.[1-4] Hence, it is in our view clinically relevant to screen already high-risk patients for the metabolic syndrome. The metabolic syndrome comprises a combination of metabolic disorders including known risk factors like dyslipidemia, hyperglycemia and hypertension, but also risk factors not routinely measured like hyperinsulinemia, decreased fibrinolysis, oxidative stress, small dense LDL-cholesterol and increased inflammation.[2,6,13] Instead of treating individual components of the metabolic syndrome, treating the underlying pathophysiological disturbance (for instance, insulin resistance) would ideally be the therapeutic option of first choice. This means initiating interventions aimed at treatment of overweight and stimulating physical activity before or in addition to conventional medical treatment of individual classical risk factors clustering in the metabolic syndrome. Future research may reveal whether early medical treatment of insulin resistance even in the absence of hyperglycemia may reduce the incidence of macrovascular complications, and may delay the development of diabetes mellitus.
In a cross-sectional study in healthy US adults, the metabolic syndrome was present in 22% (24% after age adjustment) of the participants, also using the definition of ATP III, whereas in subjects over 60 years of age this was around 40%.[7] The prevalence of the metabolic syndrome in our study in patients with manifest atherosclerotic vascular disease was 46% irrespective of age. With the same definition, the Kuopio Ischaemic Heart Disease Risk Factor Study, a prospective cohort study, reported a prevalence of 9% in a middle-aged (42-60 year) cohort of men without diabetes mellitus and without known clinical manifest vascular disease.[2] In our study, the prevalence of the metabolic syndrome in men in the age range of 42-60 years was also 46% because age was not a determinant of the prevalence.

Different definitions

Previous estimates of the prevalence of the metabolic syndrome have differed because of differences in diagnostic criteria and in study populations, hindering meaningful comparisons between populations.[8,9] The first working definition of the metabolic syndrome published by the World Health Organisation (WHO) in 1998 was based on variation in plasma glucose concentration.[12] Recently, ATP III proposed the, in our view, most useful definition for daily clinical practice. The WHO and ATP III criteria gave similar prevalences of the metabolic syndrome in the Third National Health and Nutrition Examination Survey: 25.1% and 23.9%, respectively; 86.2% were classified the same under the two approaches.[14]

Differences in men and women

In our study, the prevalence of the metabolic syndrome was different in men and women (43% versus 56%). In the Botnia study, the metabolic syndrome (WHO definition) was more prevalent in males compared to females.[1] In older persons, the prevalence is higher in women than in men. In a study by Trevisan et al. in patients aged 50 or above, women had a higher prevalence than men.[3] A study in US adults showed that the prevalence of the metabolic syndrome (ATP III criteria) differed little among men and women in the general population (24% versus 23% after age adjustment), but after 70 years of age women had a higher prevalence.[7]
The presence of the metabolic syndrome is associated with increased cardiovascular mortality compared to subjects without the syndrome (12.0% versus 2.2%).[1] Metabolic syndrome-associated mortality is higher in male patients with the syndrome than in female patients.[1] In our study, only survivors of vascular incidents were included which may have led to an overrepresentation of female patients with the metabolic syndrome. Also, the diagnostic criteria for the metabolic syndrome differ between men and women with respect to waist circumference (102 cm versus 88 cm) and HDL-cholesterol (1.04 mmol/l versus 1.29 mmol/l) and may induce differences in prevalences between sexes. Another explanation may be that other risk factors for the development of atherosclerotic diseases are more relevant in male patients. In the present study, male patients were more often past or present smokers.

Future directions

Several studies have shown that the metabolic syndrome is associated with an increased risk for the development of vascular diseases. However, it is not known whether patients with both a history of clinically manifest vascular disease and the metabolic syndrome are at a particularly increased risk for new macrovascular complications. In the Botnia study, patients with the metabolic syndrome and microalbuminuria were at markedly increased risk for cardiovascular death (relative risk 2.8) compared to patients with the metabolic syndrome but without microalbuminuria.[1] Microalbuminuria is thought to be a surrogate for endothelial dysfunction and is an early marker for increased cardiovascular risk.[15,16] This may indicate that patients with the metabolic syndrome and vascular damage or vascular dysfunction are at increased risk compared to patients with the metabolic syndrome but without vascular damage. Future research in cohorts of patients with clinically manifest vascular diseases is needed to really establish whether the metabolic syndrome is an additional risk factor in these patients.

Limitations of the study

We acknowledge some of the limitations of our study. In the present study, taking lipid-lowering medication was not included in the definition of low HDL-cholesterol or hypertriglyceridemia, because the effects of drugs on HDL and triglycerides may vary. In their study, Liese et al. also did not incorporate lipid-lowering treatment into the dyslipidemia definition in the ARIC cohort study because of the same reason.[17] It can be argued that this underestimates the presence of dyslipidemia and thus leads to an underestimation of the prevalence of the metabolic syndrome. Incorporating lipid-

lowering treatment into the definition of low HDL-cholesterol or hypertriglyceridemia resulted in our study in an overall prevalence of 53%. As mentioned before, this cohort only comprises patients who survived their vascular disease. Finally, we could not assess any conclusions about women with AAA, because of the limited number of patients.

Conclusions

In conclusion, our findings clearly indicate a high prevalence of the metabolic syndrome: 41% in CHD patients, 43% in CVD patients, 58% in PAD patients and 47% in AAA patients. Screening for metabolic syndrome in patients with clinically manifest atherosclerotic vascular diseases may identify patients with an even higher risk for the development of cardiovascular complications. This may direct secondary preventive measures, aiming at improvement of the underlying insulin resistance, in order to prevent new vascular incidents in the same or another vascular bed.

Acknowledgements

The SMART study was financially supported by a grant of the University Medical Centre Utrecht. This survey has been made possible because of the support of M. Edlinger, Data Manager of the SMART study.

References

1. Isomaa B, Almgren P, Tuomi T et al. Cardiovascular morbidity and mortality associated with the metabolic syndrome. *Diabetes Care*. 2001;24:683-689.
2. Lakka HM, Laaksonen DE, Lakka TA et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *J Am Med Assoc*. 2002;288:2709-2716.
3. Trevisan M, Liu J, Bahsas FB et al. Syndrome X and mortality: a population-based study. Risk Factor and Life Expectancy Research Group. *Am J Epidemiol*. 1998;148:958-966.
4. Isomaa B, Henricsson M, Almgren P et al. The metabolic syndrome influences the risk of chronic complications in patients with type II diabetes. *Diabetologia*. 2001;44:1148-1154.
5. Alexander CM, Landsman PB, Teutsch SM et al. NCEP-defined metabolic syndrome, diabetes, and prevalence of coronary heart disease among NHANES III participants age 50 years and older. *Diabetes*. 2003;52:1210-1214.
6. Ridker PM, Buring JE, Cook NR et al. C-reactive protein, the metabolic syndrome, and risk of incident cardiovascular events: an 8-year follow-up of 14 719 initially healthy American women. *Circulation*. 2003;107:391-397.
7. Ford ES, Giles WH, Dietz WH. Prevalence of the metabolic syndrome among US adults: findings from the third National Health and Nutrition Examination Survey. *J Am Med Assoc*. 2002;287:356-359.
8. Rantala AO, Kauma H, Lilja M et al. Prevalence of the metabolic syndrome in drug-treated hypertensive patients and control subjects. *J Intern Med*. 1999;245:163-174.
9. Liese AD, Mayer-Davis EJ, Haffner SM. Development of the multiple metabolic syndrome: an epidemiologic perspective. *Epidemiol Rev*. 1998;20:157-172.
10. Simons PC, Algra A, van de Laak MF et al. Second Manifestations of ARTerial disease (SMART) study: rationale and design. *Eur J Epidemiol*. 1999;15:773-781.
11. Executive Summary of The Third Report of The National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, And Treatment of High Blood Cholesterol In Adults (Adult Treatment Panel III). *J Am Med Assoc*. 2001;285:2486-2497.
12. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. 1. Diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med*. 1998;15:539-553.
13. Sakkinen PA, Wahl P, Cushman M et al. Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. *Am J Epidemiol*. 2000;152:897-907.
14. Ford ES, Giles WH. A comparison of the prevalence of the metabolic syndrome using two proposed definitions. *Diabetes Care*. 2003;26:575-581.
15. Garg JP, Bakris GL. Microalbuminuria: marker of vascular dysfunction, risk factor for cardiovascular disease. *Vasc Med*. 2002;7:35-43.
16. Clausen P, Jensen JS, Jensen G et al. Elevated urinary albumin excretion is associated with impaired arterial dilatory capacity in clinically healthy subjects. *Circulation*. 2001;103:1869-1874.
17. Liese AD, Mayer-Davis EJ, Tyroler HA et al. Development of the multiple metabolic syndrome in the ARIC cohort: joint contribution of insulin, BMI, and WHR. Atherosclerosis risk in communities. *Ann Epidemiol*. 1997;7:407-416.

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Advanced vascular damage due to insulin resistance or components of metabolic syndrome in patients with manifest arterial disease without known diabetes? The SMART study

Abstract

Objective

Insulin resistance and metabolic syndrome are important factors in the development of cardiovascular disease. Metabolic syndrome is associated with advanced atherosclerosis in patients with manifest arterial disease. We investigated whether advanced vascular damage in patients with manifest arterial disease without known diabetes can be explained by insulin resistance *per se*, or is mediated by the individual components of metabolic syndrome.

Methods

Cross-sectional study in 1372 patients with manifest arterial disease without known diabetes. Homeostasis model assessment of insulin resistance (HOMA-IR) was used to quantify insulin resistance. Carotid intima-media thickness (CIMT) and albuminuria were used as indicators of advanced vascular damage. The relation of HOMA-IR with CIMT and presence of albuminuria was assessed with linear and logistic regression analyses, and adjusted for single components constituting metabolic syndrome.

Results

In patients with manifest arterial disease without known diabetes, high HOMA-IR was not associated with an increased CIMT, adjusted for age and gender (quartile 4 versus quartile 1, β 0.009; 95% CI -0.024, 0.043). Prevalence of albuminuria increased across quartiles of HOMA-IR (quartile 4 versus quartile 1, age- and gender-adjusted OR 2.05; 95% CI 1.32-3.20). After adjustment for individual components of metabolic syndrome, patients with HOMA-IR levels in the highest quartile still had a 60% higher risk of prevalent albuminuria compared to patients with HOMA-IR levels in the lowest quartile (OR 1.61; 95% CI 0.91-2.85).

Conclusions

In patients with already manifest arterial disease without known diabetes, elevated insulin resistance is associated with an increased prevalence of albuminuria, only partly mediated by the components of metabolic syndrome, whereas not with an increased CIMT.

Introduction

Both insulin resistance and metabolic syndrome are recognized as important factors in the development of cardiovascular disease.[1,2] Obesity-induced insulin resistance is considered to be the major driver of the clustering of interrelated metabolic disturbances (e.g. dyslipidemia, hyperglycemia, elevated blood pressure)[3], often referred to as metabolic syndrome[4], thereby leading to an increased cardiovascular risk. Although insulin resistance may provide the unifying pathophysiological mechanism underlying metabolic syndrome[3], there is uncertainty regarding the independent role of insulin resistance in the development of atherosclerotic vascular disease.[1,5-12] In this field, studies are rather limited in patients with arterial diseases.[13,14]

Metabolic syndrome is highly prevalent in patients with manifest arterial disease (46%)[15] and is associated with advanced vascular damage[16], thereby identifying patients with an even higher risk of cardiovascular events. The high cardiovascular risk may be due to the combination of non-classical risk factors associated with insulin resistance, e.g. inflammation, hyperinsulinemia, oxidative stress, and hypercoagulability, together with the separate components of metabolic syndrome.[17-19] Nevertheless, the magnitude of the association between insulin resistance and advanced vascular damage in these high-risk patients is not yet elucidated. In addition, it is not known whether insulin resistance *per se* has an influence on the extent of advanced vascular damage or that it is mediated by the components of metabolic syndrome. Carotid intima-media thickness (CIMT)[20-22] and albuminuria[23-25] are well-established markers for generalized atherosclerosis and can therefore be used to assess the extent of advanced vascular damage. CIMT and albuminuria are indicators of different stages of the atherosclerotic process; CIMT is an indicator of advanced atherosclerosis, while albuminuria could be seen as a reflection of endothelial dysfunction.

Aim of the current cross-sectional study is (1) to investigate the relation between insulin resistance and metabolic disturbances, (2) to determine whether insulin resistance, derived by homeostasis model assessment of insulin resistance (HOMA-IR), is associated with CIMT and albuminuria in patients with manifest arterial disease without known diabetes, and (3) to evaluate to what extent this relation can be explained by the individual components of metabolic syndrome.

Methods

Study settings, participants and design

In this study, we used data from patients enrolled in the Second Manifestations of ARTerial disease (SMART) study. The SMART study is an ongoing prospective single-centre cohort study in patients with manifest arterial disease or cardiovascular risk factors.[26] Started in September 1996, patients aged 18-80, were referred to the University Medical Centre (UMC) Utrecht with a recent diagnosis of manifest arterial disease or a cardiovascular risk factor. Not approached are patients with terminal malignant disease, those not independent in daily activities (Rankin scale >3) or not sufficiently fluent in Dutch. Patients entered the SMART study if participation in the study was supported by the treating specialist and if the patients themselves consented to participate. The Medical Ethics Committee approved the study, and all participants gave their written informed consent. The rationale and design of the SMART study and a detailed description of the criteria used to define the different manifest arterial diseases were published previously.[26]
For the current study, the data of the 1730 participants with clinical manifestations of arterial disease (coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm) included between August 1, 2003 and March 1, 2007 were considered. A total of 250 patients with known type 1 or type 2 diabetes (use of oral glucose-lowering agents or insulin therapy) were excluded. Also, 108 participants were excluded due to incomplete data for glucose, insulin, albuminuria or CIMT. Finally, 1372 patients were used for the analyses.

Data acquisition

All measurements were performed on a single day at the UMC Utrecht. Medical history, use of current medication and current and past smoking habits were derived from a questionnaire described elsewhere.[26] Height, weight, waist circumference and blood pressure were measured. Blood samples were collected after an overnight fast. Total cholesterol, triglycerides, high-density lipoprotein (HDL)-cholesterol, creatinine, and high sensitive-CRP (hs-CRP) levels were measured. Plasma glucose was measured using commercial enzymatic dry chemistry kits (Johnson and Johnson). Plasma insulin was measured with an immunometric technique on an IMMULITE 1000 Analyzer (Diagnostic Products Corporation, Los Angeles, USA). The lower limit of detection was 2 mIU/l and inter-assay coefficient of variation was 9% at 7 mIU/l and <5.5% at 20-120 mIU/l. HOMA-B was used to quantify the degree of pancreatic beta-cell function and was calculated using the formula: HOMA-B = 20 x (fasting serum insulin (mIU/l)/(fasting serum glucose (mmol/l) – 3.5)).[27]

Assessment of insulin resistance

HOMA-IR was used as quantitative estimate of the degree of insulin resistance at baseline. The value for insulin resistance can be assessed by the formula: HOMA-IR = (fasting serum glucose (mmol/l) x fasting serum insulin (mIU/l)/22.5).[28] HOMA-IR correlates well with measurements obtained by means of the euglycemic clamp technique[29], therefore provides a reliable approach to estimate insulin resistance and lends itself to use in large epidemiological studies.[30]

Metabolic syndrome

Metabolic syndrome was defined according to the Adult Treatment Panel (ATP) III criteria.[4] Metabolic syndrome requires the presence of at least three of the following metabolic abnormalities: abdominal obesity (waist circumference >102 cm in men and >88 cm in women), high blood pressure (≥130 mmHg systolic or ≥85 mmHg diastolic), hypertriglyceridemia (serum triglycerides ≥1.70 mmol/l (150 mg/dl)), low HDL-cholesterol (serum HDL-cholesterol <1.04 mmol/l (40 mg/dl) in men and <1.29 mmol/l (50 mg/dl) in women), high fasting glucose (fasting serum glucose ≥5.6 mmol/l (100 mg/dl)). Patients on anti-hypertensive medication were regarded as having high blood pressure.

Assessment of Carotid IMT and albuminuria

CIMT was measured in supine position in the left and right common carotid arteries in anterolateral, posterolateral and mediolateral direction, the head turned 45 degrees away from the side being scanned. An ATL Ultramark 9 (Advanced Technology Laboratories, Bethel, WA, USA) equipped with a 10 MHz linear array transducer was used. Reference point for measurement of the CIMT was the onset of the dilatation of the carotid bulb, with loss of the parallel configuration of the near and far walls of the common carotid artery. An R-wave-triggered optimal longitudinal image of the far wall was frozen and stored on video-tape. On this image, the leading edges corresponding to the transition zone between lumen-intima and media-adventitia were traced, over a length of 1 cm proximal to the reference point and the total intima-media surface of this selected area was calculated.[26] The mean CIMT of these six measurements was calculated only, if at least four of six measurements were available. Previously, an interobserver coefficient of variation of 11.7% for CIMT measurements has been reported.[31]

A morning urine portion was collected for measuring albumin and creatinine concentrations. Creatinine was measured with a commercial enzymatic dry chemistry kit (Johnson and Johnson) and albumin was determined with an immunoturbidimetric assay (Boehringer-Mannheim). Albuminuria is defined as a ratio of >3 mg albumin/mmol creatinine.[32]

Statistical analysis

Differences in metabolic disturbances across quartiles of HOMA-IR were tested with one-way ANOVA (continuous normal distributed variables), chi-square (categorical variables) or Kruskal-Wallis test (continuous skewed variables).

The relation of HOMA-IR and separate indicators of advanced vascular damage was determined with linear regression analysis (CIMT) and logistic regression analysis (albuminuria). HOMA-IR was categorized into quartiles. Results are expressed as beta (β) coefficients with 95% confidence intervals (CI) and as odds ratios (OR) with 95% CI. Three models were used to estimate the relation between HOMA-IR as independent and CIMT and albuminuria as dependent variable. First, we estimated the relationship adjusted for variables which we considered to be confounders: age and gender. In the second model, additional adjustment was performed for waist circumference, HDL-cholesterol, triglycerides, fasting glucose and systolic blood pressure to evaluate to what extent this relationship is mediated by the single components of metabolic syndrome.

All covariates were included as continuous variables. Additionally, models with (model III) and without (model I) presence of metabolic syndrome were compared.
A trend between the number of components of metabolic syndrome and HOMA-IR was investigated with analysis of covariance (ANCOVA, general linear model procedure), and adjusted for age and gender.
To reduce bias and increase statistical efficiency, missing values (HDL-cholesterol (n = 1), triglycerides (n = 1), creatinine (n = 1), diastolic (n = 8) and systolic (n = 8) blood pressure, waist circumference (n = 48), hs-CRP (n = 6)) in the data were completed by regression imputation.[33,34] Significance was taken at the 5% level (two-sided).

Results

Study population

The mean age was 59 ± 11 years and 70% of patients were male. HOMA-IR levels (median (interquartile range)) were 2.4 (1.6-3.9) for male and 2.2 (1.4-3.2) for female. General characteristics of the study population according to quartiles of HOMA-IR are listed in *Table 1*.

Table 1. General characteristics of patients with manifest arterial disease without known diabetes according to quartiles of HOMA insulin resistance (n = 1372)

HOMA-IR	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
(Range)	(0.4-1.5)	(1.6-2.3)	(2.4-3.6)	(3.7-23.3)
	n = 344	n = 344	n = 341	n = 343
HOMA-IR	1.2 (0.9–1.4)	2.0 (1.7–2.2)	2.9 (2.6–3.2)	5.0 (4.2–6.3)
Age (years)	59 ± 11	59 ± 11	58 ± 10	60 ± 11
Male gender, n (%)	229 (67)	235 (68)	228 (67)	267 (78)
Body mass index (kg/m^2)	24 ± 3	26 ± 3	28 ± 3	29 ± 4
Ever smoking (current or past), n (%)	270 (79)	260 (76)	273 (80)	292 (85)
Creatinine clearance (Cockroft) (ml/min/1.73m^2)	77 ± 17	77 ± 21	79 ± 22	80 ± 24
Medications				
Lipid-lowering agents, n (%)	227 (66)	235 (68)	248 (73)	277 (81)
Blood pressure-lowering agents, n (%)	217 (63)	239 (70)	254 (75)	296 (86)
Manifest arterial disease*				
Cerebrovascular disease, n (%)	111 (32)	104 (30)	111 (33)	73 (21)
Coronary heart disease, n (%)	183 (53)	205 (60)	207 (61)	244 (71)
Peripheral arterial disease, n (%)	62 (18)	48 (14)	57 (17)	67 (20)
Abdominal aortic aneurysm, n (%)	23 (7)	28 (8)	16 (5)	22 (6)

All data in n (%), mean ± standard deviation or median (interquartile range).
HOMA-IR: Homeostasis model assessment determined insulin resistance (fasting serum glucose x fasting serum insulin / 22.5).
* Ever or current diagnosis, a single person can be classified into more than one disease category.

Insulin resistance and metabolic disturbances

Prevalence of metabolic syndrome was 38%. Metabolic syndrome was more prevalent in the highest HOMA-IR quartile compared with the lowest quartile (71% versus 10%) (*Table 2*). Individual components of metabolic syndrome were generally more adverse among patients with higher levels of insulin resistance. Level of hs-CRP was higher among patients with higher insulin resistance levels. In *Table 3* it is shown that HOMA-IR increased with an increment in the number of components of metabolic syndrome, adjusted for age and gender (p-value for trend <0.001).

Table 2. Metabolic risk factors in patients with manifest arterial disease without known diabetes according to quartiles of HOMA insulin resistance (n = 1372)

HOMA-IR	Quartile 1	Quartile 2	Quartile 3	Quartile 4	P-value
(Range)	(0.4-1.5)	(1.6-2.3)	(2.4-3.6)	(3.7-23.3)	
	n = 344	n = 344	n = 341	n = 343	
HOMA-IR	1.2 (0.9–1.4)	2.0 (1.7–2.2)	2.9 (2.6–3.2)	5.0 (4.2–6.3)	
Fasting insulin (mIU/l)	5 (4-6)	8 (7-9)	11 (10-13)	18 (16-22)	<0.001
HOMA-B	53 (38-67)	82 (64-100)	104 (80-129)	142 (107-190)	<0.001
hs-CRP (mg/l)*	1.3 (0.6-2.7)	1.5 (0.7-3.3)	2.0 (1.0-3.7)	2.1 (1.1-4.4)	<0.001
Metabolic syndrome components					
Metabolic syndrome (ATP III)	35 (10)	88 (26)	152 (45)	245 (71)	<0.001
Fasting glucose (mmol/l)	5.2 (4.9-5.5)	5.5 (5.1-5.8)	5.7 (5.4-6.0)	6.1 (5.7-6.9)	<0.001
Triglycerides (mmol/l)	1.04 (0.83-1.40)	1.20 (0.96-1.70)	1.39 (1.09-1.94)	1.70 (1.22-2.38)	<0.001
HDL-cholesterol (mmol/l)	1.43 (1.19-1.74)	1.33 (1.11-1.58)	1.23 (1.03-1.47)	1.13 (0.95-1.32)	<0.001
Waist circumference (cm)	86 ± 10	91 ± 11	94 ± 10	101 ± 11	<0.001
Blood pressure systolic (mmHg)	140 ± 21	143 ± 21	144 ± 22	144 ± 20	0.07

All data in n (%), mean ± standard deviation or median (interquartile range).
HOMA-IR: Homeostasis model assessment determined insulin resistance (fasting serum glucose x fasting serum insulin / 22.5).
HOMA-B: Homeostasis model assessment determined beta-cell function (20 x (fasting serum insulin/ (fasting serum glucose – 3.5))).
* high sensitive C-Reactive Protein (plasma values >15 mg/l excluded from analyses (n = 71)).

Insulin resistance and indicators of advanced vascular damage

In patients with manifest arterial disease without known diabetes, CIMT was 0.91 ± 0.24 mm in patients within the highest HOMA-IR quartile compared to 0.89 ± 0.26 mm in patients within the lowest quartile. High HOMA-IR was not associated with an increased CIMT, adjusted for age and gender (quartile 4 versus quartile 1, β 0.009; 95% CI -0.024, 0.043) (*Table 4*). A β coefficient of 0.009 indicates that CIMT is 0.009 mm higher in patients within the highest HOMA-IR quartile relative to patients within the lowest quartile, but the confidence interval includes 0 meaning there is no association and the observed β coefficient could have been obtained by chance.

Table 3. HOMA insulin resistance in relation to metabolic syndrome (ATP III) and the number of components in patients with manifest arterial disease without known diabetes (n = 1372)

	N	HOMA-IR*	P-value for trend*
Metabolic syndrome no	852	2.2 ± 0.1	
Metabolic syndrome yes	520	4.1 ± 0.1	<0.001
Components			
0	84	1.5 ± 0.2	
1	345	1.8 ± 0.1	
2	423	2.6 ± 0.1	
3	316	3.8 ± 0.1	
4	150	4.3 ± 0.2	
5	54	5.7 ± 0.3	<0.001

All data are means ± standard errors. * Adjusted for age and gender. P-value for trend for differences in HOMA-IR derived using log-transformed variables with backtransformed values presented in the Table.

Table 4. HOMA insulin resistance in relation to advanced vascular damage, as assessed by carotid intima-media thickness and albuminuria, in patients with manifest arterial disease without known diabetes (n = 1372)

		HOMA-IR			
	Model	Quartile 1	Quartile 2	Quartile 3	Quartile 4
(Range)		(0.4-1.5)	(1.6-2.3)	(2.4-3.6)	(3.7-23.3)
CIMT					
		β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
	I	0	-0.006 (-0.039, 0.028)	0.017 (-0.017, 0.050)	0.009 (-0.024, 0.043)
	II	0	-0.021 (-0.056, 0.013)	-0.009 (-0.046, 0.028)	-0.027 (-0.070, 0.016)
	III	0	-0.012 (-0.046, 0.021)	0.002 (-0.033, 0.037)	-0.017 (-0.055, 0.020)
Albuminuria					
		OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
	I	1	1.31 (0.82, 2.09)	1.22 (0.76, 1.97)	2.05 (1.32, 3.20)
	II	1	1.19 (0.73, 1.93)	1.01 (0.60, 1.71)	1.61 (0.91, 2.85)
	III	1	1.26 (0.79, 2.02)	1.12 (0.68, 1.84)	1.76 (1.07, 2.89)

CIMT: carotid intima-media thickness, β: regression coefficient, OR: odds ratio, 95% CI: 95% confidence interval.
CIMT: β indicates the difference in CIMT (mm) relative to the quartile with the lowest HOMA-IR levels.
Albuminuria: >3.0 mg/mmol: The OR indicates the risk of presence of albuminuria relative to the quartile with the lowest HOMA-IR levels.
Model I: adjusted for age and gender.
Model II: Model I, additionally adjusted for waist circumference, fasting glucose, triglycerides, HDL-cholesterol, systolic blood pressure.
Model III: Model I, additionally adjusted for presence of metabolic syndrome (ATP III).

Of patients within the highest HOMA-IR quartile, a total of 66 patients (19%) had albuminuria compared to 36 patients (10%) within the lowest HOMA-IR quartile (age- and gender-adjusted OR 2.05; 95% CI 1.32-3.20) (*Table 4*). Additional adjustment for the individual components of metabolic syndrome attenuated the association, pointing to a role in the pathogenesis of the relationship. However, patients with HOMA-IR levels in the highest quartile still had a 60% higher risk of presence of albuminuria compared to patients with HOMA-IR levels in the lowest quartile (OR 1.61; 95% CI 0.91-2.85). Additional adjustment for presence of metabolic syndrome instead of single components also attenuated the association (*Table 4*).

Discussion

Our study in patients with already manifest arterial disease without known diabetes confirms that insulin resistance clusters with metabolic abnormalities. Also, an elevated level of insulin resistance was associated with an increased prevalence of albuminuria; this association was only partly explained by components of metabolic syndrome. High insulin resistance was not related with an increased CIMT.

In general, patients with high insulin resistance are at an increased cardiovascular risk.[1,6] The mechanisms by which insulin resistance leads to atherosclerotic cardiovascular disease are not fully understood. Both direct effects of insulin on the vessel wall, such as stimulating the release of vasoconstrictor endothelin-1, and indirect effects through accelerating chronic inflammation, oxidative stress, and metabolic abnormalities (e.g. hyperglycemia, dyslipidemia, and elevated blood pressure) have been implicated to contribute to endothelial dysfunction thereby enhancing atherosclerotic cardiovascular disease.[19,35] CIMT is a well-established marker for advanced atherosclerosis and an indicator for a high cardiovascular risk.[20-22] Many[36-40] but not all studies[41-43] in the general population or in diabetic patients show positive associations of indices of insulin resistance with CIMT. Differences in studies with regard to eligible criteria, e.g. whether diabetic patients have been excluded or not, definitions for insulin resistance and control in the analyses, potentially contribute to the lack of inconsistency. In the current study, among patients with manifest arterial disease without known diabetes, those with a high insulin resistance (HOMA-IR) did not have higher age- and gender-adjusted CIMT levels. This implies that although elevated insulin resistance is a major driver of the underlying clustering of metabolic abnormalities, insulin resistance *per se* is not directly related to an increased CIMT. An explanation may be that we studied patients with already manifest arterial disease generally having larger CIMT than the general population.

Albuminuria is associated with vascular dysfunction and therefore an indicator of a high cardiovascular risk.[23-25] In the present study, insulin resistance, derived by HOMA-IR, was associated with the presence of albuminuria in patients with manifest arterial disease without known diabetes. Our findings are in agreement with several studies[44,45] that found high insulin resistance to be associated with albuminuria in patients without both established arterial disease and diabetes. In our study, classical components of metabolic syndrome constituted part of the link between insulin resistance and albuminuria, but did not fully account for the observed relation.

This indicates that insulin resistance is in part directly associated with albuminuria regardless of its relationship with hyperglycemia, arterial systolic blood pressure and other metabolic abnormalities. One of the mechanisms may be that elevated plasma insulin levels, accompanying insulin resistance, sustain glomerular hyperfiltration, and increase vascular permeability, leading to an increased albuminuria.[46] This reasoning is supported by the presence of higher creatinine clearance in patients with higher insulin resistance levels (80 ± 24 ml/min versus 77 ± 17 ml/min) (*Table 1*) compared to those with lower levels. Our findings suggest that in patients with manifest arterial disease but without known diabetes, insulin resistance alone has an adverse effect on vascular function, as reflected by albuminuria, which may therefore play an important role linking insulin resistance to atherosclerotic cardiovascular disease.

In addition, we have examined the relationship between insulin resistance and metabolic disturbances in patients with manifest arterial disease without known diabetes. Waist circumference, fasting glucose level, triglycerides level and systolic blood pressure were higher in patients within the highest HOMA-IR quartile, whereas HDL-cholesterol level was lower. Also, insulin resistance was elevated in patients with a higher number of metabolic syndrome components. These results demonstrated a clustering of metabolic abnormalities across HOMA-IR quartiles confirming the pathophysiological rationale behind the metabolic syndrome in these high-risk patients.[3]

Our data may help to further unravel the pathogenesis of the development of atherosclerotic cardiovascular diseases as a result of insulin resistance. Our findings indicate a different effect of insulin resistance on CIMT and albuminuria in patients with manifest arterial disease without known diabetes, suggesting that insulin resistance may play a role in different stages of the atherosclerotic process. In most studies, albuminuria is associated with an increased CIMT[47], however, albuminuria and an increased CIMT do not always exist together supporting a difference in pathophysiological mechanisms. In our study, the lack of an association between insulin resistance and CIMT suggests that in patients with already manifest arterial disease without known diabetes, insulin resistance alone does not further enhance atherosclerosis in the arterial wall. Additionally, we found that insulin resistance *per se* is in part directly associated with albuminuria in patients with manifest arterial disease without known diabetes. This suggests that in these high-risk patients, insulin resistance identifies patients with more generalized vascular dysfunction, as reflected by albuminuria, who may be more susceptible for progression of atherosclerosis and/or the development of instable atherosclerotic plaques both leading to clinical events. Improvement of insulin sensitivity may ameliorate the impaired vascular function thereby delaying the progression of structural vascular damage.

We acknowledge some limitations of our study. Due to the cross-sectional design, only assumptions about possible etiological relationships can be made. Furthermore, HOMA-IR was used to estimate the level of insulin resistance. The euglycemic clamp technique is the standard for measuring insulin resistance[48], however, this technique is unsuitable for epidemiologic studies. HOMA-IR seems to be a reliable tool in the assessment of insulin resistance due to its strong relation to clamp-measured insulin resistance in both patients with and without diabetes.[29,30] Also, misclassification of subjects according to insulin resistance is very rare with HOMA.[29,30] Moreover, there is

not a single clinical definition for the clustering of metabolic abnormalities. We used the ATP III-definition of metabolic syndrome[4] because it is most commonly used in studies, best related with the development of vascular diseases and easy to use in clinical practice. However, we realize that there are more definitions for the metabolic syndrome.[3]

In conclusion, in patients with already manifest arterial disease but without known diabetes, elevated insulin resistance is associated with an increased prevalence of albuminuria, only partly mediated by components of metabolic syndrome, whereas not with an increased CIMT.

Acknowledgements

The SMART study was financially supported by a grant of the University Medical Centre Utrecht, The Netherlands. The help of M. Edlinger for data coordination is greatly acknowledged.

References

1. Bonora E, Kiechl S, Willeit J et al. Insulin resistance as estimated by homeostasis model assessment predicts incident symptomatic cardiovascular disease in caucasian subjects from the general population: the bruneck study. *Diabetes Care.* 2007;30:318-324.
2. Lakka HM, Laaksonen DE, Lakka TA et al. The metabolic syndrome and total and cardiovascular disease mortality in middle-aged men. *JAMA.* 2002;288:2709-2716.
3. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365:1415-1428.
4. Grundy SM, Brewer HB, Jr, Cleeman JI et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation.* 2004;109:433-438.
5. Bonora E, Formentini G, Calcaterra F et al. HOMA-estimated insulin resistance is an independent predictor of cardiovascular disease in type 2 diabetic subjects: prospective data from the Verona Diabetes Complications Study. *Diabetes Care.* 2002;25:1135-1141.
6. Hanley AJ, Williams K, Stern MP et al. Homeostasis model assessment of insulin resistance in relation to the incidence of cardiovascular disease: the San Antonio Heart Study. *Diabetes Care.* 2002;25:1177-1184.
7. Rutter MK, Meigs JB, Sullivan LM et al. Insulin resistance, the metabolic syndrome, and incident cardiovascular events in the Framingham Offspring Study. *Diabetes.* 2005;54:3252-3257.
8. Resnick HE, Jones K, Ruotolo G et al. Insulin resistance, the metabolic syndrome, and risk of incident cardiovascular disease in nondiabetic american indians: the Strong Heart Study. *Diabetes Care.* 2003;26:861-867.
9. Meigs JB, Rutter MK, Sullivan LM et al. Impact of insulin resistance on risk of type 2 diabetes and cardiovascular disease in people with metabolic syndrome. *Diabetes Care.* 2007;30:1219-1225.
10. Hedblad B, Nilsson P, Engstrom G et al. Insulin resistance in non-diabetic subjects is associated with increased incidence of myocardial infarction and death. *Diabet Med.* 2002;19:470-475.
11. Saely CH, Aczel S, Marte T et al. The metabolic syndrome, insulin resistance, and cardiovascular risk in diabetic and nondiabetic patients. *J Clin Endocrinol Metab.* 2005;90:5698-5703.
12. Adler AI, Levy JC, Matthews DR et al. Insulin sensitivity at diagnosis of Type 2 diabetes is not associated with subsequent cardiovascular disease (UKPDS 67). *Diabet Med.* 2005;22:306-311.
13. Tenenbaum A, Adler Y, Boyko V et al. Insulin resistance is associated with increased risk of major cardiovascular events in patients with preexisting coronary artery disease. *Am Heart J.* 2007;153:559-565.
14. Yanase M, Takatsu F, Tagawa T et al. Insulin resistance and fasting hyperinsulinemia are risk factors for new cardiovascular events in patients with prior coronary artery disease and normal glucose tolerance. *Circ J.* 2004;68:47-52.
15. Gorter PM, Olijhoek JK, van der Graaf Y et al. Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm. *Atherosclerosis.* 2004;173:363-369.
16. Olijhoek JK, van der Graaf Y, Banga JD et al. The metabolic syndrome is associated with advanced vascular damage in patients with coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm. *Eur Heart J.* 2004;25:342-348.
17. Sakkinen PA, Wahl P, Cushman M et al. Clustering of procoagulation, inflammation, and fibrinolysis variables with metabolic factors in insulin resistance syndrome. *Am J Epidemiol.* 2000;152:897-907.
18. Scuteri A, Najjar SS, Muller DC et al. Metabolic syndrome amplifies the age-associated increases in vascular thickness and stiffness. *J Am Coll Cardiol.* 2004;43:1388-1395.
19. Martens FM, Visseren FL. The operative risk factors in the metabolic syndrome: is it lipids and high BP or are there direct vascular effects of insulin resistance and obesity. *Curr Diab Rep.* 2007;7:74-81.
20. Bots ML, Grobbee DE. Intima media thickness as a surrogate marker for generalised atherosclerosis. *Cardiovasc Drugs Ther.* 2002;16:341-351.

21. Lorenz MW, Markus HS, Bots ML et al. Prediction of clinical cardiovascular events with carotid intima-media thickness: a systematic review and meta-analysis. *Circulation.* 2007;115:459-467.
22. Dijk JM, van der Graaf Y, Bots ML et al. Carotid intima-media thickness and the risk of new vascular events in patients with manifest atherosclerotic disease: the SMART study. *Eur Heart J.* 2006;27:1971-1978.
23. Paisley KE, Beaman M, Tooke JE et al. Endothelial dysfunction and inflammation in asymptomatic proteinuria. *Kidney Int.* 2003;63:624-633.
24. Hillege HL, Janssen WM, Bak AA et al. Microalbuminuria is common, also in a nondiabetic, nonhypertensive population, and an independent indicator of cardiovascular risk factors and cardiovascular morbidity. *J Intern Med.* 2001;249:519-526.
25. Borch-Johnsen K, Feldt-Rasmussen B, Strandgaard S et al. Urinary albumin excretion. An independent predictor of ischemic heart disease. *Arterioscler Thromb Vasc Biol.* 1999;19:1992-1997.
26. Simons PC, Algra A, van de Laak MF et al. Second Manifestations of ARTerial disease (SMART) study: rationale and design. *Eur J Epidemiol.* 1999;15:773-781.
27. Albareda M, Rodriguez-Espinosa J, Murugo M et al. Assessment of insulin sensitivity and beta-cell function from measurements in the fasting state and during an oral glucose tolerance test. *Diabetologia.* 2000;43:1507-1511.
28. Matthews DR, Hosker JP, Rudenski AS et al. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia.* 1985;28:412-419.
29. Bonora E, Targher G, Alberiche M et al. Homeostasis model assessment closely mirrors the glucose clamp technique in the assessment of insulin sensitivity: studies in subjects with various degrees of glucose tolerance and insulin sensitivity. *Diabetes Care.* 2000;23:57-63.
30. Haffner SM, Miettinen H, Stern MP. The homeostasis model in the San Antonio Heart Study. *Diabetes Care.* 1997;20:1087-1092.
31. Kanters SD, Elgersma OE, Banga JD et al. Reproducibility of measurements of intima-media thickness and distensibility in the common carotid artery. *Eur J Vasc Endovasc Surg.* 1998;16:28-35.
32. Jones CA, Francis ME, Eberhardt MS et al. Microalbuminuria in the US population: third National Health and Nutrition Examination Survey. *Am J Kidney Dis.* 2002;39:445-459.
33. Miettinen OS. Theoretical epidemiology, principles of occurrence research in medicine. *New York: Wiley*. 1985;p. 216-244.
34. Greenland S, Finkle WD. A critical look at methods for handling missing covariates in epidemiologic regression analyses. *Am J Epidemiol.* 1995;142:1255-1264.
35. Wheatcroft SB, Williams IL, Shah AM et al. Pathophysiological implications of insulin resistance on vascular endothelial function. *Diabet Med.* 2003;20:255-268.
36. Haffner SM, D'Agostino R, Mykkanen L et al. Proinsulin and insulin concentrations in relation to carotid wall thickness: Insulin Resistance Atherosclerosis Study. *Stroke.* 1998;29:1498-1503.
37. Folsom AR, Eckfeldt JH, Weitzman S et al. Relation of carotid artery wall thickness to diabetes mellitus, fasting glucose and insulin, body size, and physical activity. Atherosclerosis Risk in Communities (ARIC) Study Investigators. *Stroke.* 1994;25:66-73.
38. Hedblad B, Nilsson P, Janzon L et al. Relation between insulin resistance and carotid intima-media thickness and stenosis in non-diabetic subjects. Results from a cross-sectional study in Malmo, Sweden. *Diabet Med.* 2000;17:299-307.
39. Rajala U, Laakso M, Paivansalo M et al. Low insulin sensitivity measured by both quantitative insulin sensitivity check index and homeostasis model assessment method as a risk factor of increased intima-media thickness of the carotid artery. *J Clin Endocrinol Metab.* 2002;87:5092-5097.
40. Bokemark L, Wikstrand J, Wedel H et al. Insulin, insulin propeptides and intima-media thickness in the carotid artery in 58-year-old clinically healthy men. The Atherosclerosis and Insulin Resistance study (AIR). *Diabet Med.* 2002;19:144-151.

41. Larsson H, Berglund G, Ahren B. Insulin sensitivity, insulin secretion, and glucose tolerance versus intima-media thickness in nondiabetic postmenopausal women. *J Clin Endocrinol Metab.* 2003;88:4791-4797.
42. Rantala AO, Paivansalo M, Kauma H et al. Hyperinsulinemia and carotid atherosclerosis in hypertensive and control subjects. *Diabetes Care.* 1998;21:1188-1193.
43. Bonora E, Tessari R, Micciolo R et al. Intimal-medial thickness of the carotid artery in nondiabetic and NIDDM patients. Relationship with insulin resistance. *Diabetes Care.* 1997;20:627-631.
44. Mykkanen L, Zaccaro DJ, Wagenknecht LE et al. Microalbuminuria is associated with insulin resistance in nondiabetic subjects: the insulin resistance atherosclerosis study. *Diabetes.* 1998;47:793-800.
45. Fujikawa R, Okubo M, Egusa G et al. Insulin resistance precedes the appearance of albuminuria in non-diabetic subjects: 6 years follow up study. *Diabetes Res Clin Pract.* 2001;53:99-106.
46. Catalano C, Muscelli E, Quinones GA et al. Effect of insulin on systemic and renal handling of albumin in nondiabetic and NIDDM subjects. *Diabetes.* 1997;46:868-875.
47. Mykkanen L, Zaccaro DJ, O'Leary DH et al. Microalbuminuria and carotid artery intima-media thickness in nondiabetic and NIDDM subjects. The Insulin Resistance Atherosclerosis Study (IRAS). *Stroke.* 1997;28:1710-1716.
48. DeFronzo RA, Tobin JD, Andres R. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am J Physiol.* 1979;237:E214-223.

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Diabet Med. 2007;24:1352-1360.

The impact of site and extent of clinically evident cardiovascular disease and atherosclerotic burden on new cardiovascular events in patients with type 2 diabetes. The SMART study

Abstract

Objective

Patients with type 2 diabetes and coronary heart disease (CHD) have an excess cardiovascular risk. The relationship of both other sites (cerebrovascular disease, peripheral arterial disease (PAD)) and the extent of clinically evident cardiovascular disease (CVD) with the occurrence of new cardiovascular events have not been investigated previously in patients with diabetes. We aimed to quantify this relationship and to assess the additional influence of atherosclerotic burden.

Methods

From 1996 to 2005, 776 patients with type 2 diabetes with (n = 458) and without (n = 318) clinically evident CVD were followed prospectively for cardiovascular events (cardiovascular death, non-fatal ischemic stroke or myocardial infarction). CVD was classified according to the site (cerebrovascular disease, CHD, PAD); the extent of atherosclerosis was expressed as the number of affected sites. Carotid intima-media thickness and albuminuria were used as markers of atherosclerotic burden.

Results

Compared with patients with diabetes without CVD, the hazard ratio (HR) for a cardiovascular event was 3.8 (95% confidence interval 1.7, 8.5), adjusted for age, gender and potential confounders, in those with cerebrovascular disease, 4.3 (1.9, 9.5) in those with CHD, and 4.6 (2.1, 10.2) in those with PAD. Findings were similar after additional adjustment for atherosclerotic burden. Adjusted HR was 3.4 (1.6, 6.9) for patients with diabetes with one affected site and 6.6 (3.0, 14.3) for those with two or more sites.

Conclusions

Patients with type 2 diabetes and cerebrovascular disease, CHD or PAD have strongly increased risks for future cardiovascular events which are comparable. This risk increases markedly with the number of different cardiovascular sites affected and is irrespective of atherosclerotic burden.

Introduction

Patients with type 2 diabetes have a two- to threefold higher risk of cardiovascular events than those without diabetes.[1,2] Moreover, cardiovascular events are the cause of death in approximately 65% of subjects with diabetes.[3] The cardiovascular risk in patients with type 2 diabetes is almost as high as that of patients with cardiovascular disease (CVD), which suggests that in patients with diabetes cardiovascular risk factors should be managed as aggressively as in CVD patients.[4-8] Others have concluded that type 2 diabetes might be a coronary heart disease (CHD) risk equivalent, but only in women[9] or for certain definitions of CHD.[10] A recent study has shown a gradation in risk of CHD in subjects with diabetes depending on the existing risk factors.[11]

Subjects with diabetes who have experienced CVD are at higher risk of new cardiovascular events than those without such a history.[7,9,12,13] Indeed, recent guidelines recognize that these patients need more aggressive lipid-lowering treatment.[14] Thus far, investigating the risk of new cardiovascular events in patients with type 2 diabetes who already have clinically apparent CVD has been confined to those with CHD.[4,5,10,12,15]

Consequently, the risk associated with arterial disease in other cardiovascular beds has not been investigated previously in a single cohort of subjects with diabetes.[16] Because the site at which atherosclerosis occurs is influenced by differences in cardiovascular risk factors[17], the risk of new events may vary among subjects with diabetes and cerebrovascular disease, CHD or peripheral arterial disease (PAD). In addition, the impact of the extent of clinically evident CVD (expressed as the number of affected sites) on variation in cardiovascular risk in subjects with diabetes is unclear.[16] Identifying patients at highest risk of recurrent events among those already at high risk might be useful in the development of new treatment strategies, such as lower treatment goals for cardiovascular risk factors, to prevent subsequent cardiovascular events.

Atherosclerotic burden (carotid intima-media thickness (CIMT)) has been associated with an increased risk of CHD in patients with diabetes.[18] As patients with diabetes and advanced CVD are likely to have a high prevalence of atherosclerotic burden, this may influence the relation between clinically evident CVD and new cardiovascular events.

The aim of the current prospective study was to quantify and compare the effect of the site of clinically evident CVD on the risk of new cardiovascular events in patients with type 2 diabetes. The impact of the number of sites clinically affected by CVD and the influence of the extent of atherosclerotic burden on the risk of new events were also investigated.

Subjects and methods

Study population

Patients were recruited from the Second Manifestations of ARTerial disease (SMART) study, an ongoing prospective single-centre cohort study in patients with CVD or cardiovascular risk factors.[19] Beginning in September 1996, patients aged 18-80 years referred to the University Medical Centre Utrecht (UMCU) with a recent diagnosis of clinically evident CVD (cerebrovascular disease, CHD, PAD or abdominal aortic aneurysm) or an increased risk for CVD (hypertension, hyperlipidemia or diabetes mellitus) were recruited. Patients with terminal malignant disease, those not independent in daily activities (Rankin scale >3) or not sufficiently fluent in Dutch were excluded. Patient entered the SMART study if participation in the study was supported by the treating specialist and if the patients themselves consented. The local Ethics Committee approved the study, and all participants gave their written informed consent. The rationale and design of the SMART study have been described in detail elsewhere.[19]

For the current prospective study, 776 patients with type 2 diabetes, with and without clinically evident CVD, who were included in the SMART study between 1 September 1996 and 1 March 2005 were enrolled (*Figure 1*). The presence of documented type 2 diabetes was ascertained by inclusion diagnosis and medical history (use of glucose-lowering medications). Not included were patients with self-reported type 1 diabetes or probable type 1 diabetes (based on diagnosis of diabetes at age ≤30 years and requiring insulin treatment) or with serum creatinine >350 µmol/l.

The presence of clinically evident CVD was based on inclusion diagnosis or cardiovascular history (*Table 1*). A cardiovascular history was assessed by a medical history questionnaire. In all analyses, patients with abdominal aortic aneurysm were included in the group of patients with PAD.

Data acquisition

Medical history, use of current medication and diabetes duration were derived from a questionnaire described elsewhere.[19] Duration of diabetes was calculated as years since first diagnosis of diabetes. All patients underwent a non-invasive standardized diagnostic protocol on a single day at the UMCU, including physical examination (height, weight and blood pressure), CIMT measurements and fasting measurements of lipid profile (total cholesterol, high-density lipoprotein (HDL)-cholesterol, triglycerides), glucose and creatinine.[19] Low-density lipoprotein-cholesterol was calculated with Friedewald`s formula. A morning urine sample was collected for measuring albumin and creatinine concentrations.

Atherosclerotic burden

CIMT and albuminuria can be used as markers of generalized atherosclerosis and as indicators of cardiovascular risk.[20-22] The extent of atherosclerotic burden was therefore assessed with measurement of CIMT and determination of albuminuria. CIMT was measured at the left and right common carotid arteries. The arteries were examined in anterolateral, posterolateral and mediolateral direction with an ATL Ultramark

9 (Advanced Technology Laboratories, Bethel, WA, USA) equipped with a 10-MHz linear array transducer as previously described elsewhere.[19] The mean CIMT of the six measurements was used for analysis. Albuminuria was defined as a ratio of >3 mg albumin/mmol creatinine in a morning urine sample.

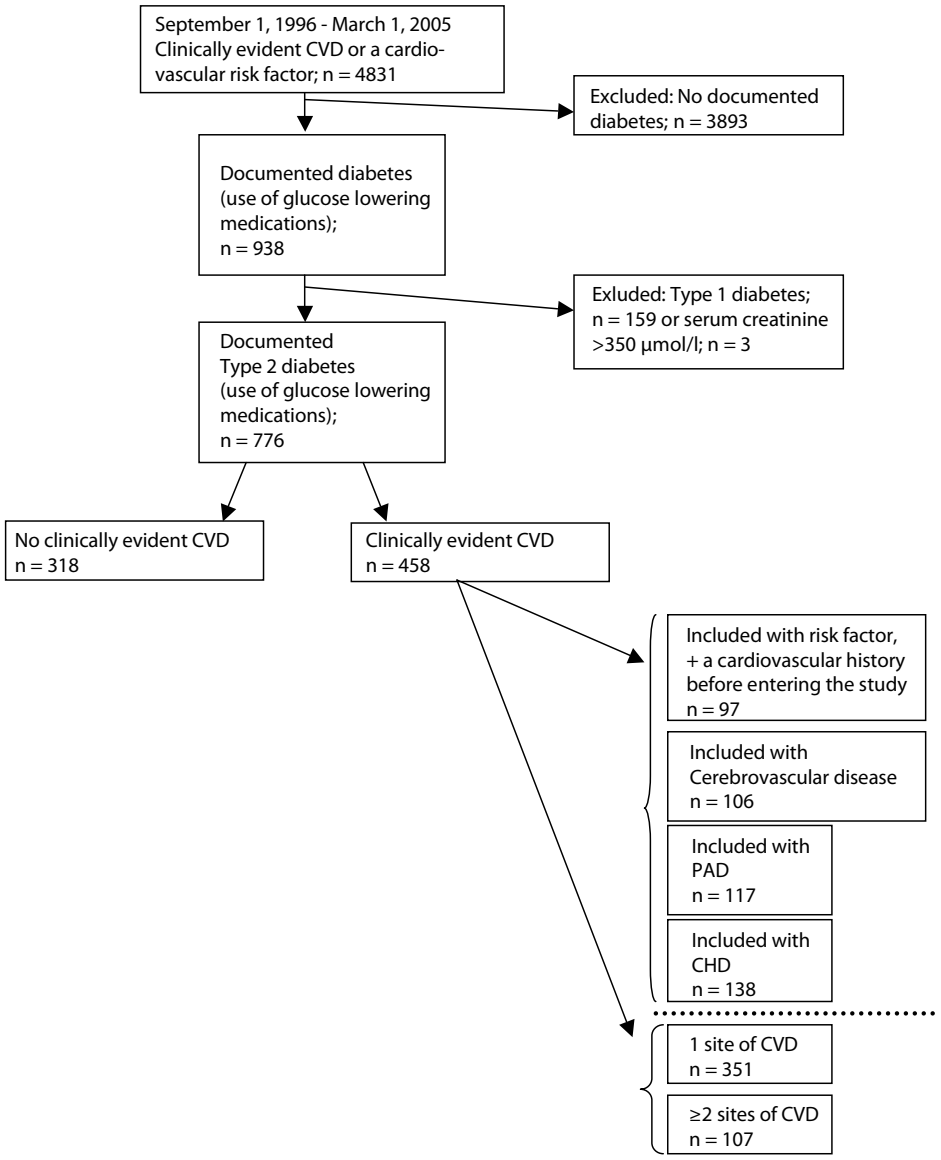


Figure 1. Study population. CVD, cardiovascular disease; PAD, peripheral arterial disease; CHD, coronary heart disease.

Table 1. Classification of clinically evident CVD categories based on inclusion diagnosis and cardiovascular history

Clinically evident CVD	Inclusion diagnosis	Cardiovascular history
Cerebrovascular disease	Cerebral ischemia, transient ischemic attack, amaurosis fugax, minor ischemic stroke, or retinal infarction	Stroke
Coronary heart disease	Patients with coronary artery stenosis on coronary angiography referred for elective PTCA or CABG	Myocardial infarction, angina pectoris, CABG or PTCA
Peripheral arterial disease	Intermittent claudication, rest pain, gangrene, ulcers, resting ABPI ≤0.90	Peripheral arterial disease, arterial surgery, PTA leg, or amputation leg
Abdominal aortic aneurysm	Distal aortic anteroposterior diameter ≥3.0 cm and/or distal/proximal ratio ≥1.5 cm	Abdominal aortic aneurysm, surgery for abdominal aortic aneurysm

CABG, coronary artery bypass graft; CVD, cardiovascular disease; PTCA, percutaneous transluminal coronary angioplasy; PTA, percutaneous transluminal angioplasty; ABPI, ankle brachial pressure index.

Follow-up

Biannually, patients were asked to complete a questionnaire on hospital admissions and out-patient clinic visits in the preceding 6 months. Events of interest for this study were ischemic stroke, myocardial infarction and cardiovascular death. Definitions of events are given in *Table 2*.[19] The primary outcome was the composite of these cardiovascular events and was defined as the first fatal or non-fatal cardiovascular event during follow-up. When a possible event was recorded by a participant, hospital discharge letters and results of relevant laboratory and radiology examinations were collected. With this information, all events were audited by three members of the Outcome Event Committee, comprising physicians from different departments. Follow-up duration (years) was defined as the period between study inclusion and first cardiovascular event, date of loss to follow-up, date of death not due to cardiovascular causes or the preselected date of 1 March 2005. Twenty-five of the 776 participants (3%) were lost to follow-up due to migration or discontinuation of the study.

Statistical analysis

Cumulative absolute risks for the incidence of a new cardiovascular event were computed with Kaplan-Meier survival analysis. Cox proportional hazard models were built to estimate the hazard ratios (HRs) with corresponding 95% confidence intervals (CIs) of the site of CVD (CHD, PAD or cerebrovascular disease) and the number of different sites affected for the occurrence of new cardiovascular events. If a patient had multiple events, the first was taken. Patients were censored if they were lost to follow-up or died due to non-cardiovascular causes. Subjects with diabetes without clinically evident CVD served as the reference group. In order to determine the impact of site of CVD on the risk of new cardiovascular events, subjects with diabetes and clinically evident CVD were categorized into four groups: those with a cardiovascular history but

Table 2. Definitions of events

Cardiovascular event	Cardiovascular death (as defined below) Ischemic stroke (as defined below) Myocardial infarction (as defined below)
Cardiovascular death	Sudden death: unexpected cardiac death occurring within 1 hour after onset of symptoms, or within 24 hours given convincing circumstantial evidence Death from ischemic stroke Death from intracerebral hemorrhage (hemorrhage on CT scan) Death from congestive heart failure Death from myocardial infarction Death from rupture of abdominal aortic aneurysm Cardiovascular death from other cause, such as sepsis following stent placement
Ischemic stroke	Definite: relevant clinical features that have caused an increase in impairment of at least one grade on the modified Rankin scale, accompanied by a fresh ischemic infarction on a repeat CT scan Probable: clinical features that have caused an increase in impairment of at least one grade on the modified Rankin scale; no CT documentation needed
Myocardial infarction	Myocardial infarction: at least two of the following criteria 1. chest pain for at least 20 minutes, not disappearing after administration of nitrates 2. ST elevation >1 mm in two following leads or a left bundle branch block on the ECG 3. CK elevation of at least two times the normal value of CK and a MB-fraction >5% of the total CK

CT, computer tomography; ECG, electrocardiogram; CK, creatinine kinase; MB, myocardial band.

no inclusion diagnosis of CVD, and those with an inclusion diagnosis of cerebrovascular disease, CHD or PAD. Four models were constructed, the first with adjustment for age and gender. In model II, additional adjustments were performed for other potentially confounding variables, namely diabetes duration, fasting glucose, use of insulin, and oral glucose-lowering agents. In model III, we additionally adjusted for body mass index, total cholesterol, HDL-cholesterol, systolic blood pressure and ever smoking to assess the effect of site of clinically evident CVD on the risk of new cardiovascular events independent of cardiovascular risk factors. Furthermore, the influence of atherosclerotic burden was determined by comparing the models with (model IV) and without (model II) adjustment for CIMT (continuously) and albuminuria.

For assessing the effect of the extent of CVD (number of different sites of clinically evident CVD), a participant was considered to have one site of clinically evident CVD regardless of the previous number of clinically evident episodes at that site. The four models mentioned above were constructed.
Variables measured on a continuous scale were used as such in the regression models, except for diabetes duration, which was categorized into quintiles because of the skewed distribution. Total cholesterol and fasting glucose were log-transformed to obtain normally distributed variables. The proportional hazards assumption was satisfied on the basis of logminlog plots.

Results

Baseline characteristics

In *Table 3*, the baseline characteristics of 776 patients with type 2 diabetes are listed: 318 patients (41%) without clinically evident CVD, 97 (13%) patients with a cardiovascular history and 361 patients (47%) with an inclusion diagnosis of clinically evident CVD stratified according to the cardiovascular site. Median duration of diabetes was equally distributed in patients with different sites of clinically evident CVD (range 4-5 years).
Of the subjects with diabetes and clinically evident CVD (n = 458), 77% had CVD at one site (n = 351) and 23% at two or more different sites (n = 107) (*Table 4*). Subjects with CVD at two or more different sites were older (64 ± 9 vs. 61 ± 10 vs. 54 ± 11 years; ANOVA p-value <0.0001) and had a longer duration of diabetes (6.0 (2.0-13.0) vs. 4.0 (1.0-10.0) vs. 2.0 (0.5-6.0) years; Kruskal-Wallis p-value <0.0001) than subjects with CVD at one site or without CVD. Atherosclerotic burden was most often present in patients with clinically evident CVD at two or more different sites (n = 107); 34 patients (32%) had albuminuria and the mean CIMT was 1.14 ± 0.34 mm. Mean CIMT was 1.21 ± 0.42 mm in patients with albuminuria (n = 34) and 1.11 ± 0.30 mm in those without albuminuria (n = 73).

Follow-up

During a median (interquartile range) follow-up of 2.9 (1.4-5.4) years, 2631 person-years, 87 patients experienced a first fatal or non-fatal cardiovascular event: 37 cardiovascular deaths, 31 myocardial infarctions (five occurred during percutaneous coronary intervention) and 19 ischemic strokes (two occurred during carotid endarterectomy). The number of non-cardiovascular deaths was 31 (4%).
The cumulative 3-year risk of a new cardiovascular event was 3.8% (95% CI 1.3, 6.2) in patients with diabetes without clinically evident CVD, and 15.2% (11.4, 18.9) in those with clinically evident CVD. The corresponding HR for new vascular events was 4.0 (95% CI 2.0, 8.1), adjusted for age, gender, diabetes duration, fasting glucose, use of insulin and oral glucose-lowering agents.

Site of clinically evident CVD and risk of new cardiovascular events

The cumulative 3-year risk of new cardiovascular events was 15.6% (95% CI 6.5, 24.8) in subjects with diabetes with a cardiovascular history but no inclusion diagnosis of CVD, 14.8% (95% CI 7.5, 22.0) in those with cerebrovascular disease, 13.9% (7.3, 20.1)

Table 3. Baseline characteristics in patients with type 2 diabetes (n = 776) with and without clinically evident CVD according to categories of CVD

	No clinically evident CVD	Cardiovascular history*	Inclusion diagnosis of cerebrovascular disease	Inclusion diagnosis of CHD	Inclusion diagnosis of PAD
	n = 318	n = 97	n = 106	n = 138	n = 117
Age (years)[1]	54 ± 11	59 ± 10	64 ± 9	61 ± 9	63 ± 10
Male gender, n (%)	188 (59)	59 (61)	81 (76)	105 (76)	89 (76)
BMI (kg/m^2)[1]	30 ± 6	29 ± 5	27 ± 4	29 ± 4	27 ± 4
Ever smoking, n (%)	208 (65)	69 (71)	88 (83)	103 (75)	96 (82)
Diabetes duration (years)[2]	2.0 (0.5-6.0)	4.0 (0.5-10.0)	4.0 (1.0-10.0)	5.0 (1.5-10.0)	4.0 (1.0-12.0)
Systolic blood pressure (mmHg)[1]	142 ± 20	145 ± 26	156 ± 20	142 ± 20	148 ± 20
Diastolic blood pressure (mmHg)[1]	85 ± 11	83 ± 13	84 ± 11	79 ± 10	78 ± 10
Fasting glucose (mmol/l)[2]	8.8 (7.1–11.5)	8.6 (6.9–10.9)	8.6 (6.6–10.7)	8.4 (7.1–10.7)	8.3 (7.0–10.3)
Triglycerides (mmol/l)[2]	1.93 (1.27–2.74)	1.72 (1.22–2.51)	1.65 (1.14–2.45)	1.72 (1.28–2.48)	1.86 (1.33–2.91)
HDL-cholesterol (mmol/l)[2]	1.13 (0.91–1.38)	1.11 (0.96–1.31)	1.06 (0.88-1.32)	1.05 (0.89–1.25)	1.05 (0.87-1.30)
LDL-cholesterol (mmol/l)[2]	3.1 (2.4-3.9)	3.1 (2.4-3.6)	3.0 (2.2-3.7)	2.6 (2.2-3.2)	3.2 (2.5-4.1)
Medication use					
Blood pressure-lowering agents, n (%)	150 (47)	71 (73)	63 (59)	111 (80)	62 (53)
Anti-platelet agents, n (%)	30 (9)	48 (50)	82 (77)	97 (70)	49 (42)
Lipid-lowering agents, n (%)	80 (25)	51 (53)	48 (45)	87 (63)	41 (35)
Oral glucose-lowering agents, n (%)	192 (60)	57 (59)	69 (65)	70 (51)	60 (51)
Use of insulin, n (%)	62 (20)	32 (33)	15 (14)	34 (25)	29 (25)

All data in number (percentages), or as indicated: [1]mean ± S.D. or [2]median (interquartile range).
* Patients without an inclusion diagnosis of clinically evident CVD, but with a cardiovascular history before entering the study.
† Patients with an inclusion diagnosis or a cardiovascular history of CHD, PAD or cerebrovascular disease. Disease groups are not mutually exclusive.
CVD, cardiovascular disease; PAD, peripheral arterial disease; CHD, coronary heart disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CIMT, carotid intima-media thickness.

Table 3. Continued

	No clinically evident CVD	Cardiovascular history*	Inclusion diagnosis of cerebrovascular disease	Inclusion diagnosis of CHD	Inclusion diagnosis of PAD
	n = 318	n = 97	n = 106	n = 138	n = 117
Atherosclerotic burden					
CIMT (mm)[1]	0.84 ± 0.22	0.98 ± 0.32	1.10 ± 0.38	0.97 ± 0.31	1.08 ± 0.35
Albuminuria >3.0 mg/mmol, n (%)	72 (23)	28 (29)	31 (29)	18 (13)	36 (31)
Presence of clinically evident CVD†					
Cerebrovascular disease, n (%)	-	31 (32)	106 (100)	8 (6)	24 (21)
CHD, n (%)	-	59 (61)	25 (24)	138 (100)	28 (24)
PAD, n (%)	-	23 (24)	13 (12)	8 (6)	117 (100)

All data in number (percentages), or as indicated: [1]mean ± S.D. or [2]median (interquartile range).
*Patients without an inclusion diagnosis of clinically evident CVD, but with a cardiovascular history before entering the study.
†Patients with an inclusion diagnosis or a cardiovascular history of CHD, PAD or cerebrovascular disease. Disease groups are not mutually exclusive.
CVD, cardiovascular disease; PAD, peripheral arterial disease; CHD, coronary heart disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CIMT, carotid intima-media thickness.

Table 4. Baseline characteristics in patients with type 2 diabetes (n = 776) according to the number of sites of clinically evident CVD

	No clinically evident CVD	Clinically evident CVD at one site*	Clinically evident CVD at two or more sites*
	n = 318	n = 351	n = 107
Age (years)[1]	54 ± 11	61 ± 10	64 ± 9
Male gender, n (%)	188 (59)	248 (71)	86 (80)
BMI (kg/m^2)[1]	30 ± 6	28 ± 4	27 ± 3
Ever smoking, n (%)	208 (65)	262 (75)	94 (88)
Diabetes duration (years)[2]	2.0 (0.5-6.0)	4.0 (1.0-10.0)	6.0 (2.0-13.0)
Systolic blood pressure (mmHg)[1]	142 ± 20	146 ± 22	153 ± 20
Diastolic blood pressure (mmHg)[1]	85 ± 11	81 ± 11	80 ± 11
Fasting glucose (mmol/l)[2]	8.8 (7.1–11.5)	8.6 (6.8–10.7)	8.3 (7.1–10.6)
Triglycerides (mmol/l)[2]	1.93 (1.27–2.74)	1.70 (1.23–2.48)	2.00 (1.46–2.81)
HDL-cholesterol (mmol/l)[2]	1.13 (0.91–1.38)	1.09 (0.91–1.29)	1.02 (0.85-1.26)
LDL-cholesterol (mmol/l)[2]	3.1 (2.4-3.9)	2.9 (2.3-3.6)	3.0 (2.3-3.6)
NCEP metabolic syndrome, n (%)	247 (78)	256 (73)	87 (81)
Medication use			
Blood pressure-lowering agents, n (%)	150 (47)	237 (68)	70 (65)
Anti-platelet agents, n (%)	30 (9)	205 (58)	71 (66)
Lipid-lowering agents, n (%)	80 (25)	159 (45)	68 (64)
Oral glucose-lowering agents, n (%)	192 (60)	204 (58)	52 (49)
Use of insulin, n (%)	62 (20)	76 (22)	34 (32)
Atherosclerotic burden			
CIMT (mm)[1]	0.84 ± 0.22	1.00 ± 0.34	1.14 ± 0.34
Albuminuria >3.0 mg/mmol, n (%)	72 (23)	79 (23)	34 (32)
Presence of clinically evident CVD*			
Cerebrovascular disease, n (%)	-	96 (27)	73 (68)
CHD, n (%)	-	169 (48)	81 (76)
PAD, n (%)	-	86 (25)	75 (70)

All data in number (percentages), or as indicated: [1]mean ± S.D. or [2]median (interquartile range).
* Patients with an inclusion diagnosis or a cardiovascular history of CHD, PAD and/or cerebrovascular disease.
CVD, cardiovascular disease; PAD, peripheral arterial disease; CHD, coronary heart disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; CIMT, carotid intima-media thickness; NCEP, National Cholesterol Educational Program.

in those with CHD, and 18.2% (10.2, 26.1) in those with PAD. As shown in *Table 5*, the corresponding HRs for new cardiovascular events, adjusted for potential confounders (model II), were similar regardless of the site of CVD, compared with data for patients with diabetes without CVD.
Atherosclerotic burden did not influence the relationship between different sites of CVD and new cardiovascular events, because adjustments for CIMT and albuminuria did not materially affect the strengths of the HRs (model II vs. model IV).

Extent of clinically evident CVD and risk of new cardiovascular events

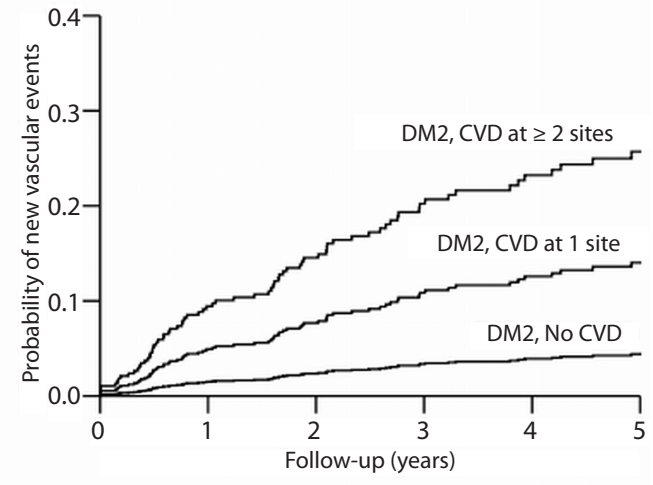
The cumulative 3-year risk of new cardiovascular events was 11.5% (95% CI 7.7, 15.3) in those with clinically evident CVD at one site and 25.9% (16.6, 35.3) in those with clinically evident CVD at two or more sites. Compared with patients with diabetes without CVD, those with CVD at one site had a HR for a cardiovascular event of 3.4 (95% CI 1.6, 6.9) adjusted for potential confounders (model II), those with CVD at two or more sites an adjusted HR of 6.6 (3.0, 14.3) (*Table 5*). After adjustment for atherosclerotic burden, the associations were not materially altered (HR 3.3 (1.5, 6.8); HR 6.8 (3.1, 14.9)) (model IV).

Table 5. Hazard ratios (HR) for new cardiovascular events in patients with type 2 diabetes and clinically evident CVD according to the site of CVD and the number of different affected sites of CVD

	N	No. of events	Model I	Model II	Model III	Model IV
			HR (95% CI)	HR (95% CI)	HR (95% CI)	HR (95% CI)
Site of clinically evident CVD						
No clinically evident CVD	318	10	Reference	Reference	Reference	Reference
Cardiovascular history*	97	13	3.8 (1.6-8.6)	3.5 (1.5-8.1)	3.3 (1.4-7.8)	3.4 (1.4-7.9)
Cerebrovascular disease†	106	21	3.8 (1.7-8.4)	3.8 (1.7-8.5)	3.7 (1.6-8.6)	3.7 (1.6-8.6)
CHD†	138	20	4.4 (2.0-9.7)	4.3 (1.9-9.5)	4.0 (1.8-8.9)	4.4 (2.0-9.9)
PAD†	117	23	4.6 (2.1-9.9)	4.6 (2.1-10.2)	4.5 (2.0-10.2)	4.2 (1.8-9.3)
Extent of clinically evident CVD						
No clinically evident CVD	318	10	Reference	Reference	Reference	Reference
Clinically evident CVD at 1 site‡	351	47	3.6 (1.8-7.2)	3.4 (1.6-6.9)	3.2 (1.6-6.7)	3.3 (1.5-6.8)
Clinically evident CVD at ≥2 sites‡	107	30	6.3 (2.9-13.4)	6.6 (3.0-14.3)	6.3 (2.9-13.9)	6.8 (3.1-14.9)

Model I, adjusted for age and gender.
Model II, Model I with additional adjustment for diabetes duration (quintiles), (log) fasting glucose, use of insulin, and oral glucose-lowering agents.
Model III, Model II with additional adjustment for body mass index, (log) total cholesterol, HDL-cholesterol, systolic blood pressure, and ever smoking.
Model IV, Model II with additional adjustment for CIMT and albuminuria.
* Patients without an inclusion diagnosis of clinically evident CVD, but with a cardiovascular history before entering the study.
† Patients with an inclusion diagnosis of clinically evident CVD.
‡ Patients with an inclusion diagnosis or a cardiovascular history of CHD, PAD, and/or cerebrovascular disease.
Median (interquartile range) follow-up was 2.9 (1.4-5.4) years. CVD, cardiovascular disease; PAD, peripheral arterial disease; CHD, coronary heart disease; HDL, high-density lipoprotein; CIMT, carotid intima-media thickness.

Figure 2 displays a Cox proportional hazard survival curve according to the extent of clinically evident CVD and adjusted for potential confounders. The incidence of cardiovascular events increased with the number of sites of clinically evident CVD.



No. at Risk						
DM2, No CVD	318	264	222	157	125	102
DM2, CVD at 1 site	351	280	221	164	126	90
DM2, CVD at ≥2 sites	107	86	67	55	42	30

Figure 2. Probability of a new cardiovascular event stratified for type 2 diabetes (DM2) and number of sites of clinically evident cardiovascular disease (CVD). Adjusted for age, gender, diabetes duration, fasting glucose, use of insulin, and oral glucose-lowering agents.

Discussion

In the present study, patients with type 2 diabetes and cerebrovascular disease, CHD or PAD had a similar three- to fourfold higher risk of new cardiovascular events compared with patients with diabetes without CVD. Moreover, it was found that subjects with diabetes and clinically evident CVD at two or more sites had a sixfold higher risk of new events than those without CVD. This risk was not influenced by the extent of atherosclerotic burden.

Previous studies have investigated the cardiovascular risk in subjects without diabetes or CHD, those with diabetes, those with CHD and those with both diabetes and CHD.[4,7,12,15] In the present study, the risk of cardiovascular events was quantified and compared in a single cohort of patients with diabetes with and without clinically evident CVD, stratified by the cardiovascular site affected. In a 10-year follow-up study, subjects with diabetes and CVD (stroke, CHD or PAD) had a sixfold higher age-adjusted risk of cardiovascular events than those without diabetes or CVD.[9] The same relative risk was found when patients with diabetes and only myocardial infarction were studied. The 10-year age-adjusted risk of CHD mortality in subjects with diabetes who

previously had a myocardial infarction was higher (HR 10.6; 6.9, 16.0) than in those with a prior stroke (HR 3.9; 2.0, 7.6)[13], compared with those without diabetes.

In the current study, the risk of cardiovascular events was increased in patients with type 2 diabetes with already clinically evident CVD irrespective of the site (cerebrovascular disease, CHD or PAD). A possible explanation may be that we studied patients with advanced atherosclerotic disease. Atherosclerosis is considered to be a generalized process, particularly in a high-risk population. Therefore, these patients may already have had widespread (sub)clinical atherosclerosis at other sites of CVD and were prone to new cardiovascular events.[23,24] Atherosclerotic plaques become manifest at sites with a higher plaque vulnerability, but are present in all arterial beds.[25] It is therefore likely that subjects with diabetes with already clinically evident CVD have a similar cardiovascular risk regardless of the site of the atherosclerotic manifestation.

In addition, the risk of new cardiovascular events according to the extent of clinically evident CVD has not been quantified previously in subjects with diabetes. Notably, patients with diabetes have a higher number of affected coronary vessels than those without[26], and increased severity of CHD is associated with a poorer long-term prognosis.[27] Interestingly, a recent community-based study has shown that multiple disease locations increase the 1-year event rate of cardiovascular events by a factor of approximately 2 in subjects without diabetes.[16] In concordance with this study, we have shown that patients with diabetes with two or more sites of clinically evident CVD have a twofold higher risk of new cardiovascular events compared with those with one site of CVD. The association remained strong even after additional adjustment for age, diabetes duration, fasting glucose, and traditional cardiovascular risk factors, indicating that these factors do not explain the excess risk.

Atherosclerotic burden, as measured by CIMT or albuminuria, did not influence the relationships between site and extent of clinically evident CVD and the occurrence of new cardiovascular events in subjects with diabetes. This indicates that although the presence of atherosclerotic burden contributes to disease progression, it does not incorporate additional prognostic information above the cardiovascular damage already sustained in patients with diabetes and evident CVD. An explanation for this might be that clinically evident CVD already reflects the arterial damage brought about by cardiovascular risk factors and atherosclerotic burden. Measures of atherosclerotic burden do not indicate higher risk above the presence of evident CVD and are therefore not of use for risk stratification in patients with diabetes and clinically evident CVD.

Our findings indicate that the prognostic implications for the occurrence of new cardiovascular events for different sites of CVD seem to be equal in subjects with diabetes. Moreover, these data support the view that there is variability in cardiovascular risk in patients with diabetes.[11] Counting the number of cardiovascular sites affected may improve risk stratification in patients with diabetes. It is an easy way in clinical practice to identify patients at highest risk of recurrent events from the generally high-risk diabetes population, who may gain from lower treatment goals for dyslipidemia than currently recommended.[28]

The present study had a number of potential limitations. First, misclassification of clinically evident CVD may have occurred. However, this will only lead to an underestimation of the effect of pre-existing CVD. Second, HbA_{1c} was not measured routinely in the screening programme, and thus we could not adjust for glycemic

control. Third, we were unable to do subanalyses on specific cardiovascular outcomes due to the limited number of events. Fourth, although the median follow-up was 2.9 years (1.4-5.4), a longer follow-up would provide more information about the impact of different clinically evident CVD and its extent on the prognosis of patients with diabetes. Nevertheless, our results suggest there is no reason to expect that the effects of clinically evident CVD on the risk of cardiovascular events would change with longer follow-up (*Figure 1*). Lastly, the atherosclerotic burden was not assessed in the coronary bed. However, CIMT can be used as a reliable marker of generalized atherosclerotic burden in the coronary and other cardiovascular beds.[20,21]

In conclusion, these prospective findings indicate that patients with type 2 diabetes and clinically evident CVD have a three- to fourfold higher risk of experiencing new cardiovascular events than patients with diabetes without CVD, irrespective of the site of CVD. This risk increases markedly with the number of different sites of CVD and is not influenced by atherosclerotic burden. These data may contribute to the identification of patients with diabetes at the highest risk of new cardiovascular events.

Acknowledgements

The help of M. Edlinger for data coordination is gratefully acknowledged. The SMART study was financially supported by a grant of the University Medical Centre Utrecht.

References

1. Kannel WB, McGee DL. Diabetes and glucose tolerance as risk factors for cardiovascular disease: the Framingham study. *Diabetes Care.* 1979;2:120-126.
2. Stamler J, Vaccaro O, Neaton JD et al. Diabetes, other risk factors, and 12-yr cardiovascular mortality for men screened in the Multiple Risk Factor Intervention Trial. *Diabetes Care.* 1993;16:434-444.
3. Vinik A, Flemmer M. Diabetes and macrovascular disease. *J Diabetes Complications.* 2002;16:235-245.
4. Haffner SM, Lehto S, Rönnemaa T et al. Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. *N Engl J Med.* 1998;339:229-234.
5. Lotufo PA, Gaziano JM, Chae CU et al. Diabetes and all-cause and coronary heart disease mortality among US male physicians. *Arch Intern Med.* 2001;161:242-247.
6. Evans JM, Wang J, Morris AD. Comparison of cardiovascular risk between patients with type 2 diabetes and those who had had a myocardial infarction: cross sectional and cohort studies. *BMJ.* 2002;324:939-942.
7. Cho E, Rimm EB, Stampfer MJ et al. The impact of diabetes mellitus and prior myocardial infarction on mortality from all causes and from coronary heart disease in men. *J Am Coll Cardiol.* 2002;40:954-960.
8. Eberly LE, Cohen JD, Prineas R et al. Impact of incident diabetes and incident nonfatal cardiovascular disease on 18-year mortality: the multiple risk factor intervention trial experience. *Diabetes Care.* 2003;26:848-854.
9. Becker A, Bos G, de Vegt F et al. Cardiovascular events in type 2 diabetes: comparison with nondiabetic individuals without and with prior cardiovascular disease. 10-year follow-up of the Hoorn Study. *Eur Heart J.* 2003;24:1406-1413.
10. Juutilainen A, Lehto S, Rönnemaa T et al. Type 2 diabetes as a 'coronary heart disease equivalent': an 18-year prospective population-based study in Finnish subjects. *Diabetes Care.* 2005;28:2901-2907.
11. Howard BV, Best LG, Galloway JM et al. Coronary heart disease risk equivalence in diabetes depends on concomitant risk factors. *Diabetes Care.* 2006;29:391-397.
12. Hu FB, Stampfer MJ, Solomon CG et al. The impact of diabetes mellitus on mortality from all causes and coronary heart disease in women: 20 years of follow-up. *Arch Intern Med.* 2001;161:1717-1723.
13. Ho JE, Paultre F, Mosca L. Is diabetes mellitus a cardiovascular disease risk equivalent for fatal stroke in women? Data from the Women's Pooling Project. *Stroke.* 2003;34:2812-2816.
14. Grundy SM, Cleeman JI, Merz CN et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation.* 2004;110:227-239.
15. Lee CD, Folsom AR, Pankow JS et al. Cardiovascular events in diabetic and nondiabetic adults with or without history of myocardial infarction. *Circulation.* 2004;109:855-860.
16. Steg PG, Bhatt DL, Wilson PW et al. One-year cardiovascular event rates in outpatients with atherothrombosis. *JAMA.* 2007;297:1197-1206.
17. Asakawa H, Tokunaga K, Kawakami F. Comparison of risk factors of macrovascular complications. Peripheral vascular disease, cerebral vascular disease, and coronary heart disease in Japanese type 2 diabetes mellitus patients. *J Diabetes Complications.* 2000;14:307-313.
18. Yamasaki Y, Kodama M, Nishizawa H et al. Carotid intima-media thickness in Japanese type 2 diabetic subjects: predictors of progression and relationship with incident coronary heart disease. *Diabetes Care.* 2000;23:1310-1315.
19. Simons PC, Algra A, van de Laak MF et al. Second Manifestations of ARTerial disease (SMART) study: rationale and design. *Eur J Epidemiol.* 1999;15:773-781.
20. Bots ML, Hofman A, De Jong PT et al. Common carotid intima-media thickness as an indicator of atherosclerosis at other sites of the carotid artery. The Rotterdam Study. *Ann Epidemiol.* 1996;6:147-153.
21. Hodis HN, Mack WJ, LaBree L et al. The role of carotid arterial intima-media thickness in predicting clinical coronary events. *Ann Intern Med.* 1998;128:262-269.

22. Gerstein HC, Mann JF, Yi Q et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *JAMA.* 2001;286:421-426.
23. Kuller LH, Velentgas P, Barzilay J et al. Diabetes mellitus: subclinical cardiovascular disease and risk of incident cardiovascular disease and all-cause mortality. *Arterioscler Thromb Vasc Biol.* 2000;20:823-829.
24. Cerne A, Kranjec I. Atherosclerotic burden in coronary and peripheral arteries in patients with first clinical manifestation of coronary artery disease. *Heart Vessels.* 2002;16:217-226.
25. Davies MJ. Acute coronary thrombosis--the role of plaque disruption and its initiation and prevention. *Eur Heart J.* 1995;16 Suppl. L:3-7.
26. Fallow GD, Singh J. The prevalence, type and severity of cardiovascular disease in diabetic and non-diabetic patients: a matched-paired retrospective analysis using coronary angiography as the diagnostic tool. *Mol Cell Biochem.* 2004;261:263-269.
27. Figueras J, Domingo E, Hermosilla E. Long-term prognosis of clinical variables, coronary reserve and extent of coronary disease in patients with a first episode of unstable angina. *Int J Cardiol.* 2005;98:27-34.
28. Shepherd J, Barter P, Carmena R et al. Effect of lowering LDL cholesterol substantially below currently recommended levels in patients with coronary heart disease and diabetes: the Treating to New Targets (TNT) study. *Diabetes Care*. 2006;29:1220-1226.

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Intra-abdominal fat and metabolic syndrome are associated with larger infrarenal aortic diameters in patients with manifest arterial disease. The SMART study

Abstract

Objective

Abdominal obesity and its associated metabolic consequences are major determinants for the development of vascular disease. Fat tissue in close proximity of arteries may also directly affect atherogenesis. Aim was to examine whether intra-abdominal fat accumulation is an independent determinant of infrarenal aortic diameter in patients with manifest arterial disease. In addition, the relationship between metabolic syndrome and infrarenal aortic diameter was assessed in this patient group.

Methods

Cross-sectional study in 2726 patients with manifest arterial disease enrolled in the SMART study (Second Manifestations of ARTerial disease). Intra-abdominal fat was measured with ultrasonography and by measuring waist circumference. Metabolic syndrome was defined according to Adult Treatment Panel III. The maximal anteroposterior diameter of the infrarenal aorta was measured using ultrasonography. The relation between intra-abdominal fat, as well as metabolic syndrome, and infrarenal aortic diameter was determined with linear regression analyses.

Results

Infrarenal aortic diameter (mm) increased across quartiles of intra-abdominal fat derived by ultrasonography (quartile 4 vs. quartile 1, β 1.38; 95% CI 0.76-2.01) and across quartiles of waist circumference (quartile 4 vs. quartile 1, β 1.56; 95% CI 0.93-2.19) after adjustment for age, gender, height and smoking. Patients with metabolic syndrome had slightly larger infrarenal aortic diameters (β 0.70; 95% CI 0.27-1.13, adjusted for age, gender, height and smoking) compared to those without metabolic syndrome.

Conclusions

Intra-abdominal fat accumulation and metabolic syndrome are associated with larger infrarenal aortic diameters in patients with manifest arterial disease. These data indicate a role for intra-abdominal fat in the development of larger aortic diameters.

Introduction

Abdominal obesity is associated with an increased risk of atherosclerotic vascular disease.[1] Intra-abdominal fat accumulation causes an altered adipocyte function, leading to high systemic plasma levels of tumor necrosis factor-alpha (TNF-α), interleukine-6 (IL-6), and free fatty acids, as well as low plasma concentrations of adiponectin, all involved in accelerating atherosclerosis[2] and contributing to the development of insulin resistance.[3] Insulin resistance is considered to be an essential feature in the development of vascular risk factors (e.g. elevated blood pressure, dyslipidemia, and hyperglycemia).[4] The clustering of all these metabolic abnormalities, often referred to as metabolic syndrome[5], is most often the result of obesity-induced insulin resistance and is associated with a high cardiovascular risk.[6]

Patients with an enlarged diameter of the abdominal aortic artery are at high risk for cardiovascular morbidity and mortality.[7,8] In addition to proteolytic degradation and remodeling of the elastic media[9,10], atherosclerosis is recognized as an important feature in the process of aortic dilatation.[11] Indeed, vascular risk factors, such as smoking[12-15], elevated blood pressure[15-17], body weight[16-18] and dyslipidemia[13-15,17], are associated with an enlarged diameter of the abdominal aorta in the general population. Inflammatory cytokines appear to be involved as well[18-21] by mediating connective tissue destruction of the aortic wall.[22]

Intra-abdominal fat accumulation and its associated metabolic abnormalities may contribute to the dilatation of the abdominal aortic artery by accelerating aortic wall degeneration. Since obesity-related insulin resistance can induce elevated blood pressure by activation of the sympathetic nerve system, increased renal sodium retention and endothelial dysfunction[23], this could be one of the underlying mechanisms. Moreover, obesity-induced insulin resistance and high plasma levels of inflammatory cytokines might aggravate inflammation in the peri-aortic fat, which is in close proximity to the aortic adventitia and locally secretes inflammatory cytokines.[24]

Although increased intra-abdominal fat and metabolic syndrome are highly prevalent in patients with manifest arterial disease[25], it is not known whether they are related to abdominal aortic diameter in these high-risk patients. Aim of the current etiologic study was to examine whether intra-abdominal fat accumulation is an independent determinant of infrarenal aortic diameter in patients with manifest arterial disease. In addition, the relationship between metabolic syndrome and infrarenal aortic diameter was assessed in these patients.

Methods

Study settings, participants and design

In this study, we used data from patients enrolled in the Second Manifestations of ARTerial disease (SMART) study. The SMART study is an ongoing prospective single-centre cohort study in patients with manifest atherosclerotic arterial disease or cardiovascular risk factors.[26] Started in September 1996, patients aged 18-80, were referred to the University Medical Centre (UMC) Utrecht with a recent diagnosis of manifest arterial disease or a cardiovascular risk factor. Not approached are patients with terminal malignant disease, those not independent in daily activities (Rankin scale >3) or not sufficiently fluent in Dutch. Patients entered the SMART study if participation in the study was supported by the treating specialist and if the patients themselves consented to participate. The Medical Ethics Committee approved the study, and all participants gave their written informed consent. The rationale and design of the SMART study and a detailed description of the criteria used to define the different manifest arterial diseases were published previously.[26]
For the current study, analyses were based on the inclusion period from May 2000 to March 2007 and were limited to patients with manifest arterial disease (coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm) (n = 3020). A total of 64 patients with previous surgery for abdominal aortic aneurysm were excluded. Also, 195 patients were excluded because of incomplete data for waist circumference and intra-abdominal fat. Subsequently, 35 patients were excluded due to poor visualization of the aorta. Finally, 2726 patients were used for the analyses.

Data acquisition

All patients underwent a non-invasive standardised diagnostic protocol on a single day at the UMC Utrecht. Medical history, use of current medication, and current and past smoking habits were derived form a standardised health questionnaire described elsewhere.[26] Physical examination (weight, height, waist circumference, diastolic and systolic blood pressure) was performed. Height and weight were measured while participants wore indoor clothes and no shoes. Blood pressure was measured two times in sitting position at the right and left upper arm with a non-random sphygmomanometer. The mean value of the two blood pressure measurements was taken as the blood pressure. Blood samples were collected after an overnight fast. Total cholesterol, triglycerides, high-density lipoprotein (HDL)-cholesterol, glucose and creatinine levels were measured. Low-density lipoprotein (LDL)-cholesterol was calculated by use of Friedewald`s formula if triglyceride plasma level was <4.5 mmol/l. The left and right ankle-brachial pressure indexes (ABPI) at rest were determined.[26]

Definitions

Metabolic syndrome was defined according to the Adult Treatment Panel (ATP) III criteria.[5] Metabolic syndrome requires the presence of at least three of the following metabolic abnormalities: abdominal obesity (waist circumference >102 cm in men and >88 cm in women), high blood pressure (≥130 mmHg systolic or ≥85 mmHg

diastolic), hypertriglyceridemia (serum triglycerides ≥1.70 mmol/l (150mg/dl)), low HDL-cholesterol (serum HDL-cholesterol <1.04 mmol/l (40 mg/dl) in men and <1.29 mmol/l (50 mg/dl) in women), high fasting glucose (fasting serum glucose ≥5.6 mmol/l (100 mg/dl)). Patients on glucose-lowering agents or anti-hypertensive medication were regarded as having high fasting glucose or high blood pressure, respectively. Presence of documented diabetes mellitus was ascertained by medical history (use of glucose-lowering medications). Ever smoking was defined as current or past smoking including currently smoking patients, those who recently stopped and smoked in the past. A reduced ABPI was defined as a resting ABPI ≤0.9.[26]

Assessment of intra-abdominal fat

Intra-abdominal fat was estimated anthropometrically and ultrasonographically. Waist circumference was measured halfway between the lower rib and the iliac crest and was taken in standing position. B-mode ultrasound of the abdomen was obtained to measure intra-abdominal fat and performed by well-trained registered vascular technologists in a certified vascular laboratory. Ultrasonographic measurements were made in supine position using an ATL HDI 3000 (Philips Medical Systems, Eindhoven, Netherlands) with a C 4-2 transducer. There was no bowel prep performed before the ultrasound measurement. Intra-abdominal fat was ultrasonographically measured as the distance between the peritoneum and the lumbar spine or psoas muscles using electronic calipers. A strict protocol, including the position of and pressure on the transducer, was used. The transducer was placed on a straight line drawn between the left and right midpoints of the lower rib and the iliac crest. Measurements were performed at the end of a quiet inspiration, applying minimal pressure without displacement or compression of the abdominal cavity. The distance was measured three times at three different positions.[27] Previously, the ultrasound protocol for measuring intra-abdominal fat was compared with computed tomography (CT) at our center.[27] Ultrasonographic measurements were strongly associated with CT measurements of intra-abdominal fat: Pearson`s correlation coefficient was 0.81 ($p < 0.001$). Also, an inter-observer coefficient of variation of 5.4% was found for ultrasound measurements of intra-abdominal fat, indicating good reproducibility.

Assessment of infrarenal aortic diameter

B-mode ultrasound scanning was carried out to measure the maximal anteroposterior diameter of the infrarenal aorta using the same ATL HDI 3000 as for intra-abdominal fat. The measurements were performed in transverse sections, taking special care to perform the diameter measurements perpendicular to the longitudinal axis of the vessel. The echo-free lumen of the vessel was measured between the inner edge of the anterior wall and the inner edge of the posterior wall.[26] Ultrasonography is a reliable method to assess the infrarenal aortic diameter.[28,29]

Statistical analysis

The association between intra-abdominal fat and infrarenal aortic diameter was quantified using linear regression analysis. Intra-abdominal fat was categorized into quartiles, and the lowest quartile was considered as reference. Results are expressed

as beta (β) coefficients with 95% confidence intervals (CI). Three models were used to estimate the relation between intra-abdominal fat as independent and infrarenal aortic diameter as dependent variable. In the first model adjustments were made for age and gender. In the second model, additional adjustments were performed for other potentially confounding variables, namely height and ever smoking. In the third model, additional adjustment was performed for intermediate variables (triglycerides, HDL-cholesterol, fasting glucose, systolic blood pressure) to investigate whether they explained the association between intra-abdominal fat and infrarenal aortic diameter. The covariates were included as continues variables, except gender and ever smoking were included as categorical variables. Potential effect modification of a reduced ABPI and presence of diabetes[30] on the relationship between intra-abdominal fat and infrarenal aortic diameter was investigated by entering the cross-products of a reduced ABPI, as well as diabetes, and intra-abdominal fat (continuously) in the regression models. In assessing the potential modifying effect of a reduced ABPI, diabetic patients (n = 321) were excluded because ABPI assessment may be inadequate in these patients because of medial arterial calcification.[31] Interaction was considered present when the p-value of the interaction term in the model was <0.05.

Linear regression analysis was also performed with metabolic syndrome and the number of components of metabolic syndrome as independent variables respectively and infrarenal aortic diameter as dependent variable, and adjusted for age, gender, height and ever smoking.

To reduce bias and increase statistical efficiency, missing values (fasting glucose (n = 33), total cholesterol (n = 23), triglycerides (n = 30), HDL-cholesterol (n = 30), creatinine (n = 26), diastolic (n = 10) and systolic (n = 10) blood pressure, and ABPI (n = 17)) in the data were completed by regression imputation.[32,33]

Results

Baseline characteristics

In *Table 1* the baseline characteristics of the study population are listed according to quartiles of ultrasonographically measured intra-abdominal fat. The distribution of characteristics was comparable across quartiles of waist circumference (data not shown). Mean age was 59 ± 10 years, and 74% of patients were male. Mean intra-abdominal fat was 9.3 ± 2.6 cm and mean waist circumference was 95 ± 12 cm. Prevalence of metabolic syndrome was 46%; metabolic syndrome was more prevalent in the highest quartile of ultrasonographically measured intra-abdominal fat compared to the lowest quartile (76% versus 21%). Individual components of metabolic syndrome were generally more adverse among patients with more intra-abdominal fat.

Table 1. Baseline characteristics in patients with manifest arterial disease according to quartiles of intra-abdominal fat (n = 2726)

Intra-abdominal fat (cm)	**Quartile 1**	**Quartile 2**	**Quartile 3**	**Quartile 4**
(Range)	(2.7-7.5)	(7.6-9.0)	(9.1-10.9)	(11.0-20.6)
	n = 724	n = 646	n = 707	n = 649
Intra-abdominal fat (cm)	6.2 ± 1.0	8.3 ± 0.4	9.9 ± 0.5	12.8 ± 1.6
Age (years)	57 ± 11	59 ± 10	60 ± 10	60 ± 10
Male gender, n (%)	403 (56)	478 (74)	585 (83)	559 (86)
Body mass index (kg/m^2)	24 ± 3	26 ± 3	28 ± 3	30 ± 4
Ever smoking (current or past), n (%)	534 (74)	509 (79)	598 (85)	550 (85)
Diabetes mellitus*	54 (8)	52 (8)	88 (12)	127 (20)
Creatinine clearance (ml/min/1.73m2)†	76 ± 21	77 ± 21	78 ± 23	82 ± 25
LDL-cholesterol (mmol/l)‡	2.6 (2.0-3.3)	2.7 (2.1-3.5)	2.7 (2.1-3.4)	2.7 (2.1-3.3)
Use of lipid-lowering agents, n (%)	416 (58)	396 (61)	464 (66)	416 (64)
Use of blood pressure-lowering agents, n (%)	419 (58)	418 (65)	501 (71)	465 (72)
Metabolic syndrome				
Metabolic syndrome ATP III, n (%)	152 (21)	235 (36)	384 (54)	496 (76)
Fasting glucose (mmol/l)	5.5 (5.1-5.9)	5.7 (5.3-6.2)	5.9 (5.4-6.6)	6.2 (5.7-7.5)
Triglycerides (mmol/l)	1.15 (0.90-1.53)	1.35 (1.03-1.93)	1.53 (1.15-2.09)	1.79 (1.28-2.60)
HDL-cholesterol (mmol/l)	1.36 (1.11-1.70)	1.23 (1.04-1.48)	1.16 (0.96-1.40)	1.11 (0.91-1.33)
Waist circumference (cm)	85 ± 10	93 ± 8	98 ± 8	106 ± 9
Blood pressure systolic (mmHg)	139 ± 22	142 ± 21	144 ± 21	145 ± 21
Blood pressure diastolic (mmHg)	81 ± 11	82 ± 11	84 ± 11	84 ± 11
Manifest arterial disease§				
Cerebrovascular disease, n (%)	264 (37)	171 (27)	193 (27)	146 (23)
Coronary artery disease, n (%)	384 (53)	410 (64)	451 (64)	457 (70)
Peripheral arterial disease, n (%)	127 (18)	113 (18)	120 (17)	123 (19)
Abdominal aortic aneurysm, n (%)	35 (5)	38 (6)	49 (7)	56 (9)

All data in n (%), mean ± S.D. or median (interquartile range).
* Patients on glucose-lowering agents.
† According to Cockroft-Gault.
‡ Calculated by use of Friedewald`s formula.
§ Ever or current diagnosis, a single person can be classified into more than one disease category.

Intra-abdominal fat measurements in relation to infrarenal aortic diameter

Mean infrarenal aortic diameter was 18 ± 6 mm; male patients had larger infrarenal aortic diameters than female patients (19 ± 6 mm versus 15 ± 4 mm). Infrarenal aortic diameter was 19 ± 7 mm in patients within the highest intra-abdominal fat quartile compared to 16 ± 5 mm in patients within the lowest quartile. Infrarenal aortic diameter (mm) increased across quartiles of intra-abdominal fat (quartile 4 versus quartile 1,

β 1.46; 95% CI 0.84-2.09) and across quartiles of waist circumference (quartile 4 versus quartile 1, β 1.83; 95% CI 1.20-2.46) after adjustment for age, and gender (*Table 2*). This indicates that patients within the highest quartile of intra-abdominal fat or waist circumference had respectively 1.46 mm and 1.83 mm larger infrarenal aortic diameters than those within the lowest quartile. Height and ever smoking did not materially change the relationship of intra-abdominal fat (quartile 4 versus quartile 1, β 1.38; 95% CI 0.76-2.01), and waist circumference (quartile 4 versus quartile 1, β 1.56; 95% CI 0.93-2.19) with infrarenal aortic diameter. Additional adjustment for intermediate variables (triglycerides, HDL-cholesterol, fasting glucose, and systolic blood pressure) did not markedly change the associations.

Table 2. Intra-abdominal fat in relation to infrarenal aortic diameter (mm) in patients with manifest arterial disease (n = 2726)

	Intra-abdominal fat (cm)			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
(Range)	(2.7-7.5)	(7.6-9.0)	(9.1-10.9)	(11.0-20.6)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
I	0	0.36 (-0.25; 0.98)	0.45 (-0.16; 1.06)	1.46 (0.84; 2.09)
II	0	0.31 (-0.30; 0.92)	0.39 (-0.22; 1.00)	1.38 (0.76; 2.01)
III	0	0.27 (-0.35; 0.88)	0.35 (-0.27; 0.97)	1.38 (0.72; 2.04)
	Waist circumference (cm)			
	Quartile 1	Quartile 2	Quartile 3	Quartile 4
(Range)	(59-88)	(89-95)	(96-102)	(103-149)
	β (95% CI)	β (95% CI)	β (95% CI)	β (95% CI)
I	0	0.51 (-0.11; 1.13)	0.80 (0.17; 1.42)	1.83 (1.20; 2.46)
II	0	0.39 (-0.23; 1.00)	0.55 (-0.08; 1.18)	1.56 (0.93; 2.19)
III	0	0.30 (-0.32; 0.92)	0.49 (-0.15; 1.14)	1.53 (0.87; 2.19)

All data: Beta regression coefficients (β) with 95% confidence interval (CI).
β indicates the difference in infrarenal aortic diameter (mm) relative to the quartile with the lowest intra-abdominal fat or waist circumference.
Model I: age and gender adjusted.
Model II: Model I, additional adjusted for height, and ever smoking.
Model III: Model II, additional adjusted for triglycerides, HDL-cholesterol, fasting glucose, systolic blood pressure.

The magnitude of the positive association between intra-abdominal fat (ultrasonography) and infrarenal aortic diameter was higher in the 450 patients with a reduced ABPI (Model II: β 0.46; 95% CI 0.21-0.71), than in the 1955 patients without a reduced ABPI (Model II: β 0.19; 95% CI 0.09-0.30), p-value for interaction = 0.02. The relationship between intra-abdominal fat (ultrasonography) and infrarenal aortic diameter was not modified by the presence of diabetes (p-value for interaction = 0.3).

Metabolic syndrome in relation to infrarenal aortic diameter

Patients with metabolic syndrome had slightly larger infrarenal aortic diameters (mm) compared to those without metabolic syndrome (β 0.70; 95% CI 0.27-1.13, adjusted for age, gender, height and ever smoking) (*Table 3*). Patients with five components of metabolic syndrome had larger infrarenal aortic diameters than those with less components (adjusted β 1.74; 95% CI 0.46-3.01).

Table 3. Metabolic syndrome and its components in relation to infrarenal aortic diameter in patients with manifest arterial disease (n = 2726)

		Infrarenal aortic diameter (mm)	
		Model I	Model II
	N	β (95% CI)	β (95% CI)
Metabolic syndrome present*	1267	0.72 (0.29; 1.15)	0.70 (0.27; 1.13)
Number of components			
0	131	0	0
1	518	0.42 (-0.68; 1.52)	0.51 (-0.58; 1.60)
2	810	0.72 (-0.34; 1.79)	0.79 (-0.26; 1.85)
3	680	0.93 (-0.15; 2.01)	1.02 (-0.05; 2.09)
4	400	1.60 (0.47; 2.73)	1.66 (0.53; 2.78)
5	187	1.80 (0.51; 3.08)	1.74 (0.46; 3.01)

* According to ATP III.
All data: Beta regression coefficients (β) with 95% confidence interval (CI).
β indicates for presence of metabolic syndrome: the difference in infrarenal aortic diameter (mm) between patients with and without metabolic syndrome; and for components of metabolic syndrome: the difference relative to patients without any component of metabolic syndrome.
Model I: age and gender adjusted.
Model II: Model I, additional adjusted for height, and ever smoking.

Discussion

In this study, intra-abdominal fat accumulation is associated with larger infrarenal aortic diameters in patients with manifest atherosclerotic arterial disease. Moreover, among these high-risk patients, infrarenal aortic diameter was slightly larger in patients with metabolic syndrome compared to those without metabolic syndrome, and increased with an increment in the number of metabolic syndrome components.
Our findings are in agreement with several studies, that found weight[17], waist circumference[17,18] and body mass index[16,17] to be associated with larger aortic diameters or presence of abdominal aortic aneurysm in the general population. In contrast, a recent study did not find a difference in body mass index in middle-aged men with and without abdominal aortic aneurysm.[20] Because waist circumference is considered to be a crude measure of abdominal visceral obesity, we accurately measured intra-abdominal fat using ultrasonography.[34] In addition, we measured waist circumference

because of its usefulness in clinical practice. In the current study, both intra-abdominal fat and waist circumference were associated with larger infrarenal aortic diameters in patients with atherosclerotic arterial disease.

Several factors may explain the increased diameters of the infrarenal aortic artery among patients with more intra-abdominal fat. Intra-abdominal fat is associated with the development of interrelated metabolic abnormalities clustering in the metabolic syndrome, all involved in accelerating atherosclerotic disease.[4] The dilatation of the abdominal aortic artery is a complex process in which atherosclerosis plays a leading role.[11] Indeed, we found that patients with metabolic syndrome, the clustering of metabolic abnormalities associated with obesity, had larger infrarenal aortic diameters than those without. This was in line with previous studies that found metabolic abnormalities, such as elevated blood pressure, and dyslipidemia, to be associated with abdominal aortic diameter[16,17] or abdominal aortic aneurysm[13-15,20] in the general population. However, these studies provided conflicting results particular for elevated blood pressure.

Furthermore, the increased diameters of the infrarenal aortic artery among patients with more intra-abdominal fat could be due to the increased insulin resistance associated with obesity. In an insulin resistant state, abdominal fat secretes in the systemic circulation large quantities of inflammatory cytokines, such as TNF-α and IL-6, all involved in the process of atherosclerosis[2] thereby contributing to aortic dilatation.[11] In addition to accelerating atherosclerosis, inflammatory processes likely mediate the connective tissue destruction of the aortic wall.[22] Adipocytokines, such as TNF-α, cause the release of proteases that weaken the aortic matrix[21] and inhibit the synthesis of collagen.[35]

An accumulation of intra-abdominal fat may also directly affect the aortic wall from 'outside to inside' by releasing a large number of pro-inflammatory adipocytokines (TNF-α, IL-6) in close proximity to the aortic artery. Macrophages attracted by abdominal fat-derived signals may subsequently traverse across the peritoneum to the aortic wall and elicit a local inflammatory response. Additionally, intra-abdominal fat may generate cytokine signals to the aortic wall through the peri-aortic fat. Human peri-aortic fat was found to be a source of cytokines and chemokines with potential chemotactic activity.[24] Also, the amount of peri-aortic fat markedly increased by a high-fat diet in rodents, implying a role for periaortic fat in obesity-associated vascular disease.[24,36] In the current study, we found that an accumulation of intra-abdominal fat, accurately measured by ultrasound, was associated with larger aortic diameters. Although the precise underlying mechanisms were not determined in this study, our data suggest that intra-abdominal fat plays a role in the process of aortic dilatation. Thus, interventions aimed at lowering waist may help to modify the chronic inflammatory state, insulin resistance and associated hypertension and dyslipidemia and therefore delay the enlargement of the aortic diameter.

In addition, several studies indicate that patients with diabetes are less likely to have an enlargement of the aortic diameter than those without diabetes.[30,37] These studies proposed that glycemia-associated alterations in vascular matrix may protect against dilatation of the aortic wall due to the fact that high glucose accelerates synthesis of collagen[38], and deposition of advanced glycation endproducts may impair matrix re-modeling and proteolysis.[39] However, in the present study the relation of intra-

abdominal fat with infrarenal aortic diameter was not different in patients with and without diabetes. Also, several studies reported an association between abdominal aortic dilatation and peripheral arterial occlusive disease[40], and abdominal obesity is highly prevalent in patients with peripheral arterial disease.[25] In line with this, we found that the association between intra-abdominal fat and infrarenal aortic diameter was stronger in patients with a reduced ABPI.

We acknowledge study limitations. Due to the cross-sectional design, only assumptions about possible etiological relationships can be made. Furthermore, CT has been considered to be the most accurate and reproducible technique for measuring intra-abdominal fat.[41] However, ultrasonography has been proposed as a valid alternative technique to accurately measure intra-abdominal fat.[27,42] Moreover, we used the ATP III-definition of metabolic syndrome[5] because it is most commonly used in studies, best related with the development of vascular diseases and easy to use in clinical practice. However, we realize that there are more definitions for the metabolic syndrome.[4]

In conclusion, intra-abdominal fat accumulation and metabolic syndrome are associated with larger infrarenal aortic diameters in patients with manifest atherosclerotic arterial disease. These data indicate a role for intra-abdominal fat in the development of larger aortic diameters.

Acknowledgements

The help of M. Edlinger for data coordination is greatly acknowledged. The SMART study was financially supported by a grant of the University Medical Centre Utrecht.

References

1. Yusuf S, Hawken S, Ounpuu S et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet.* 2005;366:1640-1649.
2. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res.* 2005;96:939-949.
3. Pittas AG, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. *J Clin Endocrinol Metab.* 2004;89:447-452.
4. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365:1415-1428.
5. Grundy SM, Brewer HB, Jr, Cleeman JI et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation.* 2004;109:433-438.
6. Wilson PW, D'Agostino RB, Parise H et al. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation.* 2005;112:3066-3072.
7. Norman P, Le M, Pearce C et al. Infrarenal aortic diameter predicts all-cause mortality. *Arterioscler Thromb Vasc Biol.* 2004;24:1278-1282.
8. Brady AR, Fowkes FG, Thompson SG et al. Aortic aneurysm diameter and risk of cardiovascular mortality. *Arterioscler Thromb Vasc Biol.* 2001;21:1203-1207.
9. Wassef M, Baxter BT, Chisholm RL et al. Pathogenesis of abdominal aortic aneurysms: a multidisciplinary research program supported by the National Heart, Lung, and Blood Institute. *J Vasc Surg.* 2001;34:730-738.
10. Annabi B, Shedid D, Ghosn P et al. Differential regulation of matrix metalloproteinase activities in abdominal aortic aneurysms. *J Vasc Surg.* 2002;35:539-546.
11. Reed D, Reed C, Stemmermann G, Hayashi T. Are aortic aneurysms caused by atherosclerosis? *Circulation.* 1992;85:205-211.
12. Pleumeekers HJ, Hoes AW, van der Does E et al. Aneurysms of the abdominal aorta in older adults. The Rotterdam Study. *Am J Epidemiol.* 1995;142:1291-1299.
13. Naydeck BL, Sutton-Tyrrell K, Schiller KD et al. Prevalence and risk factors for abdominal aortic aneurysms in older adults with and without isolated systolic hypertension. *Am J Cardiol.* 1999;83:759-764.
14. Alcorn HG, Wolfson SK, Jr, Sutton-Tyrrell K et al. Risk factors for abdominal aortic aneurysms in older adults enrolled in The Cardiovascular Health Study. *Arterioscler Thromb Vasc Biol.* 1996;16:963-970.
15. Singh K, Bonaa KH, Jacobsen BK et al. Prevalence of and risk factors for abdominal aortic aneurysms in a population-based study : The Tromso Study. *Am J Epidemiol.* 2001;154:236-244.
16. Paivansalo MJ, Merikanto J, Jerkkola T et al. Effect of hypertension and risk factors on diameters of abdominal aorta and common iliac and femoral arteries in middle-aged hypertensive and control subjects: a cross-sectional systematic study with duplex ultrasound. *Atherosclerosis.* 2000;153:99-106.
17. Patel AS, Mackey RH, Wildman RP et al. Cardiovascular risk factors associated with enlarged diameter of the abdominal aortic and iliac arteries in healthy women. *Atherosclerosis.* 2005;178:311-317.
18. Golledge J, Clancy P, Jamrozik K et al. Obesity, adipokines, and abdominal aortic aneurysm: Health in Men study. *Circulation.* 2007;116:2275-2279.
19. Rohde LE, Arroyo LH, Rifai N et al. Plasma concentrations of interleukin-6 and abdominal aortic diameter among subjects without aortic dilatation. *Arterioscler Thromb Vasc Biol.* 1999;19:1695-1699.
20. Lindblad B, Borner G, Gottsater A. Factors associated with development of large abdominal aortic aneurysm in middle-aged men. *Eur J Vasc Endovasc Surg.* 2005;30:346-352.
21. Newman KM, Jean-Claude J, Li H et al. Cytokines that activate proteolysis are increased in abdominal aortic aneurysms. *Circulation.* 1994;90(5 Pt 2):II224-227.
22. Shah PK. Inflammation, metalloproteinases, and increased proteolysis: an emerging pathophysiological paradigm in aortic aneurysm. *Circulation.* 1997;96:2115-2117.

23. Reaven GM, Lithell H, Landsberg L. Hypertension and associated metabolic abnormalities--the role of insulin resistance and the sympathoadrenal system. *N Engl J Med.* 1996;334:374-381.
24. Henrichot E, Juge-Aubry CE, Pernin A et al. Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis? *Arterioscler Thromb Vasc Biol.* 2005;25:2594-2599.
25. Gorter PM, Olijhoek JK, van der Graaf Y et al. Prevalence of the metabolic syndrome in patients with coronary heart disease, cerebrovascular disease, peripheral arterial disease or abdominal aortic aneurysm. *Atherosclerosis.* 2004;173:363-369.
26. Simons PC, Algra A, van de Laak MF et al. Second Manifestations of ARTerial disease (SMART) study: rationale and design. *Eur J Epidemiol.* 1999;15:773-781.
27. Stolk RP, Wink O, Zelissen PM et al. Validity and reproducibility of ultrasonography for the measurement of intra-abdominal adipose tissue. *Int J Obes Relat Metab Disord.* 2001;25:1346-1351.
28. Akkersdijk GJ, Puylaert JB, Coerkamp EG et al. Accuracy of ultrasonographic measurement of infrarenal abdominal aortic aneurysm. *Br J Surg.* 1994;81:376.
29. Yucel EK, Fillmore DJ, Knox TA et al. Sonographic measurement of abdominal aortic diameter: interobserver variability. *J Ultrasound Med.* 1991;10:681-683.
30. Le MT, Jamrozik K, Davis TM et al. Negative association between infra-renal aortic diameter and glycaemia: the Health in Men Study. *Eur J Vasc Endovasc Surg.* 2007;33:599-604.
31. Brooks B, Dean R, Patel S et al. TBI or not TBI: that is the question. Is it better to measure toe pressure than ankle pressure in diabetic patients? *Diabet Med.* 2001;18:528-532.
32. Miettinen OS. Theoretical epidemiology, principles of occurrence research in medicine. *New York: Wiley*. 1985;p. 216-44.
33. Greenland S, Finkle WD. A critical look at methods for handling missing covariates in epidemiologic regression analyses. *Am J Epidemiol.* 1995;142:1255-1264.
34. Stolk RP, Meijer R, Mali WP et al. Ultrasound measurements of intraabdominal fat estimate the metabolic syndrome better than do measurements of waist circumference. *Am J Clin Nutr.* 2003;77:857-860.
35. Reginato AM, Sanz-Rodriguez C, Diaz A et al. Transcriptional modulation of cartilage-specific collagen gene expression by interferon gamma and tumour necrosis factor alpha in cultured human chondrocytes. *Biochem J.* 1993;294(Pt 3):761-769.
36. Stern N, Marcus Y. Perivascular fat: innocent bystander or active player in vascular disease? *J Cardiometab Syndr.* 2006;1:115-120.
37. Lederle FA, Johnson GR, Wilson SE et al. The aneurysm detection and management study screening program: validation cohort and final results. Aneurysm Detection and Management Veterans Affairs Cooperative Study Investigators. *Arch Intern Med.* 2000;160:1425-1430.
38. Lam S, Verhagen NA, Strutz F et al. Glucose-induced fibronectin and collagen type III expression in renal fibroblasts can occur independent of TGF-beta1. *Kidney Int.* 2003;63:878-888.
39. Rittie L, Berton A, Monboisse JC et al. Decreased contraction of glycated collagen lattices coincides with impaired matrix metalloproteinase production. *Biochem Biophys Res Commun.* 1999;264:488-492.
40. van den Bosch MA, van der Graaf Y, Eikelboom BC et al. Distal aortic diameter and peripheral arterial occlusive disease. *J Vasc Surg.* 2001;34:1085-1089.
41. Rossner S, Bo WJ, Hiltbrandt E et al. Adipose tissue determinations in cadavers--a comparison between cross-sectional planimetry and computed tomography. *Int J Obes.* 1990;14:893-902.
42. Hirooka M, Kumagi T, Kurose K et al. A technique for the measurement of visceral fat by ultrasonography: comparison of measurements by ultrasonography and computed tomography. *Intern Med.* 2005;44:794-799.

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Atherosclerosis. 2007 Sep 18; [Epub ahead of print].

Quantification of epicardial and peri-coronary fat using cardiac computed tomography; reproducibility and relation with obesity and metabolic syndrome in patients suspected of coronary artery disease

Abstract

Objective

Adipose tissue surrounding coronary arteries may contribute to the development of coronary atherosclerosis given its localisation and potential for local production of inflammatory cytokines. We compared various measurements for quantifying epicardial adipose tissue (EAT) and peri-coronary fat using cardiac computed tomography (CT). Additionally, we estimated their relationship with obesity and metabolic syndrome in patients suspected of coronary artery disease (CAD).

Methods

EAT and peri-coronary fat measurements were performed on cardiac multi-slice CT scans in 60 patients (aged 50-70 yrs) referred for coronary angiography. EAT was measured as thickness on the right ventricular free wall, as area at the base of the ventricles, and as volume. Peri-coronary fat was assessed as thickness and cross-sectional area surrounding the three main coronary arteries. Linear regression analysis was used to assess the relation of EAT and peri-coronary fat with obesity and metabolic syndrome (ATP III criteria).

Results

Volumetric EAT measurements showed good reproducibility with low coefficients of variation (CVs) varying between 3.0% and 5.0%. Measurements of EAT and peri-coronary fat thickness and area were moderately reproducible (CVs 11.0%-23.4%). The amount of EAT and peri-coronary fat (per standard deviation) was related with obesity (BMI $\geq$30 kg/m^2) (β 1.24; 95% CI 0.66; 1.81) and metabolic syndrome (β 0.81; 95% CI 0.28; 1.33).

Conclusions

Volumetric quantification of EAT using cardiac CT is highly reproducible compared to more simple measurements as EAT and peri-coronary fat thickness and area. The quantity of EAT and peri-coronary fat is related with the presence of obesity and metabolic syndrome in patients suspected of CAD.

Introduction

Abdominal obesity is associated with an increased risk of coronary artery disease (CAD).[1] Dyslipidemia, hypertension and insulin resistance are important risk factors associated with obesity.[2] The clustering of these risk factors is often referred to as metabolic syndrome.[3] Moreover, abdominal adipose tissue is able to produce large quantities of tumor necrosis factor-alpha (TNF-α), interleukine-6 (IL-6), free fatty acids (FFA), plasminogen activator inhibitor-1 (PAI-1) and adipocytokines such as adiponectin.[4-6] Increased plasma concentrations of TNF-α, IL-6, FFA, and PAI-1 and decreased concentrations of adiponectin lead to accelerated atherosclerosis, plaque instability, and arterial thrombosis.[4,7,8]

Adipose tissue surrounding the coronary arteries, often called epicardial adipose tissue (EAT), may also act as an endocrine organ given the production of a comparable pattern of adipocytokines.[9,10] It could be hypothesized that adipocytokines produced by fat surrounding the coronary arteries might contribute to the amplification of vascular inflammation and pro-atherogenic processes from 'outside-to-inside' the vessel wall (*Figure 1*).[11,12]

To further clarify the possible relation between fat surrounding coronary arteries and the development of CAD, it is important to reliably quantify this adipose tissue. In a few studies adipose tissue around the heart has been measured by magnetic resonance imaging, computed tomography (CT) or echocardiography.[13-18] Furthermore, there are differences in anatomic description (pericardial versus epicardial fat) and measurement techniques (volume or thickness).[13,14,16] A measurement of adipose tissue directly surrounding the coronary arteries has not been published. Conceptually, this peri-coronary fat might be most interesting because of its close anatomic relation with the coronary arteries.

CT provides a more accurate quantification of adipose tissue due to its higher spatial resolution compared to magnetic resonance imaging and ultrasound. However, limited methods for measuring fat surrounding coronary arteries using cardiac CT have been explored.[16]

In this cross-sectional study, we compared the reproducibility of various methods for quantifying EAT and peri-coronary fat using cardiac CT in patients suspected of CAD referred for angiography. In addition, we estimated the relationship of the quantity of EAT and peri-coronary fat with obesity and metabolic syndrome and its components.

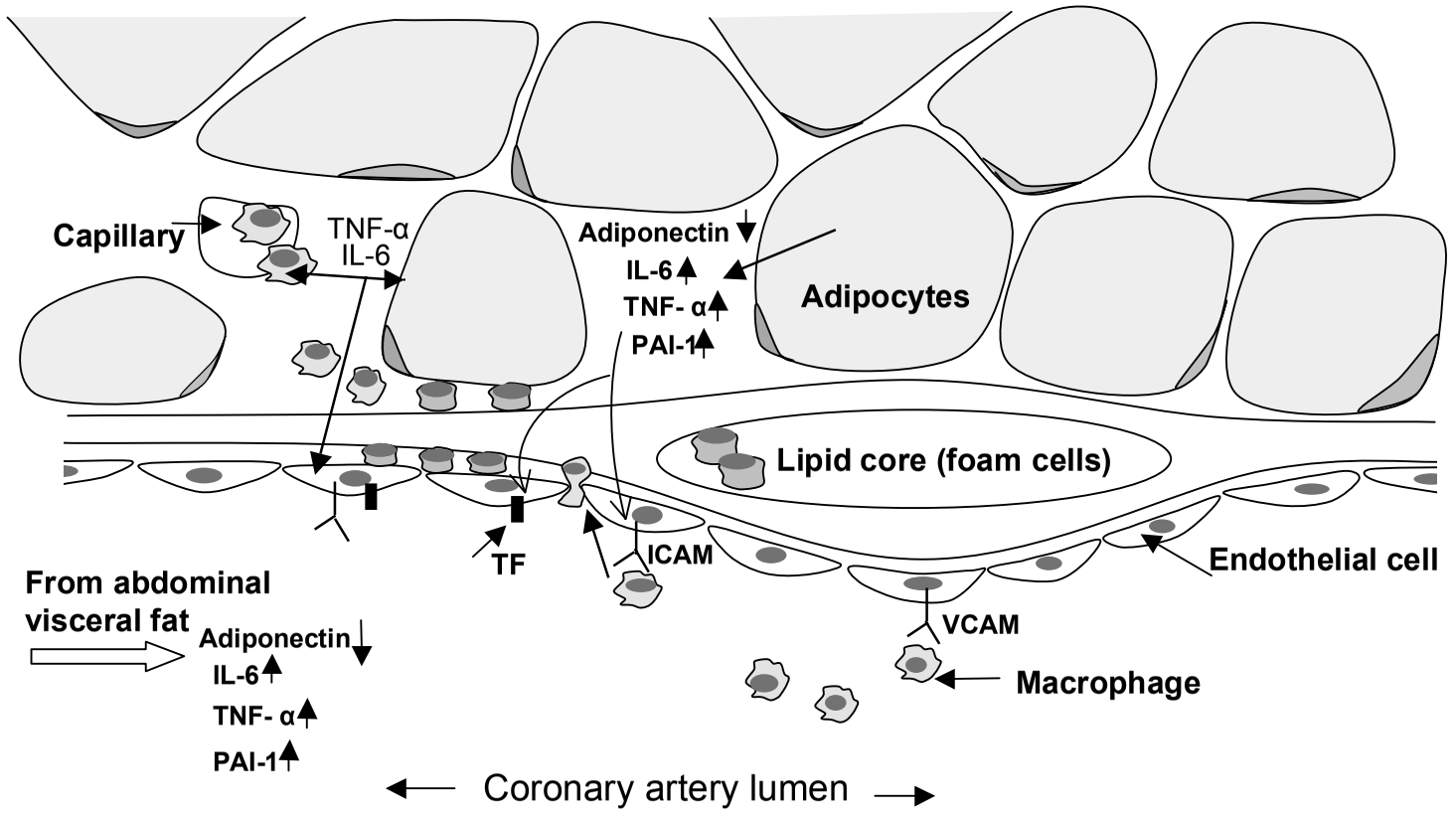

Figure 1. Potential role of adipocytes in the process of coronary atherosclerosis, plaque instability, and arterial thrombosis. TNF-α, tumor necrosis factor-alpha; IL-6, interleukine-6; PAI-1, plasminogen activator inhibitor-1; TF, tissue factor; ICAM, VCAM, endothelial adhesion molecules.

Methods

Study settings, participants and design

Patients originated from a diagnostic study at the University Medical Centre Utrecht designed to establish the diagnostic accuracy of multi-slice computed tomography coronary angiography (CTA) compared to conventional coronary angiography (CAG) in the detection of significant coronary obstruction. Patients (n = 60) were referred for diagnostic coronary angiography or percutaneous coronary intervention because of stable angina pectoris (n = 48) or unstable angina pectoris (n = 12).[19,20] The inclusion criteria were: aged 50-70 years, and stable sinus rhythm. Patients with previous PTCA or CABG, serum creatinine levels >140 μmol/l, or known iodine-based contrast allergy were not included. CTA was performed in all patients within a month before or after CAG. The Medical Ethics Committee approved the study and all participants gave their written informed consent.

Clinical information was obtained using a standardised health questionnaire. Height, body weight, and blood pressure were measured. Fasting blood was sampled to determine lipid, glucose and creatinine levels. Diabetes mellitus was defined as self-reported diabetes mellitus. Metabolic syndrome was diagnosed according to the Adult Treatment Panel III (ATP III) criteria as the presence of three or more metabolic abnormalities.[3] Because waist circumference was not available, a BMI of ≥30 kg/m^2 was used as determinant for obesity.[21] By visual assessment of CAGs by cardiologists the severity of CAD was classified as single, two or three vessel disease. A ≥50% luminal diameter stenosis was regarded as significant.[22]

CT technique and image analyses

EAT and peri-coronary fat were quantified on ECG-gated diagnostic cardiac CT scans. CT studies were performed on a 64 detector-row CT scanner (Brilliance 64, Philips Medical Systems, Cleveland, OH, USA). Scan duration time was 7-10 s. Average heart rate during image acquisition was 56 ± 10 beats/min. Standard coronary imaging protocols were applied including the use of intra-venous beta-blockers for patients with heart rates >65 beats/min (unless contraindicated) and image acquisitions were performed during a breath-hold in inspiration. Imaging parameters were: slice collimation of 64 mm x 0.625 mm, gantry rotation time of 420 ms, tube voltage of 120 kV, and tube current of 900 mAs. Contrast agent used was iopromide (Schering AG, Berlin, Germany), which was injected intravenously (1.6-2.0 g iodine/s depending on the patient's body weight).

Measurements were performed in the most motionless phase of the cardiac cycle, which was most frequently a mid-diastolic phase, with retrospective cardiac gating at 70-80% of the R-R interval. The window settings were adjusted to properly visualise the adipose tissue and the pericardium.

To establish the intra- and interobserver variability for measurement techniques of EAT and peri-coronary fat, all cardiac CT scans were analysed independently by two observers who were blinded for their own and each others results and for patient characteristics.

EAT quantification

EAT was defined as the adipose tissue between the surface of the heart and the visceral layer of the pericardium (visceral epicardium).

EAT thickness

EAT thickness (mm) was determined on the right ventricular (RV) anterior free wall. Measurements were performed at the base of the ventricles (basal level) on short-axis views of a regular Philips CT workstation (*Figure 2A*). Basal level was defined as the level at the base of the ventricles.[16] Three measurements of EAT thickness were made: inferior, centre and superior, chosen at 25%, 50% and 75% level of the RV wall, respectively, from the visceral epicardium to the outside of the myocardium and perpendicular to the surface of the heart. The mean of the three measurements (referred to as 'EAT RV thickness') was used for analyses.

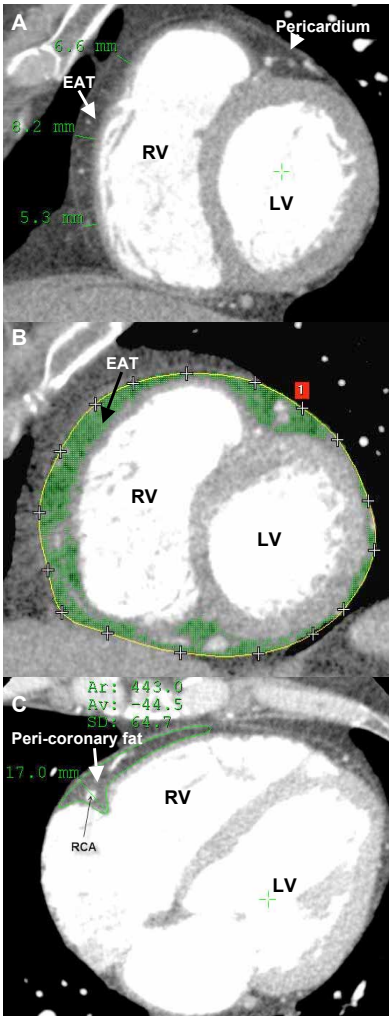

Figure 2. Epicardial adipose tissue (EAT) and peri-coronary fat measurements. (A) EAT RV thickness (green) (inferior, centre, and superior) at basal level of the ventricles. (B) Region of interest (ROI) (yellow) on a cross-sectional image to determine the EAT area (green). EAT volume is the sum of the EAT areas on cross-sectional images. (C) Peri-coronary fat thickness and area (green). (**For colour figure, see page 167**).

EAT area
EAT area (cm^2) was measured using two dedicated software programs (Extended Brilliance Workspace and Easy Vision, Philips Medical Systems, Best, The Netherlands). Measurements were performed on short-axis views of 2-mm-slice-thickness and 3-mm-intersection gaps. The stack of the short-axis views started at the apex just below the fibrous pericardium and extended until the centre of the left atrium. One section of the short-axis image, at the base of the ventricles (basal level), was used to determine 'EAT area at basal level'. An EAT area measurement was assessed by tracing a single region of interest (ROI) containing heart and EAT (*Figure 2B*). ROI's were placed at the visceral epicardium to exclude pericardial fluid. A density range between -200 and -30 Hounsfield Units was used to isolate adipose tissue.

EAT volume
EAT volume (cm^3) was obtained as the sum of the EAT areas on short-axis images using the same software programs, short-axis views and range of Hounsfield Units as for 'EAT area at basal level'. A cursor pointer was used to trace a ROI (*Figure 2B*). The computer software program produced an accurate volume of EAT by adding up the EAT areas and taking into account slice thickness and intersection gap.
Firstly, 'EAT truncated volume' was obtained as the sum of the EAT areas made of 20-25 sections extending from below the apex up to a cut-off point 1 cm (two sections) above the last visible segment of the left coronary artery (LCA). Second, 'EAT total volume' was assessed by adding up EAT areas of 4-6 sections (until the centre of the left atrium) to the 'EAT truncated volume'.

Peri-coronary fat quantification

Peri-coronary fat was defined as the adipose tissue between the surface of the heart and the visceral epicardium directly surrounding the main coronary arteries.

Peri-coronary fat thickness
Peri-coronary fat thickness (mm) was quantified on axial views of a regular Philips CT workstation. In order not to overestimate the peri-coronary fat due to obliquity, thickness measurements were performed on images in which the axial sections were perpendicular to the surface of the heart. In each of the regions of the right coronary artery (RCA), LCA and left circumflex (LCX), maximal fat thickness assessed as the largest distance from myocardium to visceral epicardium was determined (*Figure 2C*). Mean thickness of the peri-coronary fat surrounding the three coronary arteries was used for analyses ('peri-coronary fat thickness').

Peri-coronary fat area
Separately for the three coronary arteries, the cross-sectional area (cm^2) of fat surrounding the coronary artery was determined on axial sections by tracing a ROI (*Figure 2C*). In case fat area covered both the RCA and the LCA region, ROI extended halfway between both regions, and the area was measured for the involved coronary artery. Mean cross-sectional area surrounding the three main coronary arteries was used for analyses ('peri-coronary fat area').

Relationship of EAT and peri-coronary fat with obesity and metabolic syndrome

To evaluate the relation of EAT and peri-coronary fat quantity with obesity and metabolic syndrome and its components, we used (a) the most reproducible measurement (EAT truncated volume); (b) the measurement most commonly used in previous studies (EAT RV thickness); and (c) the most interesting measurement from a pathophysiological point of view (peri-coronary fat thickness).

Statistical analysis

Mean differences with 95% confidence intervals (CI) of EAT and peri-coronary fat measurements between two observers or between repeated measurements of one observer were tested with the paired t-test (two-sided). To assess the intra- and interobserver reproducibility, a coefficient of variation (CV) was calculated using the formula: (standard deviation (S.D.) of the differences between the measurements/ overall mean of the measurements) x 100. Repeatability coefficient (RC) was computed as 2 x (S.D. of the differences between two measurements). The limits of agreement were calculated as mean difference ± (1.96 x (S.D. of the differences)).
The relationship of the amount of EAT and peri-coronary fat with obesity and metabolic syndrome and its components was determined with linear regression analyses. To compare the various dimensions of fat measurements, we determined the age- and gender-adjusted regression coefficient beta (β) (95% CI) corresponding to a 1 S.D. change in fat measurement. Variables were log-transformed if they were skewly distributed. To adjust mean EAT and peri-coronary fat levels for age and gender differences between the number of components of metabolic syndrome we used analysis of covariance (ANCOVA, general linear model). To reduce bias and increase statistical efficiency, missing values (HDL-cholesterol (n = 8), triglycerides (n = 8), fasting glucose (n = 2), systolic (n = 6) and diastolic (n = 6) blood pressure) in the data were completed by regression imputation.[23,24]

Results

Baseline characteristics

Patients (n = 60) had a mean age of 61 ± 5 years (range 50-70 years) and the mean body mass index (BMI) was 27 ± 4 kg/m^2 (*Table 1*). Mean creatinine clearance (Cockroft-Gault) was 87 ± 23 ml/min/1.73m^2. Dyslipidemia (LDL-cholesterol >2.5 mmol/l or use of lipid-lowering agents) was present in 57 patients (93%).

EAT and peri-coronary fat quantification and reproducibility

The overall mean ± S.D. for intra- and interobserver measurements of EAT and peri-coronary fat are depicted in *Table 2*. Peri-coronary fat thickness was 15.9 ± 3.0 mm for the RCA region, 5.7 ± 2.1 mm for the LCA region, and 11.0 ± 2.1 mm for the LCX region.
EAT truncated volume and EAT total volume were highly reproducible (interobserver CVs 4.8% and 5.0%, respectively). EAT area at basal level and EAT RV thickness appeared

Table 1. Baseline characteristics of the study population (n = 60)

Characteristics	Data
Age (years)[1]	61 ± 5
Male gender, n (%)	44 (73)
Body mass index (kg/m^2)[1]	27 ± 4
Systolic blood pressure (mmHg)[1]	152 ± 23
Diastolic blood pressure (mmHg)[1]	82 ± 13
Creatinine clearance (ml/min/1.73m^2)*	
≥90 ml/min/1.73m^2, n (%)	23 (38)
60-89 ml/min/1.73m^2, n (%)	33 (55)
30-59 ml/min/1.73m^2, n (%)	4 (7)
Current smoking†, n (%)	21 (35)
Diabetes mellitus‡, n (%)	7 (12)
Metabolic syndrome ATP III, n (%)	22 (37)
Laboratory measurements	
Fasting glucose (mmol/l)[2]	5.9 (5.0–6.4)
Total cholesterol (mmol/l)[2]	4.4 (3.8–5.1)
LDL-cholesterol (mmol/l)§,[2]	2.25 (1.87–3.11)
HDL-cholesterol (mmol/l)[2]	1.26 (1.08–1.58)
Triglycerides (mmol/l)[2]	1.38 (1.07–2.19)
Medications	
Blood pressure-lowering agents, n (%)	53 (88)
Lipid-lowering agents, n (%)	45 (75)
Parameters of coronary artery disease	
Stable angina pectoris, n (%)	48 (80)
History of myocardial infarction, n (%)	13 (22)
Severity of coronary artery disease‖	
No significant affected vessels, n (%)	11 (18)
1-Vessel disease, n (%)	28 (47)
2-Vessel disease, n (%)	11 (18)
3-Vessel disease, n (%)	10 (17)

[1] Mean ± S.D.
[2] Median with interquartile range.
* Categorisation of creatinine clearance (Cockroft-Gault) according to K/DOQI criteria for the degree of renal insufficiency.
† Still smoking or recently (<1 month before study inclusion) stopped smoking.
‡ Self-reported diabetes mellitus.
§ Calculated by use of Friedewald's formula.
‖ According to conventional coronary angiograms, ≥50% is defined as significant stenosis.

Table 2. Distribution and intra- and interobserver reproducibility of epicardial adipose tissue (EAT) and peri-coronary fat measurements (n = 60)

	Intra-observer					Inter-observer				
	Mean ± S.D.*	Mean difference (95% CI)	(LLA; ULA)†	CV‡	RC§	Mean ± S.D.*	Mean difference (95% CI)	(LLA; ULA)†	CV‡	RC§
EAT										
EAT RV thickness (mm)	4.9 ± 2.0	0.05 (-0.10; 0.19)	(-1.06; 1.15)	11.5%	1.1	4.8 ± 1.9	0.20 (-0.01; 0.41)	(-1.39; 1.79)	16.9%	1.6
EAT area at basal level (cm^2)	35.2 ± 16.5	1.28 (0.27; 2.28)	(-6.32; 8.87)	11.0%	7.8	35.5 ± 16.6	0.61 (-0.73; 1.95)	(-9.55; 10.77)	14.6%	10.4
EAT truncated volume (cm^3)	110.2 ± 46.7	0.88 (0.02; 1.73)	(-5.60; 7.35)	3.0%	6.6	110.1 ± 46.6	1.15 (-0.21; 2.51)	(-9.16; 11.47)	4.8%	10.5
EAT total volume (cm^3)	118.2 ± 50.3	-0.13 (-1.07; 0.82)	(-7.32; 7.06)	3.1%	7.3	117.8 ± 50.2	0.68 (-0.84; 2.21)	(-10.89; 12.26)	5.0%	11.8
Peri-coronary fat										
Peri-coronary fat thickness (mm)	10.9 ± 1.9	0.54 (0.20; 0.88)	(-2.03; 3.11)	12.0%	2.6	10.9 ± 1.9	0.64 (0.27; 1.00)	(-2.15; 3.43)	13.1%	2.8
Peri-coronary fat area (cm^2)	2.3 ± 1.1	0.12 (0.03; 0.21)	(-0.56; 0.81)	15.2%	0.7	2.1 ± 1.0	0.41 (0.28; 0.53)	(-0.56; 1.38)	23.4%	1.0

* Overall mean of the measurement ± S.D.
† LLA, lower limit of agreement; ULA, upper limit of agreement.
‡ CV, coefficient of variation, calculated as S.D. of the differences between the measurements/overall mean, multiplied by 100.
§ RC, coefficient of repeatability, calculated as 2 x S.D. of the differences between the measurements.

to be moderately reproducible; CVs varied between 11.0 and 16.9%. Interobserver repeatability coefficient was 1.6 mm for EAT RV thickness with regard to an overall mean of 4.8 mm. Peri-coronary fat thickness and area measurements were moderately (CV 13.1%) and poorly reproducible (CV 23.4%), respectively (*Table 2*). Bland-Altman analysis[25] showed similar results (data not shown). For all measurements, Pearson's correlation coefficients revealed no relation between intra- and interobserver differences and the range of fat quantity (data not shown).

Table 3. Measurements of epicardial adipose tissue (EAT) and peri-coronary fat in relation to obesity and metabolic syndrome and its components (n = 60)

	EAT truncated volume (1 S.D. = 46.17 cm^3)	**EAT RV thickness** (1 S.D. = 1.88 mm)	**Peri-coronary fat thickness** (1 S.D. = 1.86 mm)
	β (95% CI)*	β (95% CI)*	β (95% CI)*
Continuous variables			
BMI per 1.0 kg/m^2	0.17 (0.11; 0.22)	0.14 (0.08; 0.20)	0.19 (0.13; 0.24)
Systolic blood pressure per 10 mmHg	-0.05 (-0.17; 0.06)	-0.02 (-0.14; 0.09)	0.02 (-0.09; 0.14)
Fasting glucose per 1.0 mmol/l†	0.33 (-0.50; 1.16)	-0.05 (-0.88; 0.78)	0.38 (-0.46; 1.22)
Triglycerides per 0.5 mmol/l†	0.16 (-0.34; 0.67)	-0.13 (-0.63; 0.38)	0.25 (-0.26; 0.76)
HDL-cholesterol per 0.1 mmol/l	-0.01 (-0.09; 0.07)	-0.05 (-0.13; 0.03)	-0.06 (-0.13; 0.02)
Dichotomous variables			
Diabetes mellitus‡	0.36 (-0.44; 1.16)	0.77 (-0.01; 1.55)	0.39 (-0.42; 1.20)
Metabolic syndrome ATP III	0.70 (0.18; 1.23)	0.30 (-0.24; 0.85)	0.81 (0.28; 1.33)
BMI ≥30 kg/m^2	1.18 (0.61; 1.75)	0.88 (0.27; 1.49)	1.24 (0.66; 1.81)
≥130 mmHg systolic or ≥85 mmHg diastolic and/or BP lowering agents	0.50 (-0.54; 1.54)	0.30 (-0.74; 1.35)	0.34 (-0.72; 1.40)
Fasting glucose ≥5.6 mmol/l or diabetes mellitus‡	0.48 (-0.03; 0.99)	0.19 (-0.33; 0.71)	0.31 (-0.21; 0.83)
Triglycerides ≥1.70 mmol/l	-0.01 (-0.57; 0.56)	-0.29 (-0.84; 0.27)	0.19 (-0.38; 0.76)
HDL-cholesterol <1.04 mmol/l (men) or <1.29 mmol/l (women)	-0.08 (-0.70; 0.53)	0.10 (-0.52; 0.71)	0.18 (-0.45; 0.80)

β: Beta regression coefficient. For continuous variables, β indicates the absolute change in S.D. of the corresponding fat measurement associated with an increase of one unit in the metabolic syndrome component (e.g. increase of 1 kg/m^2 in BMI leads to a change in EAT truncated volume of 0.17 S.D., meaning 0.17 x 46.17 = 7.85 cm^3). For dichotomous variables, β should be interpreted as follows: β x S.D. is the difference between groups (e.g. patients with metabolic syndrome have a 0.70 x 46.17 = 32.32 cm^3 higher EAT truncated volume than patients without metabolic syndrome).

* Adjusted for age, and gender.

† β (95% CI) derived using log-transformation.

‡ Self-reported diabetes mellitus.

Relationship of EAT and peri-coronary fat with obesity and metabolic syndrome

An age- and gender-adjusted association was found for the quantitative estimates of EAT and peri-coronary fat (per S.D.) with obesity (BMI ≥30 kg/m^2) (for EAT RV thickness: β 0.88, 95% CI (0.27; 1.49), for EAT truncated volume: 1.18, (0.61; 1.75) and for peri-coronary fat thickness: 1.24, (0.66; 1.81) (*Table 3*). A comparable age- and gender-adjusted relation with metabolic syndrome was found for EAT truncated volume (β 0.70, 95% CI (0.18; 1.23) and peri-coronary fat thickness (0.81, (0.28; 1.33)). EAT RV thickness was less and non-significantly associated with metabolic syndrome (0.30, (-0.24; 0.85)) (*Table 3*). Fat surrounding the coronary arteries (mean ± standard error) gradually increased with the number of components (0 or 1–4 or 5): EAT truncated volume (90.5 ± 13.1 to 144.6 ± 15.6 cm^3, p-value for trend 0.038), and peri-coronary fat thickness (9.7 ± 0.5 to 12.1 ± 0.6 mm, p-value for trend 0.015).

Discussion

Quantifying EAT volume with cardiac CT scanning is highly reproducible compared to more simple measurement techniques, such as EAT and peri-coronary fat thickness and area. The amount of this adipose tissue was associated with obesity and metabolic syndrome in patients clinically suspected of CAD.

Adipose tissue surrounding the coronary arteries (EAT or peri-coronary fat) has the potential for local production of inflammatory cytokines.[9,10,26] An increased quantity or dysfunction of this adipose tissue may contribute to a pro-atherogenic environment on the outside of coronary arteries affecting vascular function. Thus far, the direct contribution of fat surrounding coronary arteries to the development of CAD is still under investigation.[17,27] For investigating this relationship it is important to establish a reliable quantification of this adipose tissue.

CT technique allows for the precise measurement of fat surrounding coronary arteries, because the fibrous pericardium can be easily visualised due to the high spatial resolution. So far, limited methods for measuring this adipose tissue using cardiac CT have been published.[16] Previous studies evaluated the use of cardiac CT for the assessment of pericardial fat volume instead of EAT volume and used the centre of the right pulmonary artery[13] and the atrial appendage[15] as cut-off points for the extent of volume. Pericardial fat includes EAT and paracardial fat separated by the fibrous pericardium. Conceptually, measuring EAT or peri-coronary fat (surrounding the coronary arteries) might be more important than quantifying pericardial fat.[13,15] In the present study, we have used various dimensions (thickness, area and volume) to quantify EAT and peri-coronary fat with cardiac CT in a relevant population.

Our results demonstrate that volumetric quantification of EAT using cardiac CT is feasible and yields superior reproducibility compared to thickness and area measurements. This is consistent with a recent study showing that a magnetic resonance imaging-based volumetric approach was highly reproducible (CV 6%).[18] In our study, the most reproducible measurement was the EAT truncated volume. Because the cut-off point was set at 1 cm above the last segment of the LCA, all fat volume surrounding the coronary arteries was evaluated. It should be noted that volumetric assessment is time consuming, requires an advanced cardiac imaging workstation and should be

done by a skilled observer with sufficient knowledge of cardiac anatomy. Recently, EAT thickness measurement on the RV wall has been performed on short-axis views of CT-scans to evaluate EAT distribution instead of reliability.[16] In the present study, we established a moderate reproducibility for EAT RV thickness measured with CT. This is probably due to the little variation in recognising basal level and tracing the cursor pointer to measure thickness in millimetres. However, this method is more easy to perform and less time consuming compared to the labour-intensive determination of EAT volume with cardiac CT. Previous studies mainly measured EAT thickness using echocardiography.[14,17] The ability to measure EAT with echocardiography is modest compared to the use of CT; it can not give an adequate window of all cardiac segments and moreover is highly dependent on acoustic windows, which are often inadequate for subtle assessments in obese patients.[16] Remarkably, a low interobserver CV (3%) for echocardiographic EAT RV thickness was reported.[14] EAT is accurately visualised on CTA, but in contrast to echocardiography, CTA requires the use of an intravenous contrast agent and a radiation dose of 5-15 mSv.

In line with previous findings[28], the amount of EAT and peri-coronary fat was strongly associated with obesity in patients suspected of CAD. Adipose tissue is a major driver of insulin resistance, an essential pathophysiological feature for the development of metabolic disorders, including e.g. hyperglycemia, hypertension and low HDL-cholesterol.[2,29] Presence of metabolic syndrome[3] could therefore be seen as an estimation of the insulin resistance state. In an obesity-related insulin resistant state, adipocytes produce large quantities of pro-atherogenic factors (e.g. TNF-α, IL-6, PAI-1).[4-6] In agreement with previous studies showing that echocardiographic EAT thickness was related with metabolic abnormalities[28], and insulin resistance[30] in obese subjects, our results indicate that EAT and peri-coronary fat accumulation cluster with metabolic syndrome and its components in patients suspected of CAD. This may implicate that adipose tissue directly surrounding coronary arteries may be involved in pathophysiological processes leading to the development of coronary atherosclerosis seen in obesity, insulin resistance and metabolic syndrome.

Some limitations of this study have to be taken into account. This is a cross-sectional study, which means that only assumptions about possible etiological relationships can be made. Moreover, due to the sample size, the confidence intervals are quite wide indicating that the point estimates for the relationship of EAT and peri-coronary fat with obesity and metabolic syndrome and its components are less precise. Furthermore, waist circumference was not measured, thus we could not evaluate the relationship between EAT/peri-coronary fat and abdominal obesity. However, when waist circumference is missing, a BMI ≥30 kg/m^2 is recommended as determinant for obesity to assess the presence of metabolic syndrome[21], and both are indicators of a high cardiovascular risk in patients with cardiovascular disease.[31] Finally, data were obtained in routine medical care. Markers of dysfunction of fat surrounding coronary arteries (IL-6, TNF-α, PAI-1) were not measured in fat samples, and leptin and fasting insulin were not measured. Future studies are needed to reveal the etiologic relation between the quantity and the quality of EAT or peri-coronary fat and CAD.

In conclusion, volumetric quantifications of EAT using cardiac CT were highly reproducible compared to more simple thickness and area measurements of EAT and peri-coronary fat. The quantity of adipose tissue surrounding coronary arteries is related with obesity and metabolic syndrome in patients clinically suspected of CAD.

Acknowledgements

The help of K. Benders, A.J. Borgen and J. op den Brouw for data collection is greatly acknowledged. Funding: University Medical Centre Utrecht, Utrecht, The Netherlands, the Bekalis Foundation, Brussels, Belgium and ZonMw, The Hague, The Netherlands.

References

1. Yusuf S, Hawken S, Ounpuu S et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet.* 2005;366:1640-1649.
2. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care.* 1991;14:173-194.
3. Grundy SM, Cleeman JI, Daniels SR et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation.* 2005;112:2735-2752.
4. Sharma AM. Adipose tissue: a mediator of cardiovascular risk. *Int J Obes Relat Metab Disord.* 2002;26 Suppl 4:S5-7.
5. Eriksson P, Reynisdottir S, Lonnqvist F et al. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. *Diabetologia.* 1998;41:65-71.
6. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* 1993;259:87-91.
7. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation.* 2002;105:1135-1143.
8. Shioji K, Moriguchi A, Moriwaki S et al. Hypoadiponectinemia implies the development of atherosclerosis in carotid and coronary arteries. *J Cardiol.* 2005;46:105-112.
9. Mazurek T, Zhang L, Zalewski A et al. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation.* 2003;108:2460-2466.
10. Baker AR, Silva NF, Quinn DW et al. Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol.* 2006;5:1.
11. Shimokawa H, Ito A, Fukumoto Y et al. Chronic treatment with interleukin-1 beta induces coronary intimal lesions and vasospastic responses in pigs in vivo. The role of platelet-derived growth factor. *J Clin Invest.* 1996;97:769-776.
12. Miyata K, Shimokawa H, Kandabashi T et al. Rho-kinase is involved in macrophage-mediated formation of coronary vascular lesions in pigs in vivo. *Arterioscler Thromb Vasc Biol.* 2000;20:2351-2358.
13. Wheeler GL, Shi R, Beck SR et al. Pericardial and visceral adipose tissues measured volumetrically with computed tomography are highly associated in type 2 diabetic families. *Invest Radiol.* 2005;40:97-101.
14. Iacobellis G, Assael F, Ribaudo MC et al. Epicardial fat from echocardiography: a new method for visceral adipose tissue prediction. *Obes Res.* 2003;11:304-310.
15. Taguchi R, Takasu J, Itani Y et al. Pericardial fat accumulation in men as a risk factor for coronary artery disease. *Atherosclerosis.* 2001;157:203-209.
16. Abbara S, Desai JC, Cury RC et al. Mapping epicardial fat with multi-detector computed tomography to facilitate percutaneous transepicardial arrhythmia ablation. *Eur J Radiol.* 2006;57:417-422.
17. Chaowalit N, Somers VK, Pellikka PA et al. Subepicardial adipose tissue and the presence and severity of coronary artery disease. *Atherosclerosis.* 2006;186:354-359.
18. Flüchter S, Haghi D, Dinter D et al. Volumetric assessment of epicardial adipose tissue with cardiovascular magnetic resonance imaging. *Obesity (Silver Spring).* 2007;15:870-878.
19. Goldman L, Hashimoto B, Cook EF et al. Comparative reproducibility and validity of systems for assessing cardiovascular functional class: advantages of a new specific activity scale. *Circulation.* 1981;64:1227-1234.
20. Braunwald E. Unstable angina. A classification. *Circulation.* 1989;80:410-414.
21. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* 1998;15:539-553.

22. Scanlon PJ, Faxon DP, Audet AM et al. ACC/AHA guidelines for coronary angiography. A report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on Coronary Angiography). Developed in collaboration with the Society for Cardiac Angi ography and Interventions. *J Am Coll Cardiol.* 1999;33:1756-1824.
23. Miettinen OS. Regression analysis. In: Theoretical epidemiology, principles of occurrence research in medicine. *New York: Wiley*. 1985;p. 216-244.
24. Greenland S, Finkle WD. A critical look at methods for handling missing covariates in epidemiologic regression analyses. *Am J Epidemiol.* 1995;142:1255-1264.
25. Bland JM, Altman DG. Statistical methods for assessing agreement between two methods of clinical measurement. *Lancet.* 1986;1:307-310.
26. Prati F, Arbustini E, Labellarte A et al. Eccentric atherosclerotic plaques with positive remodelling have a pericardial distribution: a permissive role of epicardial fat? A three-dimensional intravascular ultrasound study of left anterior descending artery lesions. *Eur Heart J.* 2003;24:329-336.
27. Jeong JW, Jeong MH, Yun KH et al. Echocardiographic epicardial fat thickness and coronary artery disease. *Circ J.* 2007;71:536-539.
28. Iacobellis G, Ribaudo MC, Assael F et al. Echocardiographic epicardial adipose tissue is related to an thropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. *J Clin Endocrinol Metab.* 2003;88:5163-5168.
29. Jazet IM, Pijl H, Meinders AE. Adipose tissue as an endocrine organ: impact on insulin resistance. *Neth J Med.* 2003;61:194-212.
30. Iacobellis G, Leonetti F. Epicardial adipose tissue and insulin resistance in obese subjects. *J Clin Endocrinol Metab.* 2005;90:6300-6302.
31. Dagenais GR, Yi Q, Mann JF et al. Prognostic impact of body weight and abdominal obesity in women and men with cardiovascular disease. *Am Heart J.* 2005;149:54-60.

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Epicardial and peri-coronary fat in relation to coronary atherosclerosis and coronary artery calcification in patients undergoing coronary angiography

Abstract

Objective

Fat surrounding coronary arteries might aggravate coronary artery disease (CAD). Aim was to investigate the relationship between epicardial adipose tissue (EAT) and peri-coronary fat and coronary atherosclerosis and coronary artery calcification (CAC) in patients suspected of CAD, and whether this relationship is modified by total body weight.

Methods

Cross-sectional study in 128 patients with angina pectoris (61 ± 6 years) undergoing coronary angiography. EAT volume and peri-coronary fat thickness were measured with cardiac computed tomography (CT). Severity of coronary atherosclerosis was assessed by the number of stenotic (≥50%) coronary vessels; extent of CAC was determined by Agatston score. Patients were stratified for median total body weight (body mass index (BMI) 27 kg/m^2).

Results

Overall, EAT and peri-coronary fat were not associated with severity of coronary atherosclerosis and extent of CAC. In patients with low BMI, those with multi-vessel disease had higher EAT volume (99.7 vs. 66.5 cm^3, p-value = 0.04) and peri-coronary fat thickness (9.8 vs. 8.4 mm, p-value = 0.06) compared to those without CAD. Also, patients with severe CAC had more EAT volume (108.0 vs. 68.7 cm^3, p-value = 0.02) and peri-coronary fat thickness (10.0 vs. 8.2 mm, p-value = 0.01) compared to those with minimal or absent CAC.

Conclusions

EAT and peri-coronary fat were not associated with severity of coronary atherosclerosis and CAC in patients suspected of CAD. However, in those with low BMI, high EAT and peri-coronary fat are related to more severe coronary atherosclerosis and CAC. Fat surrounding coronary arteries may be involved in the process of coronary atherosclerosis, although this is different for patients with low and high BMI.

Introduction

Obesity-induced insulin resistance is considered to be an essential feature in the development of metabolic abnormalities (e.g. hypertension, inflammation, and dyslipidemia) and the associated increased risk of coronary artery disease (CAD).[1-3] This clustering of risk factors is often referred to as metabolic syndrome.[4] Moreover, abdominal fat is able to produce large quantities of tumor necrosis factor-alpha (TNF-α), interleukine-6 (IL-6), free fatty acids and plasminogen activator inhibitor-1 (PAI-1) all involved in accelerating atherosclerosis, plaque instability and arterial thrombosis.[5-7] Adiponectin is solely produced by adipocytes and has anti-atherosclerotic properties.[8] The production of adiponectin is reduced in obesity and in insulin resistance.[9] Inflammatory cytokines, such as TNF-α, contribute to the development of insulin resistance and hypoadiponectinemia[8,10,11], both associated with an increased cardiovascular risk.[3,12,13]

It could be hypothesized that an increased quantity and/or dysfunctional adipose tissue around the coronary arteries contributes to the development of CAD. Since this adipose tissue, often called epicardial adipose tissue (EAT), produces adipocytokines (such as TNF-α, IL-6)[14,15] and is in close contact with the adventitia of the coronary arteries, it potentially aggravates inflammation in the vessel wall and stimulates the progression of atherosclerosis from 'outside to inside'. Indeed, levels of adiponectin in adipose tissue around the heart were lower in patients with CAD compared to those without CAD.[16] Nevertheless, it is still unclear whether this small quantity of adipose tissue by itself or in combination with abdominal fat contributes to the pathogenesis of CAD.[17,18]

As body mass index (BMI) and abdominal fat are strongly associated with EAT[18,19], and are important determinants for the development of coronary atherosclerosis[2,20], the influence of fat mass around the heart on the development of CAD may differ in patients with low and high BMI.[21]

Previously, the association between EAT thickness and CAD has been evaluated using echocardiography.[17,18] However, computed tomography (CT) is more accurate to quantify adipose tissue accumulation due to its higher spatial resolution.[22]

Aim of the present study is to investigate the relationship between EAT volume and peri-coronary fat thickness, quantified using cardiac CT, and the severity of coronary atherosclerosis and extent of coronary artery calcification (CAC) in patients suspected of CAD referred for coronary angiography (CAG), and whether this relationship is modified by body weight.

Methods

Study settings, participants and design

Patients originated from a diagnostic study at the University Medical Centre Utrecht designed to establish the diagnostic accuracy of multi-slice computed tomography coronary angiography (CTA) compared to conventional CAG in the detection of significant coronary obstruction. Patients (n = 128) were referred for diagnostic CAG or percutaneous coronary intervention because of stable angina pectoris (n = 100) or unstable angina pectoris (n = 28)[23,24] as clinically indicated at the discretion of the referring cardiologist. Subjects were divided by median BMI (cut off value: 27 kg/m^2) into patients with low and high total body weight (BMI). The inclusion criteria were: age 50-70 years, and stable sinus rhythm. Patients with previous PTCA or CABG, serum creatinine levels >140 µmol/l, or known iodine-based contrast allergy were not included. CTA was performed in all patients within a month before or after CAG. The Medical Ethics Committee approved the study and all participants gave their written informed consent.
Clinical information was obtained using a standardised health questionnaire. Height, body weight, and blood pressure were measured. Fasting blood was sampled to determine lipid, glucose and creatinine levels. Metabolic syndrome was diagnosed according to the Adult Treatment Panel III (ATP III) criteria as the presence of at least three or more metabolic abnormalities.[4] Because waist circumference was not available, a BMI of ≥30 kg/m^2 was used as determinant for obesity.[25]

CT technique and image analyses

EAT and peri-coronary fat were quantified on ECG-gated diagnostic cardiac CT scans. CT studies were performed on a 64 detector-row CT scanner (Brilliance 64, Philips Medical Systems, Cleveland, OH, USA). Scan duration time was 7-10 seconds. Standard coronary imaging protocols were applied including the use of intra-venous beta-blockers for patients with heart rates >65 beats/min (unless contraindicated) and image acquisitions were performed during a breath-hold in inspiration. Imaging parameters were: slice collimation of 64 x 0.625 mm, gantry rotation time of 420 ms, tube voltage of 120 kV, tube current of 900 mAs. Contrast agent used was iopromide (Schering AG, Berlin, Germany), which was injected intravenously (1.6-2.0 g iodine/s depending on patient's body weight).
Measurements were performed in the most motionless phase of the cardiac cycle, which was most frequently a mid-diastolic phase, with retrospective cardiac gating at 70-80% of the R-R interval. The window settings were adjusted to properly visualise the adipose tissue and the pericardium.

Quantification of EAT volume

EAT volume was defined as the total amount of adipose tissue between the surface of the heart and the visceral layer of the pericardium (visceral epicardium). EAT volume (cm^3) was measured using two dedicated software programs (Extended Brilliance Workspace and Easy Vision, Philips Medical Systems, Best, The Netherlands). Measurements were performed on short-axis views of 2-mm-slice-thickness and 3-mm-intersection gaps.

The stack of the short-axis views started at the apex just below the fibrous pericardium and extended until the centre of the left atrium. An EAT area measurement was performed by tracing a single region of interest (ROI) containing heart and EAT (*Figure 1A*) on the section obtained at each level. ROI's were placed at the visceral epicardium to exclude pericardial fluid. A density range between -200 and -30 Hounsfield Units (HU) was used to isolate the adipose tissue. Subsequently, the computer software program produced an accurate volume of EAT by adding up the EAT areas of 25-30 sections and taking into account slice thickness and intersection gap. The intra- and interobserver coefficient of variations (CVs), measured for 60 CT scans, were 3.1% and 5.0%, respectively.[26]

Quantification of peri-coronary fat thickness

Peri-coronary fat was defined as the adipose tissue between the surface of the heart and the visceral epicardium directly surrounding the three main coronary arteries. Peri-coronary fat thickness (mm) was quantified on axial views of a regular Philips CT workstation. In order not to overestimate the peri-coronary fat due to obliquity, thickness measurements were performed on images in which the axial sections were perpendicular to the surface of the heart. In each of the regions of the main coronary arteries (i.e. right coronary artery (RCA), left coronary artery (LCA) and left circumflex (LCX)), maximal fat thickness assessed as the largest distance from myocardium to visceral epicardium was determined (*Figure 1B*). Mean thickness of the peri-coronary fat surrounding the three coronary arteries was used for analyses ('peri-coronary fat thickness'). The intra- and interobserver CVs, measured for 60 CT scans, were 12.0% and 13.1%, respectively.[26]

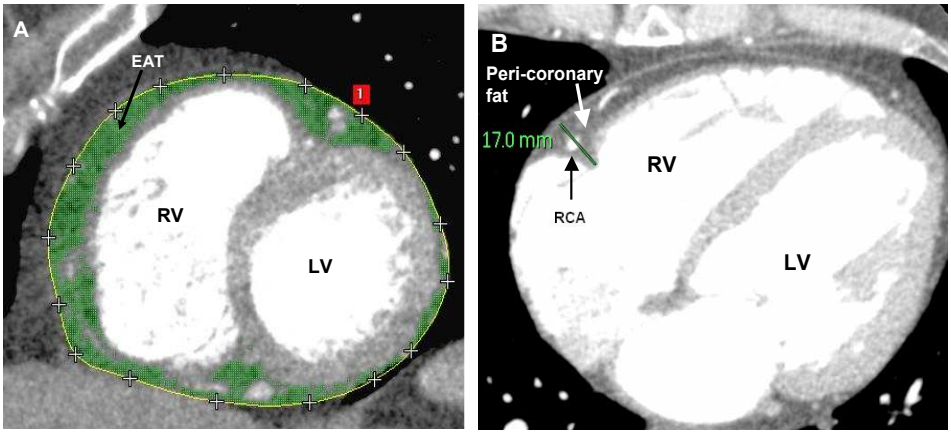

Figure 1. Epicardial adipose tissue (EAT) and peri-coronary fat measurements. (A) Region of interest (ROI) (yellow) on a cross-sectional image to determine the EAT area (green). EAT volume is the sum of the EAT areas on cross-sectional images. (B) Peri-coronary fat thickness (green). (**For colour figure, see page 167**).

Severity of coronary atherosclerosis

Conventional coronary angiograms were recorded in multiple projections for left and right coronary arteries, and reviewed for significant coronary artery obstructions by cardiologists unaware of the amount of EAT and peri-coronary fat. The severity of coronary atherosclerosis was classified as the number of coronary arteries (RCA, LCA, and LCX) with a ≥50% luminal diameter stenosis.[27] Subjects were classified as having 0, 1, 2 or 3-vessel disease. Left main artery stenosis was scored as 2-vessel disease.

Extent of CAC

CAC imaging was performed using multi-slice CT scanning. Non-contrast scans, with 3-mm-slice-thickness and an increment of 1.5 mm, were performed during a single breath-hold. The CAC score was obtained using dedicated software for calcium scoring (Heartbeat-CS, Extended Brilliance Workspace, Philips Medical Systems, Best, The Netherlands). A single experienced investigator blinded to clinical data performed the CAC scoring using the Agatston method.[28] All regions with a density >130 HU were identified as potential calcifications. To reduce the influence of noise, the minimum size of calcified lesion was set at 0.5 mm^2. The peak density in HU and the area in mm^2 of each selected region were calculated and multiplied by a weighting factor.[28] The scores of individual lesions were added to obtain the Agatston calcium score for the entire coronary tree.

CAC outcome was expressed as ordinal categories, based on cut-offs that have been widely used in the literature and are proposed by Rumberger et al.[29]: ≤10 (minimal or non-significant CAC), 11-100 (mild CAC), 101-400 (moderate CAC), 401-1000 (severe CAC), and >1000 AU (extensive CAC).[29]

Statistical analysis

Differences in mean EAT volume and peri-coronary fat thickness between patients with 0, 1, and ≥2-vessel disease, and between patients with various CAC scores were determined using analysis of covariance (ANCOVA, general linear model procedure) in order to adjust for all variables that were considered to be confounders: age (continuously), and gender. To assess the independent relation of EAT and peri-coronary fat with severity of CAD, we additionally adjusted for BMI (continuously). The modifying effect of BMI on the relationship of EAT volume and peri-coronary fat thickness with severity of coronary atherosclerosis and extent of CAC was examined by calculating the differences in mean EAT volume and peri-coronary fat thickness for the different subgroups of vessel disease and CAC scores in patients with low and high BMI (cut off point: median BMI 27 kg/m^2).

To reduce bias and increase statistical efficiency, missing values (HDL-cholesterol (n = 21), triglycerides (n = 20), fasting glucose (n = 5), creatinine (n = 5), diastolic (n = 12) and systolic (n = 12) blood pressure) in the data were completed by regression imputation.[30,31] Significance was taken at the 5% level (two-sided).

Results

Baseline characteristics

In *Table 1* the clinical characteristics of the study population are listed according to tertiles of EAT volume. The distribution of characteristics among tertiles of peri-coronary fat thickness was comparable to the distribution among tertiles of EAT volume (data not shown). The majority of patients were male (70%), and the mean age was 61 ± 6 years. Sixty-three (49%) and 29 (23%) patients had a BMI ≥27 and ≥30 kg/m^2, respectively. The prevalence of metabolic syndrome was 43%.

Table 1. General characteristics of the study population according to tertiles of EAT volume (n = 128)

EAT volume	**Tertile 1**	**Tertile 2**	**Tertile 3**
(Range)	(27.9-84.9 cm^3)	(86.1-127.1 cm^3)	(129.3-270.7 cm^3)
	n = 43	n = 43	n = 42
EAT volume (cm^3)	64 ± 14	105 ± 12	161 ± 28
Age (years)	59 ± 6	62 ± 5	61 ± 6
Male gender, n (%)	28 (65)	31 (72)	30 (71)
Current smoking*, n (%)	13 (30)	17 (40)	12 (29)
Diabetes mellitus†, n (%)	5 (12)	11 (26)	8 (19)
Creatinine clearance (ml/min/1.73m2)‡	81 ± 17	92 ± 21	93 ± 29
Metabolic syndrome ATP III, n (%)	13 (30)	20 (47)	22 (52)
BMI (kg/m^2)	26 ± 3	28 ± 3	30 ± 3
Systolic blood pressure (mmHg)	148 ± 25	155 ± 22	151 ± 19
Diastolic blood pressure (mmHg)	82 ± 14	82 ± 12	78 ± 13
Glucose (mmol/l)	5.4 (5.0-6.2)	6.0 (5.1-7.2)	5.9 (5.4-7.3)
Triglycerides (mmol/l)	1.38 (0.98-2.22)	1.65 (1.05-2.10)	1.40 (1.06-2.14)
HDL-cholesterol (mmol/l)	1.37 (1.10-1.56)	1.22 (1.01-1.42)	1.28 (1.02-1.69)
Medications			
Blood pressure-lowering agents, n (%)	38 (88)	38 (88)	39 (93)
Lipid-lowering agents, n (%)	32 (74)	35 (81)	33 (79)
Parameters of coronary artery disease			
Stable angina pectoris, n (%)	37 (86)	29 (67)	34 (81)
History of unstable angina pectoris, n (%)	2 (5)	3 (7)	3 (7)
History of myocardial infarction, n (%)	13 (30)	6 (14)	10 (24)
Family history of vascular disease, n (%)	17 (40)	19 (44)	23 (55)

All data in n (%), mean ± S.D. or median (interquartile range).
* Still smoking or recently (<1 month before study inclusion) stopped smoking.
† Self-reported diabetes mellitus.
‡ According to Cockroft-Gault.

EAT volume and peri-coronary fat thickness measurements

Mean EAT volume was 109.9 ± 44.1 cm^3 (range 27.9-270.7 cm^3); 110.2 ± 42.7 cm^3 for male and 109.1 ± 47.5 cm^3 for female patients. Average peri-coronary fat thickness around the coronary arteries was 10.4 ± 2.0 mm. Peri-coronary fat thickness was higher in the RCA region (15.9 ± 3.1 (range 8.9-25.5) mm), compared to the LCX region (10.0 ± 2.2 (range 4.3-15.8) mm); paired t-test p-value <0.001, and the LAD region (5.2 ± 1.9 (range 1.7-10.9) mm); paired t-test p-value <0.001.

EAT volume and peri-coronary fat thickness in relation to severity of coronary atherosclerosis

A total of 109 (85%) patients had a ≥50% stenosis in at least one major coronary artery. EAT volume and peri-coronary fat thickness were not associated with the number of stenotic coronary arteries, adjusted for age and gender (*Table 2*). Additional adjustment for BMI did not markedly change the results. Among patients with a BMI <27 kg/m^2, those with ≥2 vessel disease (multi-vessel disease) had a higher EAT volume (99.7 ± 6.4 cm^3) compared to those without CAD (66.5 ± 11.5 cm^3), p-value for trend = 0.04. Patients with a BMI ≥27 kg/m^2 tended to have lower EAT volumes (p-value for trend = 0.2) and peri-coronary fat thickness (p-value for trend = 0.2) in combination with a higher number of stenotic coronary arteries.

Table 2. EAT volume and peri-coronary fat thickness in relation to the number of stenotic coronary vessels (≥50% stenosis) in all patients and in subpopulations stratified by total body weight

	No vessel disease	1-vessel disease	≥ 2-vessel disease	P-value for trend
All patients, n = 128				
n (%)	19 (15)	54 (42)	55 (43)	
EAT volume (cm^3)*	104.4 ± 10.8	110.3 ± 6.1	111.4 ± 6.1	0.9
Peri-coronary fat thickness (mm)*	10.1 ± 0.5	10.3 ± 0.3	10.5 ± 0.3	0.7
Patients stratified by BMI				
BMI <27, n = 65				
n (%)	10 (15)	27 (42)	28 (43)	
EAT volume (cm^3)*	66.5 ± 11.5	87.8 ± 6.7	99.7 ± 6.4	0.04
Peri-coronary fat thickness (mm)*	8.4 ± 0.5	9.2 ± 0.3	9.8 ± 0.3	0.06
BMI ≥27, n = 63				
n (%)	9 (14)	27 (43)	27 (43)	
EAT volume (cm^3)*	154.9 ± 14.9	133.5 ± 8.2	120.0 ± 8.5	0.2
Peri-coronary fat thickness (mm)*	12.5 ± 0.6	11.5 ± 0.3	11.2 ± 0.3	0.2

EAT: epicardial adipose tissue. All data in mean ± se. * Adjusted for age and gender. Patients were divided according to total body weight based on median BMI (27 kg/m^2).

EAT volume and peri-coronary fat thickness in relation to extent of CAC

The median CAC score was 181 (37-544). EAT volume and peri-coronary fat thickness were not related to the extent of CAC in the whole study population, adjusted for age and gender (*Table 3*). Additional adjustment for BMI did not alter the relationship. In patients with a BMI <27 kg/m^2, those with severe or extensive CAC had a higher EAT volume (108.0 ± 7.4 cm^3 vs. 68.7 ± 10.4 cm^3, p-value for trend = 0.02) and peri-coronary fat thickness (10.0 ± 0.3 mm vs. 8.2 ± 0.5 mm, p-value for trend = 0.01) compared to those with minimal or absent CAC.

Table 3. EAT volume and peri-coronary fat thickness in relation to coronary artery calcification (according to Agatston) in all patients and in subpopulations stratified by total body weight

	CAC ≤ 10	CAC 11-100	CAC 101-400	CAC ≥ 401	P-value for trend
All patients, n = 128					
n (%)	21 (16)	28 (22)	37 (29)	42 (33)	
EAT volume (cm^3)*	111.8 ± 9.9	110.8 ± 8.4	102.2 ± 7.4	115.1 ± 7.0	0.6
Peri-coronary fat thickness (mm)*	10.4 ± 0.4	10.5 ± 0.4	10.0 ± 0.3	10.7 ± 0.3	0.4
Patients stratified by BMI					
BMI <27, n = 65					
n (%)	10 (15)	15 (23)	20 (31)	20 (31)	
EAT volume (cm^3)*	68.7 ± 10.4	89.8 ± 8.5	81.6 ± 7.4	108.0 ± 7.4	0.02
Peri-coronary fat thickness (mm)*	8.2 ± 0.5	9.7 ± 0.4	8.8 ± 0.3	10.0 ± 0.3	0.01
BMI ≥27, n = 63					
n (%)	11 (17)	13 (21)	17 (27)	22 (35)	
EAT volume (cm^3)*	159.9 ± 13.7	134.3 ± 11.7	129.4 ± 10.3	115.3 ± 9.7	0.1
Peri-coronary fat thickness (mm)*	12.7 ± 0.5	11.3 ± 0.5	11.4 ± 0.4	11.1 ± 0.4	0.1

EAT: epicardial adipose tissue; CAC: coronary artery calcification. All data in mean ± se. * Adjusted for age and gender. Patients were divided according to total body weight based on median BMI (27 kg/m^2).

Discussion

In this study, it is shown that EAT and peri-coronary fat, quantified using cardiac CT, are not related to the severity of coronary atherosclerosis and extent of CAC in patients suspected of CAD. However, in patients with a low BMI, EAT volume and peri-coronary fat thickness are related to a higher number of stenotic coronary vessels and more severe CAC.

Adipose tissue surrounding the heart may contribute to the progression of coronary atherosclerosis due to its proximity to coronary arteries and potential for local secretion of adipocytokines.[14,15] Accordingly, in porcine coronary arteries *in vivo*, adventitial treatment with inflammatory mediators resulted in the migration of macrophages

into the vascular wall and intima thickening.[32,33] In the present study, we indeed found that an accumulation of EAT (volume) and peri-coronary fat (thickness), measured with cardiac CT, was related to an increased severity of CAD in patients with a low BMI.

Our study provides a plural assessment of the severity of CAD, in the context of the severity of coronary atherosclerosis (the number of coronary arteries with ≥50% stenosis), which roughly estimates the luminal coronary burden, and the extent of CAC, which also measures early atherosclerotic changes before luminal stenosis occurs. Extent of CAC can be applied as an estimate of the severity of CAD; it is associated with the probability of coronary artery stenosis and related to the risk of developing CAD and the overall coronary plaque burden.[34-36] Moreover, two different estimates of fat accumulation surrounding the coronary arteries, measured with cardiac CT, were used. Namely, a volumetric method of EAT to accurately estimate the total amount of EAT. In addition, a peri-coronary fat thickness measurement to assess the fat thickness directly surrounding the coronary arteries which is conceptually most interesting.

Previous studies evaluating the relationship between EAT and angiographic CAD provided conflicting results probably due to differences in measurement techniques and study populations.[17,18] A positive correlation was shown between EAT thickness and severity of CAD in mainly non-obese patients with known CAD (mean BMI 24 ± 3 kg/m^2).[18] In contrast, another study did not detect an association between EAT thickness and angiographic CAD.[17] That study mainly included patients with obesity (BMI ≥30 kg/m^2). Both studies[17,18] used a single echocardiographic thickness measurement to estimate the EAT quantity. The present study is unique in the sense that it adds an accurate volumetric assessment of the total amount of EAT and a thickness measurement of peri-coronary fat, measured with cardiac CT, which were both associated with an increased severity of CAD in patients with a low BMI. Interestingly, it has been shown that pericardial fat (EAT and paracardial fat) volume, measured with CT, was related to angiographic CAD in patients with a BMI <25 kg/m^2.[21] Neither pericardial fat nor BMI, and visceral abdominal fat were independently correlated with CAD in patients with a BMI ≥25 kg/m^2.

In the present study, the lack of an association of EAT volume and peri-coronary fat thickness with severity of CAD in the whole study population may be the result of effect modification by body weight. In line with previous findings[21], our results indicate that there might be a differential effect of fat surrounding coronary arteries (regional body fat) on CAD in patients with low and high BMI. This may be due to several reasons. Although adipose tissue around the heart is strategically located close to the coronary arteries, it is a relatively small visceral fat depot compared to abdominal fat. Patients with a high BMI are often insulin resistant leading to higher systemic plasma levels of inflammatory cytokines thereby accelerating atherogenesis. In that case, the local production of adipocytokines by epicardial adipocytes adds little to the inflammatory process in the vessel wall. This reasoning is supported by the absence of an association between EAT thickness and angiographic CAD in a study where 76% of the patients had a BMI ≥30 kg/m^2.[17] Moreover, patients with a high BMI are generally obese and therefore also have an increased amount of EAT or peri-coronary fat. In case of the absence of insulin resistance, adipocytes do not produce large quantities of inflammatory cytokines (e.g. IL-6, TNF-α), and therefore this adipose tissue does not lead to large metabolic abnormalities (e.g. hypertension, hyperglycemia, and low HDL-

cholesterol) and adds little to the atherosclerotic process. Further studies are needed to clarify this.

We acknowledge potential limitations of the present study. Firstly, due to the cross-sectional study design only assumptions about possible etiological relationships could be made. Secondly, although the definitions for severity of CAD (the number of coronary arteries with a ≥50% luminal diameter stenosis and extent of CAC) are often used in the literature[27,28], they do not account for the presence of plaque instability which may lead to acute coronary artery stenosis. Furthermore, waist circumference was not measured; therefore we could only stratify for total body weight according to BMI. However, fat surrounding coronary arteries was related to waist circumference and BMI[18,19], which are both indicators of a high cardiovascular risk.[20] Lastly, the functional characteristics of fat surrounding the coronary arteries may be more important in the pathofysiology of CAD than the amount. Our data were obtained in routine medical care. Therefore, markers of dysfunction of fat surrounding coronary arteries (IL-6, TNF-α, PAI-1) were not measured in fat samples.

In conclusion, EAT and peri-coronary fat, quantified using cardiac CT, are not related to the severity of coronary atherosclerosis and extent of CAC in patients suspected of CAD. However, in those with a low BMI, high EAT and peri-coronary fat are related to more severe coronary atherosclerosis and CAC. EAT or peri-coronary fat may play a role in the process of coronary atherosclerosis, although this is different for patients with low and high BMI.

Acknowledgements

The help of K. Benders, A.J. Borgen, J. op den Brouw and A.S.R. van Lindert for data collection is greatly acknowledged.

Funding: University Medical Centre Utrecht, Utrecht, The Netherlands, the Bekalis Foundation, Brussels, Belgium and ZonMw, The Hague, The Netherlands.

References

1. Eckel RH, Grundy SM, Zimmet PZ. The metabolic syndrome. *Lancet.* 2005;365:1415-1428.
2. Yusuf S, Hawken S, Ounpuu S et al. Obesity and the risk of myocardial infarction in 27,000 participants from 52 countries: a case-control study. *Lancet.* 2005;366:1640-1649.
3. Haffner SM. Epidemiology of insulin resistance and its relation to coronary artery disease. *Am J Cardiol.* 1999;84(1A):11J-14J.
4. Grundy SM, Brewer HB, Jr, Cleeman JI et al. Definition of metabolic syndrome: Report of the National Heart, Lung, and Blood Institute/American Heart Association conference on scientific issues related to definition. *Circulation.* 2004;109:433-438.
5. Eriksson P, Reynisdottir S, Lonnqvist F et al. Adipose tissue secretion of plasminogen activator inhibitor-1 in non-obese and obese individuals. *Diabetologia.* 1998;41:65-71.
6. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation.* 2002;105:1135-1143.
7. Berg AH, Scherer PE. Adipose tissue, inflammation, and cardiovascular disease. *Circ Res.* 2005;96:939-949.
8. Shimada K, Miyazaki T, Daida H. Adiponectin and atherosclerotic disease. *Clin Chim Acta.* 2004;344:1-12.
9. Kadowaki T, Yamauchi T, Kubota N et al. Adiponectin and adiponectin receptors in insulin resistance, diabetes, and the metabolic syndrome. *J Clin Invest.* 2006;116:1784-1792.
10. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-alpha: direct role in obesity-linked insulin resistance. *Science.* 1993;259:87-91.
11. Pittas AG, Joseph NA, Greenberg AS. Adipocytokines and insulin resistance. *J Clin Endocrinol Metab.* 2004;89:447-452.
12. Kumada M, Kihara S, Sumitsuji S et al. Association of hypoadiponectinemia with coronary artery disease in men. *Arterioscler Thromb Vasc Biol.* 2003;23:85-89.
13. Shioji K, Moriguchi A, Moriwaki S et al. Hypoadiponectinemia implies the development of atherosclerosis in carotid and coronary arteries. *J Cardiol.* 2005;46:105-112.
14. Mazurek T, Zhang L, Zalewski A et al. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation.* 2003;108:2460-2466.
15. Baker AR, Silva NF, Quinn DW et al. Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol.* 2006;5:1.
16. Iacobellis G, Pistilli D, Gucciardo M et al. Adiponectin expression in human epicardial adipose tissue in vivo is lower in patients with coronary artery disease. *Cytokine.* 2005;29:251-255.
17. Chaowalit N, Somers VK, Pellikka PA et al. Subepicardial adipose tissue and the presence and severity of coronary artery disease. *Atherosclerosis.* 2006;186:354-359.
18. Jeong JW, Jeong MH, Yun KH et al. Echocardiographic epicardial fat thickness and coronary artery disease. *Circ J.* 2007;71:536-539.
19. Iacobellis G, Ribaudo MC, Assael F et al. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. *J Clin Endocrinol Metab.* 2003;88:5163-5168.
20. Dagenais GR, Yi Q, Mann JF et al. Prognostic impact of body weight and abdominal obesity in women and men with cardiovascular disease. *Am Heart J.* 2005;149:54-60.
21. Taguchi R, Takasu J, Itani Y et al. Pericardial fat accumulation in men as a risk factor for coronary artery disease. *Atherosclerosis.* 2001;157:203-209.
22. Rossner S, Bo WJ, Hiltbrandt E et al. Adipose tissue determinations in cadavers--a comparison between cross-sectional planimetry and computed tomography. *Int J Obes.* 1990;14:893-902.
23. Goldman L, Hashimoto B, Cook EF et al. Comparative reproducibility and validity of systems for assessing cardiovascular functional class: advantages of a new specific activity scale. *Circulation.* 1981;64:1227-1234.

24. Braunwald E. Unstable angina. A classification. *Circulation.* 1989;80:410-414.
25. Alberti KG, Zimmet PZ. Definition, diagnosis and classification of diabetes mellitus and its complications. Part 1: diagnosis and classification of diabetes mellitus provisional report of a WHO consultation. *Diabet Med.* 1998;15:539-553.
26. Gorter PM, van Lindert AS, de Vos AM et al. Quantification of epicardial and peri-coronary fat using cardiac computed tomography; reproducibility and relation with obesity and metabolic syndrome in patients suspected of coronary artery disease. *Atherosclerosis.* 2007 Sep 18;[Epub ahead of print].
27. Scanlon PJ, Faxon DP, Audet AM et al. ACC/AHA guidelines for coronary angiography. A report of the American College of Cardiology/American Heart Association Task Force on practice guidelines (Committee on Coronary Angiography). Developed in collaboration with the Society for Cardiac Angiography and Interventions. *J Am Coll Cardiol.* 1999;33:1756-1824.
28. Agatston AS, Janowitz WR, Hildner FJ et al. Quantification of coronary artery calcium using ultrafast computed tomography. *J Am Coll Cardiol.* 1990;15:827-832.
29. Rumberger JA, Brundage BH, Rader DJ et al. Electron beam computed tomographic coronary calcium scanning: a review and guidelines for use in asymptomatic persons. *Mayo Clin Proc.* 1999;74:243-252.
30. Miettinen OS. Theoretical epidemiology, principles of occurrence research in medicine. *New York: Wiley*. 1985; p. 216-244.
31. Greenland S, Finkle WD. A critical look at methods for handling missing covariates in epidemiologic regression analyses. *Am J Epidemiol.* 1995;142:1255-1264.
32. Miyata K, Shimokawa H, Kandabashi T et al. Rho-kinase is involved in macrophage-mediated formation of coronary vascular lesions in pigs in vivo. *Arterioscler Thromb Vasc Biol.* 2000;20:2351-2358.
33. Shimokawa H, Ito A, Fukumoto Y et al. Chronic treatment with interleukin-1 beta induces coronary intimal lesions and vasospastic responses in pigs in vivo. The role of platelet-derived growth factor. *J Clin Invest.* 1996;97:769-776.
34. Haberl R, Becker A, Leber A et al. Correlation of coronary calcification and angiographically documented stenoses in patients with suspected coronary artery disease: results of 1,764 patients. *J Am Coll Cardiol.* 2001;37:451-457.
35. Wong ND, Hsu JC, Detrano RC et al. Coronary artery calcium evaluation by electron beam computed tomography and its relation to new cardiovascular events. *Am J Cardiol.* 2000;86:495-498.
36. Sangiorgi G, Rumberger JA, Severson A et al. Arterial calcification and not lumen stenosis is highly correlated with atherosclerotic plaque burden in humans: a histologic study of 723 coronary artery segments using nondecalcifying methodology. *J Am Coll Cardiol.* 1998;31:126-133.

General discussion

Cardiovascular risk: insulin resistance or glucose intolerance?

From the perspective of glucose metabolism there is a classical view on the spectrum of cardiovascular risks ranging from patients with normal glucose tolerance to pre-diabetic patients (impaired fasting glucose, impaired glucose tolerance, metabolic syndrome) to patients with type 2 diabetes (*Figure 1A*). Metabolic syndrome refers to a clustering of vascular risk factors[1] in which insulin resistance and impaired beta-cell function are coexisting underlying conditions. In our view, detecting metabolic syndrome, also called pre-diabetes or cardiometabolic syndrome, or diabetes itself may not adequately estimate actual cardiovascular risk, leaving patients at high risk undetected and classifying patients at high-risk while their actual risk is moderate or even low. Focusing on the degree of insulin resistance and subsequent metabolic abnormalities (e.g. hypertension, atherogenic dyslipidemia, and inflammation) seems a more promising strategy to estimate cardiovascular risk level. Namely, insulin resistant patients with metabolic syndrome may carry multiple metabolic abnormalities that put them already at a high cardiovascular risk level, even before the onset of the clinical diagnosis of diabetes (*Figure 1B*). It is even suggested that insulin resistance and associated inflammation are common antecedents for both diabetes and vascular disease, indicating that atherosclerosis might not be simply a consequence of diabetes.[2,3] In order to effectively target aggressive intervention strategies at patients with the highest vascular risk, precise cardiovascular risk assessment is warranted. A categorization assumes that cardiovascular risk is a discrete process whereas it should be seen as a continuum. In our view, cardiovascular risk increases on a gradual scale with respect to insulin resistance rather than jumping abruptly whenever a patient shifts to a higher glycemic level. A recent study showed that the increased cardiovascular risk in individuals with impaired fasting glucose or diabetes was found to be largely attributable to the accompanying metabolic disorders rather than hyperglycemia *per se*.[4] We advocate that early recognition and coherent treatment of insulin resistance and modifiable metabolic disorders should be given more consideration in cardiovascular disease prevention than just focusing on the glycemic state. Moreover, large-scale epidemiological studies are needed to further investigate the relative contribution of coexisting metabolic disturbances and hyperglycemia *per se* to cardiovascular risk. This calls for studies investigating whether hyperglycema in metabolic syndrome or diabetes carries any additional cardiovascular risk independent of other metabolic abnormalities.

We think that the concept of metabolic syndrome[1] is an easy tool in clinical practice to identify (pre-diabetic) insulin resistant patients who are prone to develop atherosclerotic vascular disease as well as type 2 diabetes. Although a variety of opinions exists on the clinical utility of metabolic syndrome and on the definition, it is now generally accepted that risk factors commonly aggregate and cluster with insulin resistance. In this thesis, we describe that in patients with already manifest arterial disease the metabolic syndrome is highly prevalent (46%). Results from our group indicate that the presence of metabolic syndrome was indeed associated with more advanced vascular damage in these high-risk patients[5], and is associated with a higher risk of subsequent occurrence of vascular events regardless of the presence of diabetes. Nevertheless, the cardiovascular risk of patients with metabolic syndrome appears to differ according

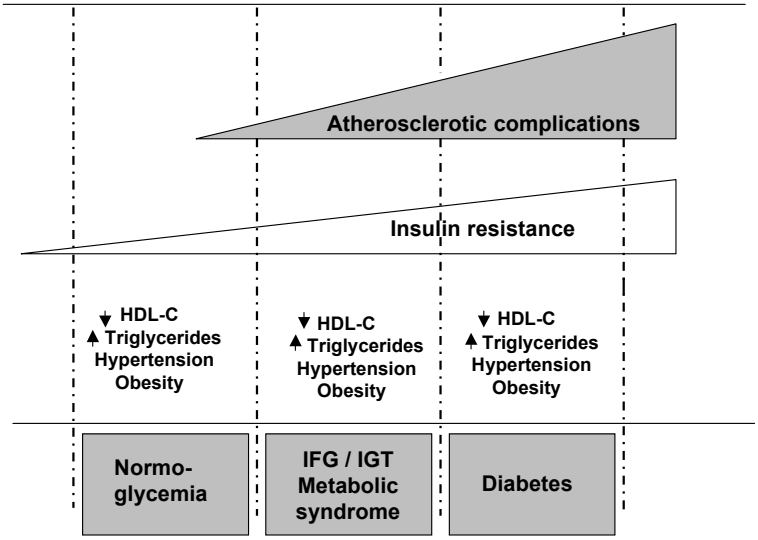

Figure 1A. Classical view of cardiovascular risk with respect to the glycemic state. IFG; impaired fasting glucose, IGT; impaired glucose tolerance, HDL-C; high-density lipoprotein cholesterol.

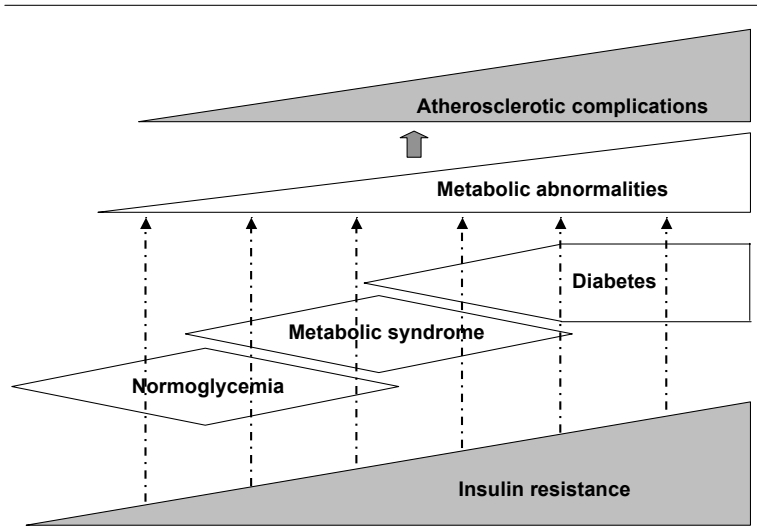

Figure 1B. Proposed view of cardiovascular risk with respect to insulin resistance and associated metabolic abnormalities

to the presence of the different components.[4,6] Thus, although metabolic syndrome can be used to select patients deserving increased attention, individualized risk assessment should be performed in these patients to focus on those who will benefit the most from aggressive intervention strategies. In one of the studies presented in this thesis (chapter 3), we showed that insulin resistance *per se* was associated with an increased prevalence of albuminuria in patients with manifest arterial disease without known diabetes. This association was only partly explained by components of metabolic syndrome. Elevated insulin resistance was not associated with an increased carotid intima-media thickness, as previously found for patients with metabolic syndrome.[5] This may indicate that insulin resistance plays a role in different stages of the atherosclerotic process. Also, metabolic syndrome may not be the sine qua non of insulin resistance. It is still an unresolved issue whether metabolic syndrome really is a useful tool to simply diagnose an insulin resistant state in individual patients.

American guidelines classify patients into low risk, moderate risk, high risk (e.g. established vascular disease or type 2 diabetes) and very high cardiovascular risk (e.g. both type 2 diabetes / metabolic syndrome and vascular disease) in order to guide therapy intensity.[7] We think that presence of insulin resistance and metabolic disorders confer additional risk of atherosclerotic vascular disease across all risk levels. The elevated cardiovascular risk among diabetic patients largely depends on concomitant metabolic abnormalities[8], implying that insulin resistance and metabolic disturbances play an important role in their cardiovascular risk and not all diabetic patients can simply be considered as high-risk patients. Findings from the Steno-2 trial showed that multifactorial risk factor intervention (insulin resistance and metabolic abnormalities) reduced the cardiovascular risk in diabetic patients; nonetheless, a considerable risk remained.[9] Among glucose-lowering agents only metformin and thiazolidinediones, both reducing insulin resistance, convincingly reduced the risk of macrovascular events in patients with type 2 diabetes.[10,11] However, there is still debate regarding the applicability of certain insulin sensitizers to vascular disease.[11] Patients with type 2 diabetes (or metabolic syndrome) and coronary heart disease obtained additional benefit from intensive lipid-lowering treatment[12,13], indicating that it is reasonable to achieve lower treatment goals for vascular risk factors, such as LDL-cholesterol <1.8 mmol/l, in these very high-risk patients.[7]

Thus, another approach to differentiate between cardiovascular risk in patients with diabetes may be the presence of clinical manifest arterial diseases. In this thesis it is shown that diabetic patients with cerebrovascular disease, peripheral arterial disease or coronary heart disease have a similar increased cardiovascular risk. We also found that among patients with type 2 diabetes and evident vascular disease there is still variability in cardiovascular risk; the incidence of vascular events increased with the number of different sites clinically affected by vascular disease, irrespective of the presence of vascular risk factors. Thus, counting the number of sites of evident vascular disease, irrespective of the type of vascular disease, may help to simply identify those patients with diabetes type 2 particularly at very high risk for new macrovascular complications from a generally high-risk diabetic population. Adding presence (and the number) of vascular diseases to the UK Prospective Diabetes Study risk model may improve individualized risk assessment for diabetic patients. However, whether diabetic patients with two or more sites of vascular diseases indeed derive incremental

benefit from lowering treatment goals for vascular risk factors should be assessed in a randomized clinical trial.

Perivascular fat: potential role in vascular disease or innocent bystander?

Impaired endothelium function is considered to be the initial step in the atherosclerotic process, driven primarily by systemic levels of risk factors such as plasma lipids, blood pressure, and inflammatory cytokines. Therefore, the extravascular compartment, comprising adventitia, vaso vasorum and perivascular fat, is often overlooked as a potential modulator of vascular function, even though arteries predisposed to atherosclerosis are covered with it. It is generally recognized that an increased quantity or inflamed intra-abdominal fat is a major risk factor for the development of vascular diseases. The observation that perivascular fat expresses chemokines and has the potential for local secretion of inflammatory cytokines is intriguing.[14-19] Combined with its proximity to the arterial wall, this may support a potential role for perivascular fat in atherogenesis[20-22], which could be of importance with respect to the current obesity epidemic. It is tempting to speculate that both local and distant inflammation contribute to the pathogenesis of atherosclerosis through the interplay (cross-talk) between various fat depots. In case of adiposity, macrophages may migrate to perivascular fat tissue and interact with other cells adjacent to the arterial wall (adipocytes, adventitial cells, smooth muscle cells) thereby enhancing vascular inflammation and promoting intima thickening from 'outside to inside'. Macrophages could enter a plaque through perivascular fat in a paracrine manner, traversing the arterial wall by diffusion, or by the vaso vasorum.[23] Accordingly, macrophage density was found to be increased in perivascular fat of coronary arteries with lipid plaques compared to perivascular fat of nonatherosclerotic arteries.[20]

Both local and distant inflammatory processes may be involved in the dilatation of the aortic artery. As presented in this thesis, more intra-abdominal fat was associated with larger infrarenal aortic diameters in patients with manifest arterial disease. This could implicate that intra-abdominal fat plays a role in the process of aortic dilatation. In our view, abdominal fat might do so by producing inflammatory cytokines, which are known to be involved in the degeneration of the aortic wall.[24] Abdominal fat may secrete large quantities of cytokines into the systemic circulation or may locally release adipocytokines thereby directly affecting the aortic wall from 'outside to inside' (partly through periaortic fat[16,21]). Among various perivascular fat depots, fat surrounding coronary arteries has received most attention. Considerable effort has been invested in the quantification of fat around the heart, particularly using echocardiography and magnetic resonance imaging, in order to assess the relationship between this fat tissue and cardiovascular risk. At the moment, there is no 'reference standard' for measuring fat surrounding coronary arteries. Our first attempt in this field was to develop a reliable quantification of fat surrounding coronary arteries using cardiac computed tomography (CT). CT is an accurate technique to quantify adipose tissue accumulation.[25,26] We found that volumetric quantifications of epicardial adipose tissue (EAT) were highly reproducible compared to thickness and area measurements of EAT

and peri-coronary fat. Nonetheless, volumetric measurements are time-consuming and automated quantification is needed.
In general, the amount of fat surrounding coronary arteries was found to be more associated with visceral adiposity than with total body weight.[27-29] In line with this finding, we showed in chapter 6 that EAT and peri-coronary fat accumulation were both associated with the clustering of vascular risk factors (metabolic syndrome) in patients undergoing coronary angiography. We assume that the amount of fat around the heart and its capacity to produce adipokines are susceptible to variation in obesity[30] and other systemic metabolic abnormalities[31], and may therefore play an additive role in pathophysiological processes leading to the development of coronary atherosclerosis seen in obesity. Namely, in an obesity-related insulin resistant state (metabolic syndrome), perivascular fat around coronary arteries might be triggered to produce large quantities of pro-atherogenic factors leading to high local concentrations around coronary arteries. Because the perivascular fat depot is relatively small compared to intra-abdominal fat, it is not likely that this fat depot adds much to systemic plasma concentrations of adipokines involved in the development of insulin resistance, although it may locally reinforce insulin resistance. Future studies should focus on investigating the mechanisms in which metabolic disturbances affect the adipokine production by perivascular fat.

Potential role for fat surrounding coronary arteries in developing coronary atherosclerosis

Several lines of evidence support an active role for fat surrounding coronary arteries in the atherosclerotic process. For instance, the degree of local inflammation is increased in fat surrounding coronary arteries compared to subcutaneous fat in patients undergoing coronary artery bypass graft[14], and was not related to plasma concentrations of inflammatory markers.[14] This means that local tissue inflammation appears to be involved in the development of coronary artery disease and that this is not reflected by plasma concentrations of inflammatory cytokines. However, it is important to recognize that this local inflammation in perivascular adipose tissue could also be a result of coronary artery disease in contrast to being the cause of coronary artery disease.

Fat surrounding coronary arteries in relation to coronary artery disease

Several clinical studies investigated the relationship between echocardiographic EAT thickness and the severity of coronary atherosclerosis in patients suspected of coronary artery disease (CAD) and provided conflicting results.[28,29,32] As presented in this thesis, we have assessed the relation between both EAT volume, as well as peri-coronary fat thickness, and the severity of CAD in patients undergoing coronary angiography. We found that only in patients with a body mass index <27 kg/m^2, the amount of EAT and peri-coronary fat was higher in patients with severe coronary atherosclerosis. This could implicate that in case of a low total body weight, patients with relatively more fat around the heart are still prone to develop coronary atherosclerosis. Although the relative absence of atherosclerosis in human intramyocardial segments of coronary arteries[20,33-35] is suspicious for a pro-atherogenic role of perivascular fat, the presence

of CAD in patients with congenital generalized lipodystrophy proves that fat around the heart is not necessary to develop coronary atherosclerosis.[36] Follow-up studies are needed to determine whether perivascular fat surrounding coronary arteries plays a causal role in the development of CAD.

In this thesis, we have approached the pathogenesis of atherosclerosis from a different perspective by focusing on the etiologic relationship between fat surrounding coronary arteries and coronary atherosclerosis which may put forward the role of perivascular fat in atherogenesis. Before embracing perivascular fat around the heart in clinical practice, further studies should be performed to determine whether this adipose tissue may provide important additional diagnostic or prognostic information or has therapeutic implications.[20] Potentially, targeting perivascular fat with specific therapies may prevent infiltration of macrophages or mast cells as have been shown in mice.[37] This may also be the case for fat surrounding the aorta.

Main conclusions of this thesis:

- In patients with manifest atherosclerotic arterial disease the metabolic syndrome is highly prevalent (46%).

- In patients with manifest arterial disease without known diabetes, elevated insulin resistance is associated with an increased prevalence of albuminuria, which is only partly explained by components of metabolic syndrome. Elevated insulin resistance is not associated with an increased carotid intima-media thickness in patients with manifest arterial disease.

- Patients with type 2 diabetes and cerebrovascular disease, coronary heart disease or peripheral arterial disease have a similar strongly increased risk for future cardiovascular events compared to diabetic patients without vascular disease.

- The cardiovascular risk in patients with type 2 diabetes increases markedly with the number of clinical manifestations of atherosclerosis at different vascular sites and is irrespective of subclinical atherosclerosis.

- The quantity of intra-abdominal fat and presence of metabolic syndrome are associated with larger infrarenal aortic diameters in patients with manifest arterial disease.

- Volumetric quantifications of EAT using cardiac CT are highly reproducible compared to more simple thickness and area measurements of EAT and peri-coronary fat.

- The quantity of adipose tissue surrounding coronary arteries (EAT and peri-coronary fat) is related with obesity and metabolic syndrome in patients undergoing coronary angiography.

- EAT and peri-coronary fat are associated with coronary atherosclerosis and coronary artery calcification in patients with a BMI <27 kg/m^2 undergoing coronary angiography.

References

1. Grundy SM, Cleeman JI, Daniels SR et al. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute Scientific Statement. *Circulation*. 2005;112:2735-2752.
2. Stern MP. Diabetes and cardiovascular disease. The "common soil" hypothesis. *Diabetes.* 1995;44:369-374.
3. Hu FB, Stampfer MJ. Is type 2 diabetes mellitus a vascular condition? *Arterioscler Thromb Vasc Biol.* 2003;23:1715-1716.
4. Liu J, Grundy SM, Wang W et al. Ten-year risk of cardiovascular incidence related to diabetes, prediabetes, and the metabolic syndrome. *Am Heart J.* 2007;153:552-558.
5. Olijhoek JK, Van der Graaf Y, Banga JD et al. The metabolic syndrome is associated with advanced vascular damage in patients with coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm. *Eur Heart J.* 2004;25:342-348.
6. Wilson PW, D'Agostino RB, Parise H et al. Metabolic syndrome as a precursor of cardiovascular disease and type 2 diabetes mellitus. *Circulation.* 2005;112:3066-3072.
7. Grundy SM, Cleeman JI, Merz CN et al. Implications of recent clinical trials for the National Cholesterol Education Program Adult Treatment Panel III guidelines. *Circulation.* 2004;110:227-239.
8. Howard BV, Best LG, Galloway JM et al. Coronary heart disease risk equivalence in diabetes depends on concomitant risk factors. *Diabetes Care.* 2006;29:391-397.
9. Gaede P, Vedel P, Larsen N et al. Multifactorial intervention and cardiovascular disease in patients with type 2 diabetes. *N Engl J Med.* 2003;348:383-393.
10. Effect of intensive blood-glucose control with metformin on complications in overweight patients with type 2 diabetes (UKPDS 34). UK Prospective Diabetes Study (UKPDS) Group. *Lancet.* 1998;352:854-865.
11. Lincoff AM, Wolski K, Nicholls SJ et al. Pioglitazone and risk of cardiovascular events in patients with type 2 diabetes mellitus: a meta-analysis of randomized trials. *JAMA.* 2007;298:1180-1188.
12. Shepherd J, Barter P, Carmena R et al. Effect of lowering LDL cholesterol substantially below currently recommended levels in patients with coronary heart disease and diabetes: the Treating to New Targets (TNT) study. *Diabetes Care.* 2006;29:1220-1226.
13. Deedwania P, Barter P, Carmena R et al. Reduction of low-density lipoprotein cholesterol in patients with coronary heart disease and metabolic syndrome: analysis of the Treating to New Targets study. *Lancet.* 2006;368:919-928.
14. Mazurek T, Zhang L, Zalewski A et al. Human epicardial adipose tissue is a source of inflammatory mediators. *Circulation.* 2003;108:2460-2466.
15. Baker AR, Silva NF, Quinn DW et al. Human epicardial adipose tissue expresses a pathogenic profile of adipocytokines in patients with cardiovascular disease. *Cardiovasc Diabetol.* 2006;5:1.
16. Henrichot E, Juge-Aubry CE, Pernin A et al. Production of chemokines by perivascular adipose tissue: a role in the pathogenesis of atherosclerosis? *Arterioscler Thromb Vasc Biol.* 2005;25:2594-2599.
17. Kremen J, Dolinkova M, Krajickova J et al. Increased subcutaneous and epicardial adipose tissue production of proinflammatory cytokines in cardiac surgery patients: possible role in postoperative insulin resistance. *J Clin Endocrinol Metab.* 2006;91:4620-4627.
18. Cheng KH, Chu CS, Lee KT et al. Adipocytokines and proinflammatory mediators from abdominal and epicardial adipose tissue in patients with coronary artery disease. *Int J Obes. (Lond)* 2007 Sep 18; [Epub ahead of print].
19. Iglesias MJ, Eiras S, Pineiro R et al. Gender differences in adiponectin and leptin expression in epicardial and subcutaneous adipose tissue. Findings in patients undergoing cardiac surgery. *Rev Esp Cardiol.* 2006;59:1252-1260.
20. Vela D, Buja LM, Madjid M et al. The role of periadventitial fat in atherosclerosis. *Arch Pathol Lab Med.* 2007;131:481-487.

21. Stern N, Marcus Y. Perivascular fat: innocent bystander or active player in vascular disease? *J Cardiometab Syndr.* 2006;1:115-120.
22. Sacks HS, Fain JN. Human epicardial adipose tissue: a review. *Am Heart J.* 2007;153:907-917.
23. Yudkin JS, Eringa E, Stehouwer CD. "Vasocrine" signalling from perivascular fat: a mechanism linking insulin resistance to vascular disease. *Lancet.* 2005;365:1817-1820.
24. Shah PK. Inflammation, metalloproteinases, and increased proteolysis: an emerging pathophysiological paradigm in aortic aneurysm. *Circulation.* 1997;96:2115-2117.
25. Rossner S, Bo WJ, Hiltbrandt E et al. Adipose tissue determinations in cadavers--a comparison between cross-sectional planimetry and computed tomography. *Int J Obes.* 1990;14:893-902.
26. Abbara S, Desai JC, Cury RC et al. Mapping epicardial fat with multi-detector computed tomography to facilitate percutaneous transepicardial arrhythmia ablation. *Eur J Radiol.* 2006;57:417-422.
27. Iacobellis G, Ribaudo MC, Assael F et al. Echocardiographic epicardial adipose tissue is related to anthropometric and clinical parameters of metabolic syndrome: a new indicator of cardiovascular risk. *J Clin Endocrinol Metab.* 2003;88:5163-5168.
28. Jeong JW, Jeong MH, Yun KH et al. Echocardiographic epicardial fat thickness and coronary artery disease. *Circ J.* 2007;71:536-539.
29. Ahn SG, Lim HS, Joe DY et al. Relationship of Epicardial Adipose Tissue by Echocardiography to Coronary Artery Disease. *Heart.* 2007 Oct 8; [Epub ahead of print].
30. Willens HJ, Byers P, Chirinos JA et al. Effects of weight loss after bariatric surgery on epicardial fat measured using echocardiography. *Am J Cardiol.* 2007;99:1242-1245.
31. Zancanaro C, Poltronieri R, Sbarbati A et al. Adipocyte morphology during hormone-induced lipid deposition and mobilization. An ultrastructural investigation in the perfused cardiac fat. *Cell Biol Int.* 1995;19:1001-1009.
32. Chaowalit N, Somers VK, Pellikka PA et al. Subepicardial adipose tissue and the presence and severity of coronary artery disease. *Atherosclerosis.* 2006;186:354-359.
33. Scher AM. Absence of atherosclerosis in human intramyocardial coronary arteries: a neglected phenomenon. *Atherosclerosis.* 2000;149:1-3.
34. Ishikawa Y, Ishii T, Asuwa N et al. Absence of atherosclerosis evolution in the coronary arterial segment covered by myocardial tissue in cholesterol-fed rabbits. *Virchows Arch.* 1997;430:163-171.
35. Ishii T, Asuwa N, Masuda S et al. The effects of a myocardial bridge on coronary atherosclerosis and ischaemia. *J Pathol.* 1998;185:4-9.
36. Chandalia M, Garg A, Vuitch F et al. Postmortem findings in congenital generalized lipodystrophy. *J Clin Endocrinol Metab.* 1995;80:3077-3081.
37. Bot I, de Jager SC, Zernecke A et al. Perivascular mast cells promote atherogenesis and induce plaque destabilization in apolipoprotein E-deficient mice. *Circulation.* 2007;115:2516-2525.

Summary

Cardiovascular disease continues to impose a heavy burden on the Western world because of its associated morbidity and mortality. There are several risk factors (e.g. smoking, hypertension, dyslipidemia, hyperglycemia, obesity) for the development of atherosclerotic vascular diseases. Among these, abdominal obesity and its associated insulin resistance are of great importance. Obesity-induced insulin resistance is a major driver behind the clustering of vascular risk factors, often referred to as metabolic syndrome. Patients with evident vascular disease or high insulin resistance (e.g. patients with metabolic syndrome or type 2 diabetes) are particularly prone to develop (new) vascular diseases. In an insulin resistant state, intra-abdominal adipose tissue secretes large quantities of inflammatory cytokines into the systemic circulation thereby accelerating atherosclerosis. Recently, there is growing evidence that perivascular fat, the adipose tissue directly surrounding the arteries, may affect atherogenesis from 'outside to inside' by the local release of a large number of pro-inflammatory factors that potentially impair vascular function and stimulate local inflammation.
The work presented in this thesis focused on the relationship between presence of insulin resistance and advanced vascular damage in patients with manifest atherosclerotic vascular disease, and on the occurrence of (new) vascular events in insulin resistant patients with and without evident vascular disease **(chapters 2, 3 and 4)**. Furthermore, the relationship between adipose tissue (abdominal fat and fat surrounding coronary arteries) and atherosclerotic vascular damage was examined **(chapters 5, 6 and 7)**.

It has been shown that in apparently healthy adults the prevalence of metabolic syndrome is 20-25%. Metabolic syndrome is expected to become more prevalent due to the increasing prevalence of obesity. It is generally recognized that patients with metabolic syndrome have a two- to threefold higher risk of developing atherosclerotic vascular disease. In **chapter 2** we demonstrated that metabolic syndrome, defined using the Adult Treatment Panel III criteria, was highly prevalent (46%) in patients with already clinical manifestations of arterial disease, especially in patients with peripheral arterial disease (58%). Overall, women had a higher prevalence compared to men (56% vs. 43%). Screening for metabolic syndrome may direct secondary preventive measures, aiming at improvement of insulin resistance, in order to prevent new vascular events in already high-risk patients. Subsequently, other studies have established that patients with arterial disease and metabolic syndrome are at even higher cardiovascular risk than those with arterial disease but without metabolic syndrome.

Although both insulin resistance and metabolic syndrome are involved in the development of atherosclerotic vascular disease, there is uncertainty regarding the independent role of insulin resistance. In this field, studies are rather limited in patients with arterial disease. In **chapter 3** we investigated whether insulin resistance *per se* (derived by homeostasis model assessment) was associated with advanced vascular damage, measured by carotid intima-media thickness (CIMT) and albuminuria, in patients with manifest arterial disease without known diabetes. Furthermore, we determined to what extent this relationship can be explained by the individual components constituting metabolic syndrome. Adjusted for age and gender, an elevated level of insulin resistance was associated with an increased prevalence of

albuminuria (quartile 4 versus quartile 1, odds ratio (OR) 2.05; 95% CI 1.32-3.20). This association was only partly mediated by components of metabolic syndrome (quartile 4 versus quartile 1, OR 1.61; 95% CI 0.91-2.85). High insulin resistance was not associated with an increased CIMT. This suggests that when patients without known diabetes already suffer from arterial disease, an elevated level of insulin resistance alone does not further enhance atherosclerosis in the arterial wall, while insulin resistance itself has an adverse effect on vascular function, as reflected by albuminuria. Thus, insulin resistance may play a role in different stages of the atherosclerotic process.

Patients with both type 2 diabetes and evident coronary heart disease have an excess risk of atherosclerotic vascular disease. In **chapter 4** the relationship of both other sites of vascular disease (cerebrovascular disease, peripheral arterial disease) as well as the extent of clinically evident vascular disease with the occurrence of new cardiovascular events was investigated in patients with type 2 diabetes. Extent of clinically evident vascular disease was expressed as the number of different sites clinically affected by vascular disease. Furthermore, we assessed the additional influence of the burden of atherosclerotic disease (measured by CIMT and albuminuria) on this relationship. Diabetic patients with cerebrovascular disease, coronary heart disease or peripheral arterial disease had a similar three- to fourfold higher risk of new vascular events, compared with diabetic patients without vascular disease. Diabetic patients with two or more clinical manifestations of atherosclerosis at different vascular sites had an even higher risk of new vascular events (adjusted hazard ratio 6.6; 95% CI 3.0-14.3). Additional adjustment for atherosclerotic burden did not influence the results. Our results suggest that counting the number of sites of evident vascular disease, irrespective of the type of vascular disease, may help to simply identify those patients with diabetes type 2 particularly at very high risk for new macrovascular complications from a generally high-risk diabetic population.

In case of obesity, an imbalanced production of pro- and anti-inflammatory cytokines by abdominal fat not only induces insulin resistance and consequently metabolic syndrome, but also leads directly to the development of endothelial dysfunction and progression of atherosclerosis. Inflammatory processes appear to be involved in the degeneration of the aortic wall. Therefore, intra-abdominal fat accumulation and its associated metabolic abnormalities may contribute to the process of aortic dilatation. In **chapter 5** the relationship between intra-abdominal fat accumulation, as well as metabolic syndrome, and infrarenal aortic diameter was assessed in patients with manifest arterial disease. Infrarenal aortic diameter (mm) was larger in patients with more intra-abdominal fat (derived by ultrasonography (quartile 4 vs. quartile 1, regression coefficient beta (β) 1.38; 95% CI 0.76-2.01); measured by waist circumference (quartile 4 vs. quartile 1, β 1.56; 95% CI 0.93-2.19)) compared to those with less intra-abdominal fat after adjustment for confounders. Patients with metabolic syndrome had slightly larger infrarenal aortic diameters (adjusted β 0.70; 95% CI 0.27-1.13) compared to those without metabolic syndrome. Thus, intra-abdominal fat may play a role in the development of larger aortic diameters.

Perivascular fat directly surrounding coronary arteries might aggravate coronary atherosclerosis due to its proximity to coronary arteries and potential for local secretion of adipocytokines. For investigating the relationship between fat surrounding coronary arteries and coronary artery disease, it is important to reliably quantify this adipose tissue. In **chapter 6**, we compared the reproducibility of various measurements (thickness, area, volume) for quantifying epicardial adipose tissue (EAT) and peri-coronary fat using 60 cardiac CT scans of patients undergoing coronary angiography (CAG). Furthermore, the relationship of the quantity of EAT and peri-coronary fat with obesity and metabolic syndrome was determined in these patients. Volumetric quantifications of EAT using cardiac CT were highly reproducible (coefficient of variations (CVs) 3%-5%) compared to thickness and area measurements of EAT and peri-coronary fat (CVs 11%-23%). EAT and peri-coronary fat accumulations were both associated with obesity and presence of metabolic syndrome. The results suggest that fat surrounding the coronary arteries may be involved in pathophysiological processes leading to the development of coronary atherosclerosis seen in obesity and metabolic syndrome.

In **chapter 7**, the relationship between the amount of EAT and peri-coronary fat and the severity of coronary atherosclerosis (number of stenotic (≥50%) coronary vessels) or extent of coronary artery calcification (CAC) was investigated in patients undergoing CAG. Furthermore, we examined whether this relationship was modified by total body weight by stratifying patients for BMI (median BMI 27 kg/m^2). EAT volume and peri-coronary fat thickness were not associated with the severity of coronary atherosclerosis and extent of CAC in the whole study population. Nonetheless, in patients with a BMI <27 kg/m^2, those with multi-vessel disease had higher EAT volume (99.7 vs. 66.5 cm^3) and peri-coronary fat thickness (9.8 vs. 8.4 mm) than those without coronary artery disease. Also, patients with severe or extensive CAC had higher EAT volume (108.0 vs. 68.7 cm^3) and peri-coronary fat thickness (10.0 vs. 8.2 mm) compared to those with minimal or absent CAC. In patients with a BMI ≥27 kg/m^2, these relationships were not present. This study suggests that fat surrounding coronary arteries may be involved in the process of coronary atherosclerosis, although this is different for patients with low and high BMI.

Lastly, in the general discussion in **chapter 8** the topic 'Cardiovascular risk: insulin resistance or glucose intolerance?' was discussed and integrated with our own study findings. Also, the topic 'Perivascular fat: potential role in vascular disease or innocent bystander?' was discussed. The main conclusions of this thesis were also presented.

Nederlandse samenvatting

Dankwoord

Curriculum Vitae

Appendix

Hart- en vaatziekte is de belangrijkste oorzaak van ziekte en sterfte in de Westerse wereld en is meestal het gevolg van slagaderverkalking (atherosclerose). Perifeer vaatlijden, cerebraal vaatlijden, coronair vaatlijden, en verwijding van de buikaorta zijn voorbeelden van klinische uitingen van atherosclerose. Bekende risicofactoren voor het ontwikkelen van atherosclerotisch vaatlijden zijn roken, hypertensie (verhoogde bloeddruk), dyslipidemie (verstoring in de samenstelling van vet- en cholesterolwaarden), hyperglycemie (verhoogde bloedsuiker), en overgewicht. Overgewicht, met name teveel buikvet, is een belangrijke factor voor het ontstaan van insulineresistentie. De term insulineresistentie betekent dat weefsels in het lichaam minder gevoelig zijn voor insuline. Insuline is een hormoon dat een grote rol speelt bij de regulatie van het glucosegehalte in het bloed en bij de vet- en eiwitstofwisseling. Insulineresistentie is een belangrijke factor voor het ontstaan van (clustering van) risicofactoren zoals hyperglycemie, hypertensie, verhoogde triglyceridenconcentratie (vetten), en verlaagde HDL-cholesterolconcentratie (goed cholesterol). De clustering van risicofactoren, die samenhangt met insulineresistentie en overgewicht, wordt vaak metabool syndroom genoemd. Patiënten met al een klinische uiting van atherosclerose of verhoogde insulineresistentie (patiënten met metabool syndroom of type 2 diabetes) hebben een verhoogd risico op (nieuwe) uitingen van vaatziekten.
Wanneer er sprake is van insulineresistentie, is vetweefsel (met name buikvet) in staat om grote hoeveelheden ontstekingsfactoren (adipocytokines) uit te scheiden in de systemische circulatie, deze kunnen atherosclerose verergeren. Er is steeds meer bewijs dat vetweefsel rondom de arteriën (perivasculair vetweefsel) het proces van atherosclerose van 'buiten naar binnen' toe kan beïnvloeden. Perivasculair vetweefsel produceert ontstekingsfactoren op lokaal niveau, die mogelijk de functie van de vaatwand kunnen beïnvloeden en lokale ontsteking kunnen versterken.
In dit proefschrift hebben wij gekeken naar de relatie tussen de aanwezigheid van insulineresistentie en de ernst van vaatschade bij patiënten met klinisch atherosclerotisch vaatlijden, en naar het optreden van (nieuwe) vaataandoeningen bij insulineresistente patiënten met en zonder klinisch evident vaatlijden **(hoofdstukken 2, 3 en 4)**. In de laatste drie hoofdstukken wordt de relatie tussen vetweefsel (buikvet en vetweefsel rondom coronairarteriën) en vaatschade behandeld **(hoofdstukken 5, 6 en 7)**.

In een ogenschijnlijk gezonde populatie is het metabool syndroom in 20-25% van de mensen aanwezig. Naar verwachting zal het aantal mensen met metabool syndroom toenemen door een toename van het aantal mensen met overgewicht / obesitas. Patiënten met metabool syndroom hebben een 2 tot 3 keer verhoogde kans op het ontwikkelen van vaatziekten. In **hoofdstuk 2** tonen wij aan dat het metabool syndroom veel voorkomt (46%) bij patiënten met klinisch vaatlijden, vooral bij patiënten met perifeer vaatlijden (58%). Het metabool syndroom was vaker aanwezig bij vrouwen (56%) dan bij mannen (43%). Screenen op metabool syndroom bij hoog risicopatiënten zou richting kunnen geven aan secundaire preventieve maatregelen met als doel de onderliggende insulineresistentie te verminderen en daardoor nieuwe uitingen van vaatziekten te kunnen voorkomen. Andere studies hebben inmiddels aangetoond dat patiënten met arterieel vaatlijden en tevens metabool syndroom een hoger cardiovasculair risico hebben dan patiënten met arterieel vaatlijden zonder metabool syndroom.

Hoewel insulineresistentie en metabool syndroom beiden een rol spelen bij de ontwikkeling van atherosclerotisch vaatlijden, is er onduidelijkheid over de onafhankelijke rol van insulineresistentie. Tot nu toe zijn er weinig studies gedaan bij patiënten met arterieel vaatlijden naar de relatie tussen insulineresistentie en vaatschade. In **hoofdstuk 3** onderzochten wij of insulineresistentie (gemeten met de HOMA-methode) geassocieerd was met meer vaatschade bij patiënten met klinisch vaatlijden zonder bekende diabetes. De ernst van vaatschade werd vastgesteld door het meten van de dikte van de vaatwand en de eiwituitscheiding door de nieren (albuminurie). Bepaald werd in welke mate deze relatie verklaard werd door de individuele componenten die deel uitmaken van het metabool syndroom. Uit deze studie bleek dat een verhoogde insulineresistentie geassocieerd was met een verhoogde aanwezigheid van albuminurie. Patiënten met meer insulineresistentie hadden nog steeds een hoger risico op de aanwezigheid van albuminurie na het corrigeren voor de componenten van het metabool syndroom. Een verhoogde insulineresistentie was niet geassocieerd met een toename van de dikte van de vaatwand. Dit suggereert dat bij patiënten met klinisch arterieel vaatlijden zonder bekende diabetes insulineresistentie zelf de structurele vaatwandschade niet verder kan verergeren, terwijl insulineresistentie wel een nadelige invloed heeft op de functie van de vaatwand (blijkend uit de mate van albuminurie). Dit zou kunnen betekenen dat insulineresistentie een rol speelt in verschillende stadia van het atherosclerotische proces.

Patiënten met type 2 diabetes en tevens coronair vaatlijden hebben een zeer hoog risico op het ontwikkelen van nieuwe vaataandoeningen. In **hoofdstuk 4** bekeken we de relatie tussen andere locaties van klinisch vaatlijden (cerebraal vaatlijden, perifeer vaatlijden) als ook de uitgebreidheid van klinisch vaatlijden en het risico op het ontwikkelen van nieuwe vaataandoeningen bij patiënten met type 2 diabetes. Ook bepaalden we de invloed van de ernst van vaatschade (gemeten met de dikte van de vaatwand en albuminurie) op deze relatie. Diabetespatiënten met cerebraal vaatlijden, coronair vaatlijden of perifeer vaatlijden hadden een overeenkomstig 3 tot 4 maal hoger risico op het ontwikkelen van een nieuwe uiting van vaatziekte in vergelijking met diabetespatiënten zonder evident vaatlijden. Patiënten met type 2 diabetes en twee of meer klinische uitingen van atherosclerose op verschillende locaties in het vaatbed hadden zelfs een 6 maal hoger risico op nieuwe vaataandoeningen. De ernst van vaatschade had geen invloed op de relatie tussen klinisch vaatlijden en nieuwe vaataandoeningen. Dus, het tellen van het aantal plekken van klinisch evident vaatlijden, ongeacht het type vaatziekte, zou binnen de hoog risicopopulatie van patiënten met type 2 diabetes, die patiënten kunnen identificeren die met name een zeer hoog risico hebben op het ontwikkelen van nieuwe vaataandoeningen.

In geval van overgewicht / obesitas, kan een veranderde productie van pro- en anti-ontstekingsfactoren (ten gevolge van niet goed functionerende vetcellen van met name buikvet) niet alleen insulineresistentie veroorzaken met als gevolg metabool syndroom, maar ook direct bijdragen aan de ontwikkeling van endotheelschade en verergering van atherosclerose. Ontstekingsfactoren blijken een rol te spelen bij het degeneratieve proces in de wand van de buikaorta. Teveel buikvet en de daaraan

gerelateerde aanwezigheid van metabole risicofactoren zouden kunnen bijdragen aan de verwijding van de wand van de buikaorta. **Hoofdstuk 5** behandelt de relatie tussen de hoeveelheid buikvet, als mede de aanwezigheid van het metabool syndroom, en de diameter van de (infrarenale) buikaorta bij patiënten met klinisch vaatlijden. Uit dit onderzoek bleek dat na het corrigeren voor 'confounders' de diameter van de buikaorta groter was bij patiënten met meer buikvet. Ook hadden patiënten met metabool syndroom grotere diameters van de buikaorta dan patiënten zonder metabool syndroom. De resultaten suggereren dat teveel buikvet een rol zou kunnen spelen bij het ontwikkelen van grotere diameters van de buikaorta.

Perivasculair vetweefsel direct rondom de coronairarteriën zou kunnen bijdragen aan de verergering van coronaire atherosclerose mede door de locatie naast de coronairarteriën en de mogelijkheid om ontstekingsfactoren (adipocytokines) te produceren. Voor het onderzoeken van de relatie tussen vetweefsel rondom de coronairarteriën en coronair vaatlijden is het belangrijk om dit vetweefsel betrouwbaar te kunnen kwantificeren. In **hoofdstuk 6** vergeleken wij de reproduceerbaarheid van verschillende CT-metingen (dikte, oppervlakte, volume) van vetweefsel rondom de coronairarteriën. Hiervoor hebben wij gebruik gemaakt van 60 CT-scans van het hart van patiënten die een coronair angiogram hebben ondergaan. Daarnaast werd de relatie tussen de hoeveelheid vetweefsel rondom de coronairarteriën (epicardiaal en pericoronair vet) en obesitas of metabool syndroom onderzocht bij deze patiënten. Volume metingen van epicardiaal vet waren goed reproduceerbaar (variatie-coëfficiënt 3%-5%) in vergelijking met simpelere meetmethoden zoals dikte en oppervlakte metingen van epicardiaal en pericoronair vet (variatie-coëfficiënt 11%-23%). Een grotere hoeveelheid epicardiaal en pericoronair vetweefsel was geassocieerd met obesitas en de aanwezigheid van het metabool syndroom. Dit suggereert dat vet direct rondom de coronairarteriën een rol kan spelen bij pathofysiologische processen die leiden tot de ontwikkeling van coronaire atherosclerose bij patiënten met obesitas en metabool syndroom.

In **Hoofdstuk 7** werd de relatie tussen de hoeveelheid epicardiaal en pericoronair vetweefsel en de ernst van coronaire atherosclerose (het aantal coronairarteriën met een vernauwing van de diameter van ≥50%) als mede de uitgebreidheid van calcificatie van de coronairarteriën onderzocht bij patiënten die een coronair angiogram hebben ondergaan. Daarnaast bekeken wij of deze relatie gemodificeerd werd door het totale lichaamsgewicht, dit door patiënten te stratificeren naar body mass index (mediaan BMI 27 kg/m^2). Epicardiaal vetvolume en pericoronaire vetdikte waren niet gerelateerd aan de ernst van coronaire atherosclerose en uitgebreidheid van coronaire calcificatie in de totale onderzoekspopulatie. Echter, bij de groep van patiënten met een BMI <27 kg/m^2, hadden patiënten met meer-vatslijden een groter epicardiaal vetvolume (99.7 versus 66.5 cm^3) en een grotere pericoronaire vetdikte (9.8 versus 8.4 mm) dan patiënten zonder coronair vaatlijden. Ook patiënten met een ernstige mate van coronaire calcificatie hadden een groter epicardiaal vetvolume (108.0 versus 68.7 cm^3) en een grotere pericoronaire vetdikte (10.0 versus 8.2 mm) dan patiënten met minimale of geen coronaire calcificatie. Bij patiënten met een BMI ≥27 kg/m^2 waren deze relaties niet aanwezig. Dit suggereert dat vet direct rondom de coronairarteriën een rol zou kunnen spelen bij het proces van coronaire atherosclerose, maar dat dit verschillend is voor patiënten met een lage of een hoge BMI.

Ten slotte wordt in de algemene discussie in **hoofdstuk 8** het onderwerp 'Cardiovasculair risico: insulineresistentie of glucose-intolerantie?' besproken and geïntegreerd met bevindingen van studies in dit proefschrift. Ook wordt het onderwerp 'Perivasculair vetweefsel: mogelijke rol in vaatziekte of onschuldige factor?' bediscussieerd. Daarnaast worden de belangrijkste conclusies van dit proefschrift genoemd.

Conclusies van dit proefschrift:

- Het metabool syndroom komt veel voor bij patiënten met klinisch arterieel vaatlijden (46%).

- Bij patiënten met klinisch vaatlijden zonder bekende diabetes, is een verhoogde insulineresistentie geassocieerd met een verhoogde prevalentie van albuminurie; dit wordt slechts gedeeltelijk verklaard door componenten van het metabool syndroom. Een verhoogde insulineresistentie is niet geassocieerd met een toename van de dikte van de vaatwand bij patiënten met klinisch vaatlijden.

- Patiënten met type 2 diabetes en coronair vaatlijden, cerebraal vaatlijden of perifeer vaatlijden hebben een sterk verhoogd en vergelijkbaar risico op een nieuwe uiting van vaatziekte, vergeleken met diabetespatiënten zonder evident vaatlijden.

- Patiënten met type 2 diabetes en een groter aantal klinisch uitingen van atherosclerose op verschillende locaties in het vaatbed hebben een nog hoger risico op nieuwe vaataandoeningen; dit wordt niet beïnvloed door de ernst van vaatschade (vaatwanddikte en albuminurie).

- De hoeveelheid buikvet en de aanwezigheid van het metabool syndroom zijn geassocieerd met een grotere diameter van de buikaorta bij patiënten met klinisch vaatlijden.

- Volume metingen van epicardiaal vetweefsel met CT zijn goed reproduceerbaar in vergelijking met simpelere meettechnieken zoals epicardiale en pericoronaire vetdikte en -oppervlakte.

- De hoeveelheid vetweefsel rondom de coronairarteriën (epicardiaal en pericoronair vet) is geassocieerd met obesitas en metabool syndroom bij patiënten die een coronair angiogram ondergaan.

- Een grotere hoeveelheid epicardiaal en peri-coronair vet gaat gepaard met ernstigere coronaire atherosclerose en uitgebreidere calcificatie van de coronairarteriën bij patiënten met een BMI <27 kg/m^2 die een coronair angiogram ondergaan.

Ook voor mij was het promotieonderzoek een wervelende tijd! Een tijd waarin je een doel probeert te bereiken, waarbij je de mensen om je heen hard nodig hebt. Dit om de uiteindelijke eindstreep te bereiken, maar ook om je promotietijd tot een leuke en onvergetelijke tijd te maken! Graag wil ik enkele mensen persoonlijk bedanken.

Prof. dr. Y. van der Graaf en **Dr. F.L.J. Visseren**. Als student ben ik begonnen met het doen van onderzoek en ben ik met jullie in contact gekomen. Jullie hebben mij toen enthousiast gemaakt voor de wetenschap! Dank voor het vertrouwen om de samenwerking voort te zetten. Mede dankzij jullie steun, begeleiding en 'teamwork' ligt er nu dit proefschrift!

Beste **Yolanda**, jij maakte van de besprekingen altijd meer dan een werkoverleg! Altijd werd er tijd gemaakt om andere zinnige zaken te bespreken, zoals bijvoorbeeld het wel en wee van de guppies. Ik heb erg veel van je geleerd en heb bewondering voor de manier waarop jij zaken oplost! Dank voor je vertrouwen en ook voor de complimentjes als ik het nodig had.

Beste **Frank**, ik weet nog goed dat jij de tijd hebt genomen om met mij te praten over mijn keuzes rondom promotie en vervolgopleiding. Ik heb jouw open visie en positiviteit toen erg gewaardeerd. Met diezelfde positiviteit en gedrevenheid heb jij mij de afgelopen jaren begeleid, ik heb erg veel van je geleerd. Bedankt!

De beoordelingscommissie bestaande uit Prof. dr. P.A.F.M. Doevendans, Prof. dr. D.E. Grobbee, Prof. dr. W.P.Th.M. Mali, Prof. dr. F.L. Moll van het UMCU en Prof. dr. J.B.L. Hoekstra van het AMC dank ik voor de bereidheid om zitting te nemen in de commissie.

Mijn collega-promovendi en kamergenoten bij de vasculaire geneeskunde.
Lieve **Gideon**, we zijn ongeveer rond dezelfde tijd gestart en eindigen nu ongeveer rond dezelfde tijd. Dat is toch wel bijzonder. Soms een beetje 'hyper', altijd behulpzaam en bovenal heel veel humor! Ik kan jouw wat cynische humor altijd erg waarderen, het heeft mijn promotietijd in ieder geval gezelliger gemaakt!
Lieve **Annemarie**, bedankt voor alle gezelligheid, kit kat chunkies, Helga-humor en starbuckies. Leuk dat je nu nog vaak in mijn inbox verschijnt. Ik blijf zeker langs komen voor een koffietje. Ga er voor de komende tijd!

Alle andere collega`s van de vasculaire geneeskunde / vasculair onderzoek. Jobien, Joris, Annemarie, Francine, Wilko, Annemieke, Corina, Inge, Sophie, Berna, Jesper, Jacub, Beate en Anneloes. Leuk om met jullie tussen de bedrijven door te lunchen, te kletsen of te stoeien met fax-apparaten. Jobien, bedankt voor je hulp bij al mijn vragen. Beate, de borrelavonden waren altijd gezellig met jou! Annemarie, met z`n vieren op een slaapkamer wanneer maak je dat nog mee?

Natuurlijk niet te vergeten mijn collega SMART-dokters, Auke, Beate, Anneloes, Arnoud, Audrey en Sefanja! Nadine en Fleur, bedankt dat jullie al mijn vragen hebben willen beantwoorden! Alle andere (ex)collega`s van het SMART team, Loes, Lies, Hetty, Ursula,

Vera, Anneke, Anja, Cindy, Kim, Desiree, Michael, Rutger, Harry en Sabita; leuk om met jullie samengewerkt te hebben en bedankt voor alles. Beste Ale, bedankt ook voor de goede samenwerking!

Cootje, bedankt voor al het regelwerk rondom mijn promotie!

Tijdens mijn promotietijd heb ik ook een aantal maanden bij de radiologie rondgelopen om het vetweefsel rondom het hart te meten. Het was leuk om weer eens rond te kijken in een heel andere 'keuken'! Prof. Prokop, Judith, Annelies, Anne en Sander, dankjulliewel voor het meedenken over de methoden. Anne, je hebt mij enorm geholpen met de vetmetingen, en dat 'monniken-werk' een stuk leuker gemaakt! Matthijs, bedankt voor je snelle reacties. Mirela, zonder jouw expertise had ik nooit alle kalkmetingen kunnen afmaken. Lisa en Audrey, gezellig dat jullie er waren als ik aan het meten was.

Roy, dank voor het verzorgen van de lay-out en de goede samenwerking!

En dan nog iedereen die ik hier toch onbedoeld vergeten ben, hartelijk dank. Graag wil ik ook degenen bedanken die mij hebben geholpen bij het lezen van de hoofdstukken.

Velen wil ik bedanken naast het promoveren.

Lieve Hanke, Marjolein, Marijke, Janna en Marlijn. Bedankt voor al de ruimte die jullie me hebben gegeven en voor jullie geduld. Ik ben blij met onze bijzondere vriendschap die al zo lang voorduurt. Dank voor jullie steun. Hanke, dankjewel voor je luisterend oor, ik zal je zeker gaan missen als je in Sint Maarten zit, maar ik kom je zeker opzoeken. Marjolein, ik ben trots op wat je allemaal hebt bereikt en overwonnen, dank voor alles. Janna, met jou aan de telefoon hangen is altijd een goede en gezellige afleiding! Marijke, ik weet zeker dat je een geweldige huisarts wordt en bovenal een fantastische moeder! Marlijn, dank ook voor je interesse en steun; geniet van je huis!

Lieve Inge, Jorinde, Kirsten, Charlotte, Lonneke J, Selma, Hanne, Lineke, Nanon, Lonneke v. D, Denise, en Angelique. Wat hebben we toch een leuke club zo met z`n dertienen. Istanboel was super, om niet meer te vergeten. Zal de film net zo hilarisch worden als die van Portugal?

Behalve deze personen, wil ik ook mijn andere vrienden en intimi bedanken!

Lieve **Inge** en **Lisa**, fijn dat jullie bij de verdediging naast mij willen staan!
Inge, leuk dat je zo enthousiast was toen ik je vroeg om mijn paranimf te zijn, terwijl je nog niet wist wat het allemaal ging inhouden. Ik waardeer altijd erg je luisterend oor en je humor als ik het even nodig heb.
Lisa, ik heb je leren kennen bij de epidemiologie opleiding en ben heel blij dat jij mij bij de verdediging wil bijstaan. Het was altijd leuk om na het harde werken een drankje te doen en tussendoor een koffie te drinken om elkaar even op te peppen!

Jan, Annelies, Marit en Ilse: ik heb jullie interesse en betrokkenheid altijd erg gewaardeerd, en vind het altijd gezellig om bij jullie te zijn!

Papa, Mama, Joanne, Janko, Oma, Gonny en Ronald, dank voor al jullie liefde en betrokkenheid. Met z`n allen in Griekenland dat is een herinnering die mij zeker bij zal blijven! Ik denk ik er met plezier aan terug.

Lieve **papa en mama**, jullie hebben mij altijd onvoorwaardelijk gesteund in alles wat ik doe, en een oppepper gegeven als ik het nodig had. Jullie staan al mijn hele leven voor me klaar, waar ik erg dankbaar voor ben. Ik ben er zeker van overtuigd dat ik zonder jullie vertrouwen dit nooit had bereikt en nooit zover was gekomen.

Lieve **oma**, ik waardeer het erg dat jij altijd op de hoogte bent waar ik mee bezig ben. Een lievere oma kan iemand zich niet wensen!

Lieve **Janko**, ik heb bewondering voor de 'relaxte' manier waarop jij je werk met onderzoek combineert. Bedankt voor je interesse en je betrokkenheid. Ook jij bent de onderzoekswereld ingedoken, waarschijnlijk sta jij over een paar jaar ook op deze plaats!

Lieve **Joanne**, je bent een lieve zus, dank voor je steun. Ik heb veel respect voor de manier waarop jij met alles omgaat en ik waardeer je vrolijkheid en humor altijd erg. Leuk dat ik nu bij jou in de stad woon!

Frans, **lieve lieve Frans**, door jou was mijn promotietijd ook nog een beetje internationaal! Het was de afgelopen jaren niet altijd makkelijk om in andere landen te wonen, maar misschien hebben we het daarom wel extra leuk nu. Ik prijs me elke dag weer gelukkig met jou, jij bent de aller-allerliefste! Jij maakt mij vrolijk en hebt mij leren relativeren, bedankt dat je er altijd voor me bent.
Ondanks dat je zelf ook een boek aan het afronden was in de late uurtjes, heb jij mij enorm gesteund tijdens het afronden van mijn promotie. Hiervoor ben ik je ontzettend dankbaar!

Petra

Januari 2008

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Appendix

Petra Monique Gorter was born May 10th, 1979, in `s Gravenhage, The Netherlands. The first four years of her life she lived in Nigeria and subsequently moved to Apeldoorn with her family and graduated from secondary school in 1997. She then started her medical training at the University Medical Centre in Utrecht. From 2001-2002 she served one year as a member of the board of the students` sorority Unitas S.R. in Utrecht. After this year, she started a research project in 2002 concerning the prevalence of metabolic syndrome in patients with manifest arterial disease under supervision of Prof. Dr. Y. van der Graaf (Clinical Epidemiology) and Dr. F.L.J. Visseren (Vascular Medicine) which is also presented in this thesis. During her medical training she went abroad for an internship of Gynecology and Obstetrics at the Regina General Hospital (Regina, Canada), and an internship of Ear, Nose and Throat at the St. Elizabeth Hospital (Willemstad, Curaçao). She graduated cum laude from medical school in February 2003, and she obtained her medical degree after finishing her internships in August 2005. In September 2005 she started the work described in this thesis at the department of Vascular Medicine, and the Julius Centre for Health Sciences and Primary Care, University Medical Centre Utrecht, again under supervision of Dr. F.L.J. Visseren and Prof. Dr. Y. van der Graaf. She obtained her Master of Science Degree in Clinical Epidemiology at the University of Utrecht in August 2007. As of January 2008 she is specialising in Dermatology at the Academical Medical Centre in Utrecht (supervised by Prof. Dr. C.A.F.M. Bruijnzeel-Koomen).

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Appendix

Members of the SMART Study Group are (alphabetically):

- A. Algra, MD, PhD, Julius Centre for Health Sciences and Primary Care and Rudolph Magnus Institute of Neuroscience, department of Neurology
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- B.C. Eikelboom, MD, PhD, department of Vascular Surgery
- Y. van der Graaf, MD, PhD, Julius Centre for Health Sciences and Primary Care
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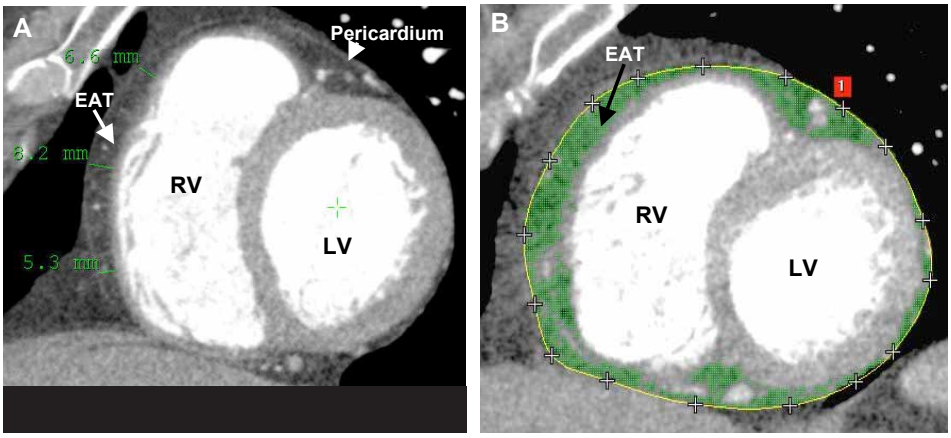
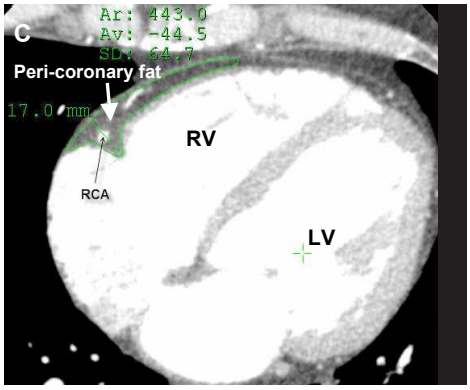
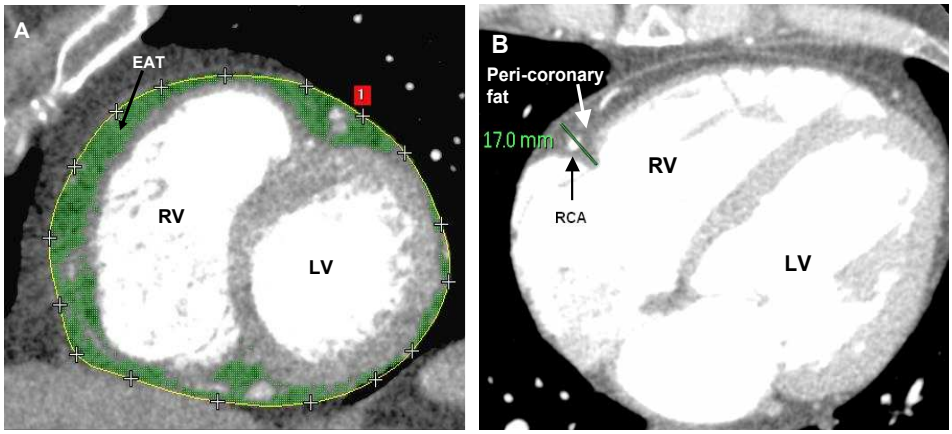
- L.J. Kappelle, MD, PhD, department of Neurology
- W.P.Th.M. Mali, MD, PhD, department of Radiology
- F.L. Moll, MD, PhD, department of Vascular Surgery
- G.E.H.M. Rutten, MD, PhD, Julius Centre for Health Sciences and Primary Care
- F.L.J. Visseren, MD, PhD, department of Internal Medicine, Vascular Medicine

Colour figures





Chapter 6, figure 2. Epicardial adipose tissue (EAT) and peri-coronary fat measurements. (A) EAT RV thickness (green) (inferior, centre, and superior) at basal level of the ventricles. (B) Region of interest (ROI) (yellow) on a cross-sectional image to determine the EAT area (green). EAT volume is the sum of the EAT areas on cross-sectional images. (C) Peri-coronary fat thickness and area (green).



Chapter 7, figure 1. Epicardial adipose tissue (EAT) and peri-coronary fat measurements. (A) Region of interest (ROI) (yellow) on a cross-sectional image to determine the EAT area (green). EAT volume is the sum of the EAT areas on cross-sectional images. (B) Peri-coronary fat thickness (green).