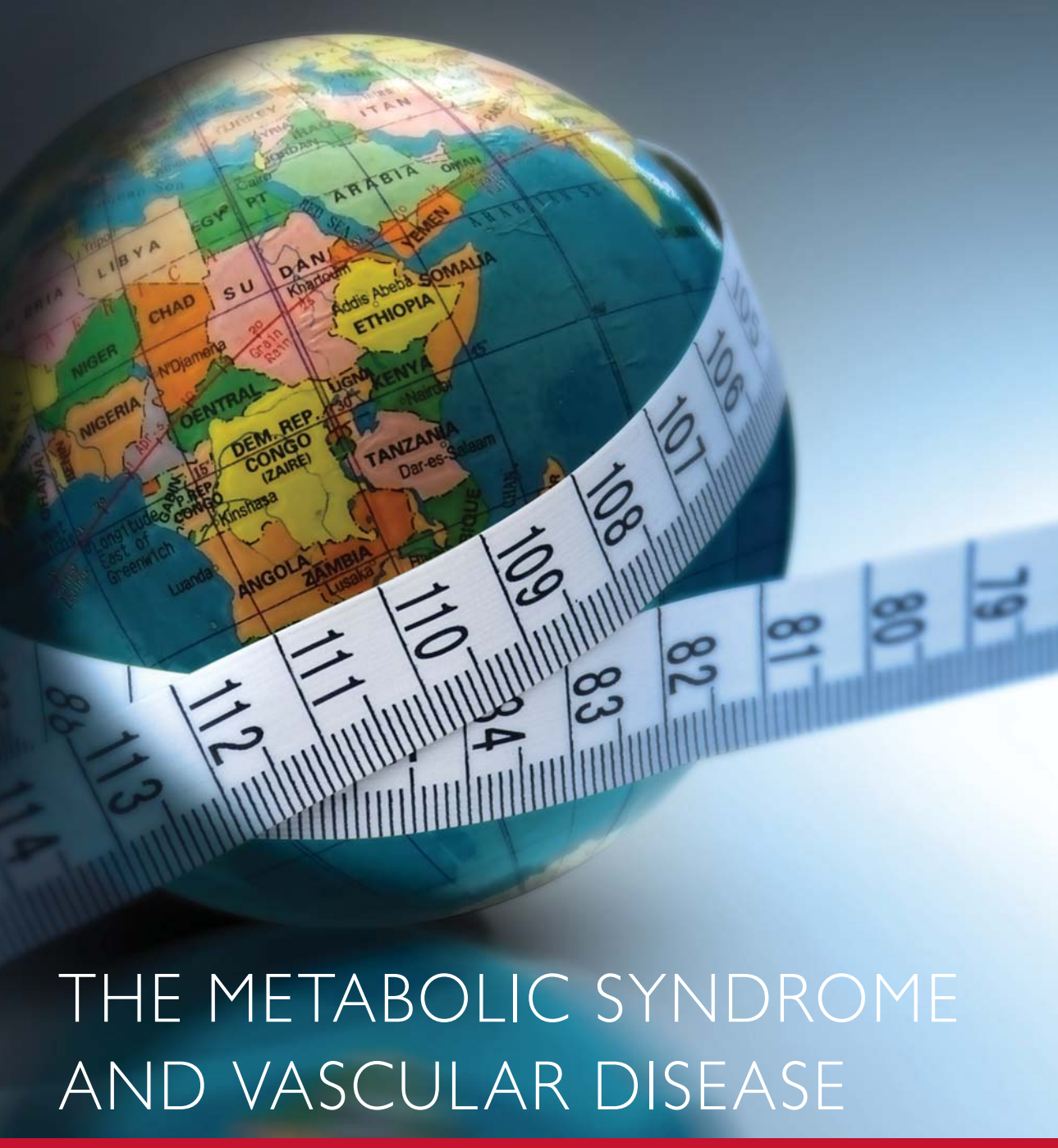

THE METABOLIC SYNDROME AND VASCULAR DISEASE

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The Metabolic Syndrome and Vascular Disease
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THE METABOLIC SYNDROME AND VASCULAR DISEASE

Het Metabool Syndroom en Vaatlijden

(met een samenvatting in het Nederlands)

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ATHEROSCLEROSIS

Presently, cardiovascular disease is the most frequent cause of mortality in the Western population. In the Netherlands, one out of three inhabitants will die due to a cardiovascular disease.[1] An important role in the pathophysiological mechanisms underlying cardiovascular disease is attributed to atherosclerosis, affecting the endothelial surface of the large and medium-size arteries throughout the human body. The process of atherosclerosis can be considered as a response to injury of the vessel wall in which a chronic proliferative inflammatory reaction is involved.[2,3] Injury of the vessel wall, caused by risk factors (as smoking, elevated LDL-cholesterol, elevated glucose levels) will induce the expression of several adhesion molecules and subsequent binding of monocytes to the endothelial surface. Monocytes, lymphocytes and lipoproteins will then migrate to the subendothelial space. By phagocytosis of oxidized lipoproteins, monocytes transform to foam cells and start to produce several (vaso)active substances as growth factors, cytokines and oxygen radicals, sustaining low grade chronic inflammation. In addition, lymphocytes, activated by the presence of local antigens will contribute to this inflammatory reaction. In order to repair, this process will be followed by the migration and proliferation of smooth muscle cells forming a fibrous cap over the lipid core. These atherosclerotic plaques can expand and remain asymptomatic for a very long time. However, when the lumen of the vessel wall is significantly narrowed or in case of thrombus formation on the plaque or even plaque rupture this will result in various clinical symptoms depending on the vessels involved.

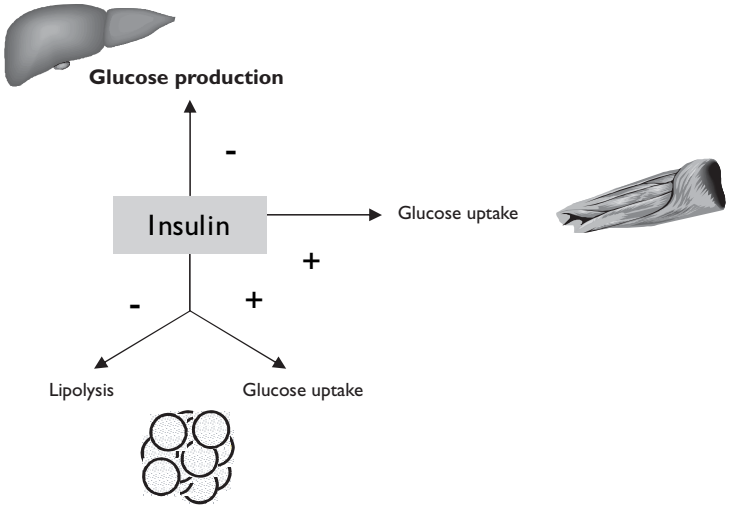
Various factors contribute to the process of atherosclerosis, among them elevated blood pressure, dyslipidemia, smoking, elevated blood glucose levels and obesity. The clustering of several of these risk factors is known as the metabolic syndrome.[4] Although the exact pathophysiological pathways of the clustering are not fully understood, insulin resistance and abdominal obesity are involved.

INSULIN

Physiological functions

Insulin is a hormone with important endocrinological functions. By stimulating the uptake of glucose in skeletal muscle cells and adipocytes and inhibiting the hepatic glucose production, insulin can be regarded as the most important regulator of the plasma glucose level. In addition, insulin stimulates the lipogenesis and glycogen- and protein synthesis in adipose tissue, liver and skeletal muscle cells and inhibits glycogenolysis, lipolysis and protein breakdown (figure 1). Furthermore, insulin is involved in the regulation of cell growth and differentiation.

Figure 1 Physiological functions of insulin



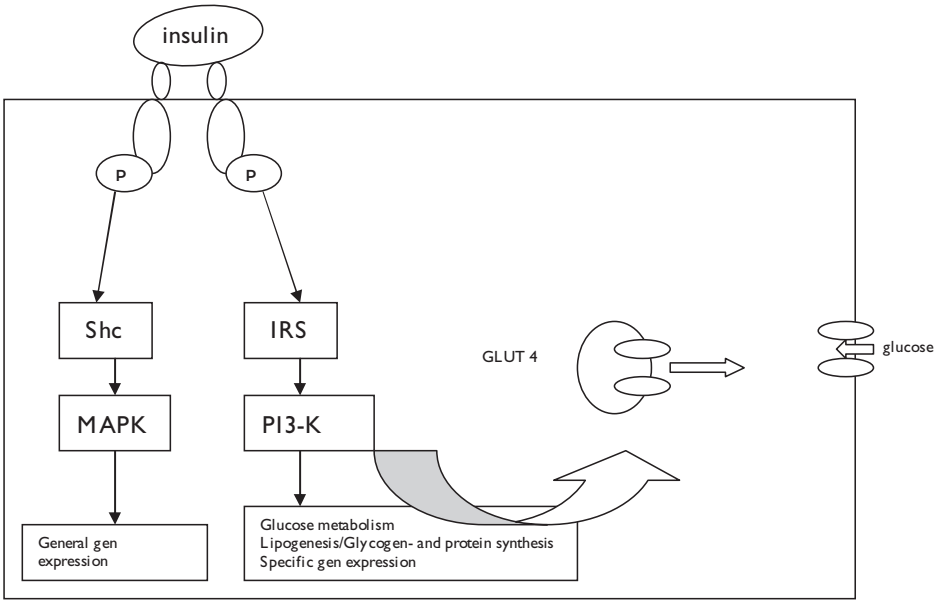
Insulin resistance and adiposity

Insulin resistance is characterized by an insufficient response of target organs on physiological insulin plasma concentrations. Although the last years the knowledge about the regulatory function of insulin, the binding of insulin to, and the function of, the insulin-receptor and the intracellular signalling cascade is definitely increased, the pathophysiological mechanism of insulin resistance on molecular level is still not fully understood.

After binding of insulin to the insulin-receptor, a complex signalling transduction pathway will be activated.[5,6] The receptor for insulin is embedded in the plasma membrane and is composed of two alpha subunits and two beta subunits linked by disulfide bonds. Binding of insulin to the alpha subunits induces the autophosphorylation of the beta subunits, thereby activating the catalytic activity of the receptor. This results in the phosphorylation of several intracellular proteins, among them Insulin Receptor Substrate (IRS) proteins. The signalling transduction takes place by these phosphorylated substrates, serving as docking stations for more distal functioning effectors, as mitogen-activated protein (MAP) kinase and phosphatidylinositol 3 kinase (PI3-K). The insulin-receptor signalling transduction system has two important pathways with each different effects. Activation

of the pathway in which MAP kinase is involved plays an important role in gene expression. However, the metabolic effects of insulin are mainly performed by the pathway in which PI3-K has a prominent role. In skeletal muscle cells and adipocytes, activation of the latter pathway induces the translocation of the insulin-sensitive glucose transporter (GLUT 4) to the cell surface providing the ability of glucose uptake. Insulin also stimulates glycogen and protein synthesis by activation of this pathway and has inhibitory effects on glycogenolysis and lipolysis (figure 2).

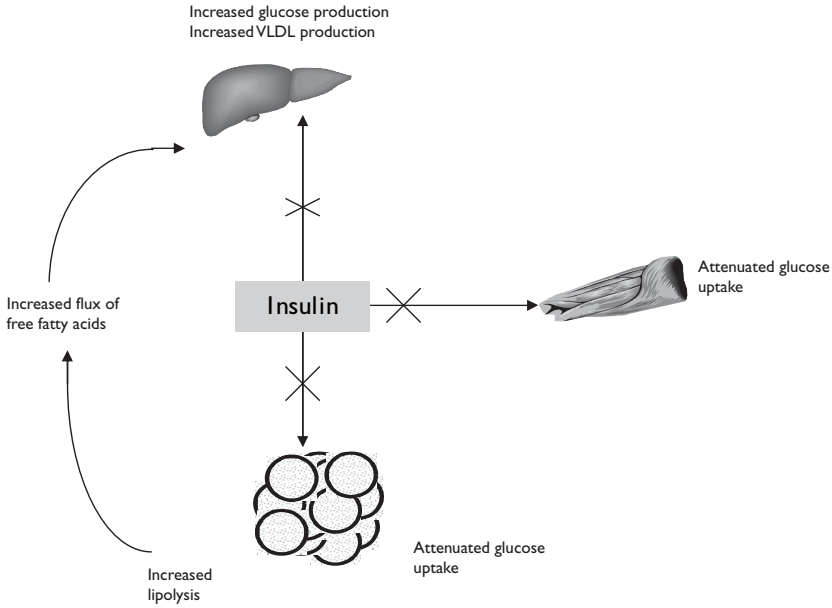
Figure 2 Simplified signalling transduction pathways of the insulin receptor



P	Phosphorylation
IRS	Insulin Receptor Substrate proteins
MAPK	mitogen-activated protein kinase
PI3-K	phosphatidylinositol 3 kinase
GLUT 4	insulin-sensitive glucose transporter
SHC	src homology 2/collagen homology containing protein

Theoretically, insulin resistance can be caused by a disturbed insulin-receptor binding, by receptor defects itself or by a disruption in the signalling transduction. The latter is now considered as the most important cause of insulin resistance. Impaired signalling transduction could be caused by the imbalanced production of adipokines.[7] Adipocyte tissue is not longer regarded as an inert storage depot, but as an organ with important endocrinological functions.[8] Adipocytes can produce substances, adipokines, involved in energy homeostasis. In case of adiposity the production of several inflammatory cytokines as TNF-α, leptin and interleukin-6 is increased, whereas the production of adiponectin is diminished. This can result in decreased insulin-sensitivity in **adipocytes**, resulting in a diminished uptake of glucose (figure 3).

Figure 3 Insulin resistance



The inhibition of insulin on lipolysis in adipocytes can also be suppressed leading to an increased efflux of free fatty acids. In **skeletal muscle cells** the uptake of glucose will decline because of disturbed signalling transduction after insulin-receptor binding. In the **liver**, the increased amount of free fatty acids suppresses the inhibitory function of insulin on gluconeogenesis, resulting in an increased hepatic glucose production.

Metabolic and hemodynamic consequences of insulin resistance

Insulin resistance leads to an increased production of insulin by the pancreatic ß-cells. If this compensatory mechanism fails, (postprandial) hyperglycaemia will result. The increased flux of free fatty acids to the liver induces an increased hepatic triglyceride production and VLDL-particles excretion.[9] In an insulin resistant state, there could be an attenuated activity of lipoprotein lipase, an enzyme involved in lipolysis of VLDL-particles, resulting in delayed degradation of VLDL-particles and thus contributing to hypertriglyceridemia. Reduced breakdown of VLDL particles leads to reduced availability of surface proteins and phospholipids involved in HDL-particle formation.

In addition, by the action of Cholesterol Ester Transfer Protein (CETP) an elevated plasma concentration of VLDL-particles results in an increased concentration of triglycerides in the HDL-particle. HDL-particles with a high concentration of triglycerides are faster cleared by the liver which results in a decreased HDL-cholesterol plasma concentration.[10] Insulin resistance can induce elevated blood pressure by several mechanisms, including activation of the sympathetic nerve system, increased renal sodium retention and endothelial dysfunction in resistant arteries.[11]

METABOLIC SYNDROME

Definitions

As outlined above, the clustering of several cardiovascular risk factors as hyperglycaemia, hypertension, dyslipidemia and abdominal obesity is known as the metabolic syndrome.[4] Insulin resistance and abdominal obesity play prominent roles in the pathophysiological pathway of the metabolic syndrome. However, inflammation, endothelial dysfunction and an imbalanced autonomic nerve system have been suggested to contribute by others whereby cause or consequence can not always be distinguished.[12,13]

Due to these different visions regarding pathophysiology and practical clinical use, various definitions for the metabolic syndrome based on different criteria are presently used. In 1998 (revised in 1999), the first working definition for the metabolic syndrome was introduced by the World Health Organisation (Table 1).[14] This definition requires the proven presence of insulin resistance. For practical reasons, a new definition was introduced in 2001 by the National Cholesterol Education program Expert Panel (NCEP).[4] According to this definition, the metabolic syndrome can be diagnosed if at least 3 out of the following 5 (metabolic) abnormalities are present: elevated fasting glucose levels, elevated blood pressure, low plasma HDL-cholesterol levels, elevated plasma triglycerides and central obesity. Since the obligatory presence of insulin resistance is abandoned, this definition is easy to use for daily clinical practice. In 1999 a European definition was introduced by the European Group for the Study of Insulin Resistance (EGIR) as response to the WHO definition.[15] This group prefers the term "Insulin Resistance Syndrome" because of the co-occurrence of non-metabolic characteristics as hypertension. Like the WHO definition, this definition is based on the presence of insulin resistance, but patients with type 2 diabetes are excluded. Very recently, a new definition for the metabolic syndrome was introduced by the International Diabetes Federation, based on the obligatory presence of an increased waist circumference elegantly paying attention to the elementary role of abdominal obesity in the development of the metabolic syndrome.[16]

Prevalence of the metabolic syndrome

The metabolic syndrome is highly prevalent and the coming years this prevalence is expected to increase due to an increased prevalence of overweight/obesity. Among healthy subjects the age adjusted prevalence is 20-25%.[17,18] Among patients with type 2 diabetes mellitus the metabolic syndrome is present in 80%.[19]

Metabolic syndrome and insulin resistance/type 2 diabetes mellitus

Patients with the metabolic syndrome are at a 3 to 4 fold increased risk for the development of type 2 diabetes mellitus.[20-23] Contrary to the WHO definition, the NCEP definition for the metabolic syndrome does not require the demonstration of insulin resistance with a hyperinsulinemic-euglycaemic clamp, a glucose tolerance test or the presence of diabetes. However, more than 95% of metabolic syndrome patients identified by the NCEP definition are insulin resistant as measured

by the gold standard for insulin resistance: the hyperinsulinemic-euglycaemic clamp.[24] In addition, the NCEP definition predicted diabetes mellitus as well as the WHO definition.[25]

Table 1 Definitions of the metabolic syndrome

WHO 1999	NCEP (ATP III) 2001	EGIR 1999†	IDF
Diabetes or IGT¶ or insulin resistance § + 2 of the following:	Combination of 3 or more of the following parameters:	Insulin resistance or hyperinsulinemia** + 2 of the following parameters:	Central obesity # + 2 of the following parameters:
Albumin excretion ≥ 20 µg/min, or albumin:creatinine ratio ≥ 20 mg/g	Fasting glucose ≥ 6.1 mmol/l and/or medication	Fasting glucose ≥ 6.1 mmol/l	Fasting glucose ≥ 5.6 mmol/l and/or medication
BMI‡> 30 kg/m^2 and/or WHR* > 0.9 (♂), > 0.85 (♀)	Waist > 102 cm (♂), > 88 cm (♀)	Triglycerides > 2.0 mmol/l and/or HDL < 1.0 mmol/l and/or medication	Triglycerides ≥ 1.7 mmol/l or specific treatment
Triglycerides ≥ 1.7 mmol/l and/or HDL < 0.9 mmol/l (♂), or < 1.0 mmol/l (♀)	Triglycerides ≥ 1.7 mmol/l	Waist ≥ 94 cm (♂), ≥ 80 cm (♀)	HDL < 1.03 mmol/l (♂), < 1.29 mmol/l (♀) or specific treatment
Blood pressure ≥ 140/90 mmHg and/or medication	HDL < 1.04 mmol/l (♂), < 1.29 mmol/L (♀) Blood pressure ≥ 130/85 mmHg and/or medication	Blood pressure ≥ 140/90 mmHg and/or medication	Blood pressure ≥ 130/85 mmHg and/or medication

¶ IGT: Impaired Glucose Tolerance
§ Insulin resistance: under hyperinsulinemic-euglycaemic conditions, glucose uptake below lowest quartile for background population under study
† only in patients without diabetes
** highest 25%
# Waist > 94 cm (♂), > 80 cm (♀)
‡ BMI: Body mass index
* WHR: Waist Hip Ratio

Metabolic syndrome and cardiovascular disease

Presence of the metabolic syndrome increased the risk for future cardiovascular morbidity and mortality 2 to 3 fold.[19,26,27] The increased cardiovascular risk can at least partially be explained by the risk factors clustering in the metabolic syndrome. However, a study investigating the association of the metabolic syndrome and vascular damage has shown that the metabolic syndrome is associated with more vascular damage than expected from the individual components of the metabolic syndrome only.[28] In addition, the increased cardiovascular risk of patients with the metabolic syndrome can not completely be explained by traditional models for risk predicting as the Framingham risk-score.[29] So it could be suggested that other, not routinely measured aspects of the metabolic syndrome, associated with insulin resistance as impaired fibrinolysis, oxidative stress, increased small dense LDL-cholesterol, hypercoagulability, inflammation, hyperinsulinemia and decreased adiponectin levels, may contribute to the increased cardiovascular risk.[30-33]

In an insulin resistant state, hyperinsulinemia is associated with endothelial dysfunction by the stimulatory effects of insulin on the release of endothelin which is a potent vasoconstrictor. Several other mechanisms as inflammation, an increased production of inflammatory cytokines and an elevated plasma concentration of free fatty acids are associated with endothelial dysfunction.[34,35] There is an enhanced oxidation of small dense LDL-particles which are more capable of

penetration through the endothelial surface by their relative small dimensions.[36] Various studies have demonstrated the association between small dense LDL-cholesterol and ischemic heart disease.[37,38] Low grade inflammation, as reflected by increased plasma C-reactive protein (CRP) levels could contribute to the increased cardiovascular risk observed in patients with the metabolic syndrome. An increment in the number of components constituting the metabolic syndrome was shown to be associated with an increase in the plasma CRP concentrations.[39] In addition, plasma CRP levels are significantly associated with plasma insulin concentrations.[40] CRP may not only be regarded as indicator of increased cardiovascular risk, but could also be directly involved in atherogenesis.[41] Adiponectin, produced by adipocytes, is involved in glucose metabolism and also has direct anti-atherogenic properties. Insulin resistance and type 2 diabetes have been shown to be associated with low plasma adiponectin concentration.[33,42] Decreased adiponectin levels are associated with coronary artery disease both in cross-sectional as in prospective studies.[43-46]

Controversies of the metabolic syndrome

In the present literature, a critical discussion is going on concerning the role of the metabolic syndrome in (cardiovascular) risk prediction.[47] The additive value of using the metabolic syndrome in the prediction of future cardiovascular risk compared to various alternative risk-score algorithms is not fully clear yet. Contrary to the traditional risk-score models, only predicting the risk for the development of cardiovascular disease, identification of the metabolic syndrome also implies an increased risk for the development of type 2 diabetes. Besides this, it has recently been shown that the metabolic syndrome increases the cardiovascular risk on top of the cardiovascular risk calculated with the Framingham risk-score. This latter risk-score model does not take into account plasma glucose- and triglyceride concentrations and obesity. Because of the elevated risk, the NCEP recently suggested that in patients with manifest vascular disease and the metabolic syndrome the target for treatment of LDL-cholesterol could further be lowered from 2.6 to below 1.8 mmol/l.[48]

Treatment of the metabolic syndrome has also been a topic of debate. From a pathophysiological point of view, treatment should be focused on reducing insulin resistance, which can be achieved by weight reduction and an increase in physical activity. By decreasing insulin resistance, improvement of both the individual components of the metabolic syndrome (hyperglycaemia, elevated blood pressure, decreased HDL-cholesterol, elevated triglycerides and central obesity) and the non-classical associated risk factors (impaired fibrinolysis, oxidative stress, increased small dense LDL-cholesterol, hypercoagulability, inflammation, hyperinsulinemia and decreased adiponectin levels) could be expected. Reduction of insulin resistance not only reduces the risk for cardiovascular events, but could also prevent or delay the development of type 2 diabetes in patients at increased risk.[49,50] However, it should be mentioned that studies investigating the effects of lifestyle interventions on clinical endpoints in patients diagnosed with the metabolic syndrome according to the above mentioned definitions are lacking. The same can be stated for medical interventions. Although both methformin as thiazolidinediones can improve insulin sensitivity there is still not enough evidence to treat patients with the metabolic syndrome with these agents if type 2 diabetes is not (yet) present.[49,51]

AIMS AND OUTLINE OF THE THESIS

The prevalence of the metabolic syndrome has been investigated in many populations. The age-adjusted prevalence of NCEP-defined metabolic syndrome among apparently healthy United States adults is 24%, not quite different from the prevalence in a Greece population study (20%). According to the modified WHO definition, the prevalence among French women is 12% and among French men 23%. However, studies investigating the prevalence of the metabolic syndrome in patients with manifest vascular disease are lacking. In **chapter 2** we determine the prevalence of the metabolic syndrome in patients with manifest vascular disease.

Adiponectin, produced by adipocytes, is involved in glucose metabolism and also has direct anti-atherogenic properties and plasma adiponectin levels are decreased in an insulin resistant state. Decreased adiponectin levels could contribute to both the development of the metabolic syndrome as well as to the associated cardiovascular complications. In **chapter 3** the associations between the NCEP-defined metabolic syndrome, insulin resistance and different estimates of obesity with plasma adiponectin levels are investigated in patients with coronary artery disease. Several studies demonstrated an association between elevated body iron stores and insulin resistance. The mechanistic link between excessive body iron and insulin resistance is not elucidated yet. In **chapter 4** we investigate the association between elevated body iron stores (as measured by ferritin) and the metabolic syndrome in patients with manifest vascular disease. In addition we determine the association between ferritin and adiponectin. In **chapter 5** we investigate whether the metabolic syndrome also confers an increased cardiovascular risk in patients already diagnosed with clinical manifestations of atherosclerosis by comparing the vascular damage in patients with and patients without the metabolic syndrome. The increased cardiovascular risk observed in patients with the metabolic syndrome could be explained by the individual components. However, also other aspects could be involved. Well developed coronary collaterals are associated with improved cardiovascular outcome in terms of limiting myocardial infarction size, prevention of ventricular aneurysm formation and future ischemic events in patients with coronary artery disease. It could be hypothesized that impaired coronary collateral formation contributes to the increased cardiovascular risk in metabolic syndrome patients. Aim of **chapter 6** is to determine the relation of the metabolic syndrome and insulin resistance with coronary collateral formation in patients referred for elective Percutaneous Transluminal Coronary Angioplasty (PTCA). Insulin resistance is associated with hyperlipidemia in the postprandial state. Postprandial hyperlipidemia could be regarded as a cardiovascular risk factor, as indicated by postprandial endothelial dysfunction. Inhibiting cholesterol absorption may influence postprandial lipid metabolism and may therefore have effects on postprandial endothelial function. In **chapter 7** the effects of low-dose statin and ezetimibe compared with high-dose simvastatin monotherapy on (postprandial) lipid profiles and endothelial function in patients with the metabolic syndrome are examined.

Chapter 8 deals with two unresolved issues regarding the definition of the metabolic syndrome: we investigate the influence of allowing for lipid-lowering therapy on the prevalence of the NCEP-defined metabolic syndrome and we compare the prevalence and characteristics of identified patients according to the newly proposed IDF definition with the NCEP definition for metabolic syndrome. The results presented in this thesis are summarised and discussed in **chapter 9**.

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Chapter 2

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

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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 the 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 were 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 kilogram 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 parentheses, as mean ± S.D. for normally distributed variables and as median with the interquartile range in parentheses 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. 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.

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

	Patients	Prevalence of metabolic syndrome		Abdominal obesity		High blood pressure		Hypertri-glyceridemia		Low HDL cholesterol		High fasting glucose	
	n	n	%	n	%	n	%	n	%	n	%	n	%
Total	1117	510	46	377	37	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	41	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	1923	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

[a] 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 2 for a description of the criteria used for each component of the metabolic syndrome

Table 1 Baseline characteristics in 18-80-year-old patients with CHD, CVD, PAD or AAA[a]

	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	18 (93)	61 ± 11	60 ± 11	58 ± 10	59 ± 11	69 ± 6	66 ± 10
Smoking, current or past (% (n))[b]	81 (350)	52 (48)	84 (162)	80 (53)	94 (153)	87 (60)	87 (82)	100 (6)
History of vascular diseases (% (n))[c]	6 (27)	8 (7)	26 (50)	6 (4)	30 (49)	32 (22)	40 (38)	50 (3)
Diabetes mellitus (% (n))[d]	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/m²)	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)

[a] CHD: coronary heart disease; CVD: cerebrovascular disease; PAD: peripheral arterial disease; AAA: abdominal aortic aneurysm

[b] Still smoking, recently stopped smoking or previously smoking

[c] History of vascular disease other than the inclusion diagnosis

[d] Fasting serum glucose ≥ 7.0 mmol/L or self-reported diabetes mellitus

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%).

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

No. of components												
	0		**1**		**2**		**3**		**4**		**5**	
	n	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
Total	73	7	227	20	307	27	282	25	151	14	77	7
men	60	7	118	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

[a] 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 2 for a description of the criteria used for each component of the metabolic syndrome

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.

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 diagnose 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, hyperglycaemia 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 hyperglycaemia 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 Organization (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 lead 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-cholesterol 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.

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Chapter 3

In patients with coronary artery disease the NCEP-defined metabolic syndrome, insulin resistance and obesity are all associated with decreased adiponectin levels.

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Submitted

ABSTRACT

Background and Aims Adiponectin, produced by adipocytes, is involved in glucose metabolism and also has direct anti-atherogenic properties and plasma adiponectin levels are decreased in an insulin resistant state. Aim of the present study is to investigate the associations between the metabolic syndrome, insulin resistance and obesity with adiponectin in patients with coronary artery disease.

Methods and Results Cross-sectional study in 227 patients referred for Percutaneous Transluminal Coronary Angioplasty (PTCA). Body mass index (BMI), waist circumference, waist/hip ratio, intraabdominal fat and subcutaneous fat (ultrasound) were used as estimates of obesity. Homeostasis model assessment determined-insulin resistance (HOMA-IR) and Quantitative insulin sensitivity check index (QUICKI) were used as quantitative estimates of insulin resistance. The metabolic syndrome was defined according to NCEP criteria.

Plasma adiponectin levels were lower in patients with the metabolic syndrome compared to patients without the metabolic syndrome (4.8 µg/ml versus 6.4 µg/ml, p-value < 0.001). The number of components of the metabolic syndrome was associated with a decrease of adiponectin levels (p-value for trend 0.001). Significant associations (regression coefficient B, (95% Confidence Interval)) were found for the metabolic syndrome (-0.32, (-0.46;-0.19)), insulin resistance (HOMA-IR -0.23, (-0.37;-0.09)) and QUICKI (0.20, (0.06;0.34))), for intraabdominal fat (-0.37, (-0.60;-0.14)) and BMI (-0.18, (-0.33;-0.03)) with adiponectin, adjusted for age, gender and smoking.

Conclusion In patients with coronary artery disease, the NCEP-defined metabolic syndrome, insulin resistance and obesity are all significantly associated with plasma adiponectin levels. Low plasma adiponectin levels, caused by adipocyte-dysfunction, may contribute to the increased cardiovascular risk associated with the metabolic syndrome.

INTRODUCTION

Adiponectin, a protein produced by adipocytes, has direct anti-atherogenic properties. Adiponectin is able to suppress foam cell formation,[1] to reduce proliferation and migration of smooth muscle cells,[2] to inhibit monocyte adhesion to endothelial cells[3] and to increase endothelial nitric oxide production in vitro.[4, 5] Low plasma adiponectin concentrations have been shown to be associated with the presence of insulin resistance and type 2 diabetes.[6-13] Several cross-sectional and prospective studies also reported an association between low plasma adiponectin concentrations and the presence of coronary artery disease.[12, 14-17] Infusion of adiponectin in mice improved insulin sensitivity.[18, 19]

Adiponectin is present at high concentrations in the human circulation and although it is adipose tissue-derived, plasma concentrations are higher in lean compared to obese subjects.[20] Adiponectin production is under control of the adiponectin gene located on chromosome 3q27 and adiponectin gene polymorphisms have been associated with features of the metabolic syndrome and type 2 diabetes.[21, 22]

The metabolic syndrome is a cluster of several cardiovascular risk factors and is associated with an increased risk for the development of type 2 diabetes[23-25] and the occurrence of cardiovascular morbidity and mortality.[26-30] The underlying pathophysiological pathways of the metabolic syndrome are still not fully understood, but insulin resistance and obesity are elementary components. However, several other factors could contribute to both the development as well as the consequences of the metabolic syndrome. Low plasma adiponectin concentration could be among them since it is involved in both the development of diabetes and the metabolic syndrome as well as in cardiovascular complications.

Although the associations between insulin resistance and clinical estimates of obesity with adiponectin have been investigated in different populations, limited information is available about these associations in patients with coronary artery disease. Besides this, the association between the metabolic syndrome defined according to the definition proposed by the National Cholesterol Education Program Panel (NCEP), and plasma adiponectin levels in these patients has never been explored. Aim of the present study is to investigate the associations between the NCEP-defined metabolic syndrome, insulin resistance and different estimates of obesity with plasma adiponectin levels in patients with coronary artery disease.

METHODS

Study settings, participants, and design

Patients originated from the Second Manifestations of ARTerial disease study (SMART), an ongoing prospective cohort study at the University Medical Centre Utrecht designed to establish the prevalence of concomitant arterial diseases and risk factors for atherosclerosis in a high risk population.[31] In this study participants are screened non-invasively for other manifestations of atherosclerotic diseases and risk factors than the inclusion diagnosis. The local Ethics Committee approved the study and all patients gave their written informed consent. For the present cross-sectional study, based on a case-cohort study investigating determinants and prognostic value of coronary collateral formation, 227 patients referred for elective Percutaneous Transluminal Coronary Angioplasty (PTCA) and included in the SMART study between January 1, 1998 and July 8, 2002 were enrolled.

Data collection

At inclusion, patients were asked to complete a standardized questionnaire requesting information about medical history, symptoms of and risk factors for cardiovascular disease, presence of vascular disease in first-degree relatives and medication use. At the time of enrolment a standardized diagnostic protocol was performed including physical examination (weight, height, waist and hip circumference, systolic and diastolic blood pressure), laboratory tests to determine fasting lipid, serum insulin, serum glucose, adiponectin, creatinine and high sensitive-CRP (hs-CRP) levels and ultrasonography of the abdomen for intraabdominal and subcutaneous fat measurements. Weight and height were measured in patients without heavy clothing and shoes. Body mass index (BMI) was calculated as weight to height squared. Waist circumference was measured halfway between the lower rib and the iliac crest. Waist/hip ratio was calculated as waist circumference in centimetres divided by hip circumference in centimetres. Insulin was measured with an immunometric assay (Diagnostic Products Corporation, Los Angeles, USA), adiponectin with a quantitative enzyme immunoassay technique (R&D Systems, Minneapolis, USA). Since abdominal fat measurements were only performed since May 1, 2000, information on subcutaneous and intraabdominal fat was available in 84 patients. Ultrasonographically measurements were performed using an HDI 3000 (Philips Medical Systems, Eindhoven, Netherlands) with a C 4-2 transducer according to a strict protocol including the position of and pressure on the transducer which has been described in detail previously.[32] Briefly, for ultrasound measurements of intraabdominal fat, the distance between peritoneum and lumbar spine was measured at 3 different positions. For subcutaneous fat measurements the distance between the skin to the linea alba was measured.

Severity of coronary artery disease was defined by visual assessment of the pre-PTCA coronary angiograms (single, two or three vessel disease).

Definitions

The metabolic syndrome was defined according to the definition proposed by the NCEP since it is based on for daily clinical practice easily measurable components.[33] Subjects with the com-

bination of any three or more of the following criteria met the definition for metabolic syndrome: abdominal obesity (waist circumference > 102 cm in men or > 88 cm in women), high blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic or use of blood pressure lowering agents), 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 or < 1.29 mmol/l (50 mg/dL) in women), high fasting glucose (fasting glucose ≥ 6.1 mmol/l (110 mg/dL) or use of glucose lowering agents). If waist circumference was not available (58 patients) a BMI ≥ 30 kg/m^2 was used as determinant for obesity[34] which revealed two more patients with the metabolic syndrome (103 patients versus 101 patients when BMI was not substituted). Diabetes mellitus was defined as self-reported diabetes or a fasting glucose ≥ 7.0 mmol/L (126 mg/dL) in patients with no history of diabetes mellitus. Smoking is defined as currently smoking or smoking in the past year. Homeostasis model assessment determined insulin resistance (HOMA-IR) and Quantitative insulin sensitivity check index (QUICKI) were used as quantitative estimates of insulin resistance. HOMA-IR was calculated using the formula: HOMA-IR = (fasting serum glucose x fasting serum insulin)/22.5,[35] and QUICKI according to the equation: (1/(log fasting serum glucose + log fasting serum insulin).[36]

Data analyses

Results are adjusted for age, gender and smoking habits, since these parameters could both be associated with the determinants under investigation as well with adiponectin concentrations. To adjust mean adiponectin levels for age, sex and smoking differences between patients with and without the metabolic syndrome and between the number of components of the metabolic syndrome we used analysis of covariance (ANCOVA, general linear model procedure).

To investigate the relationships between the metabolic syndrome, insulin resistance, different parameters of obesity and severity of coronary artery disease with adiponectin levels by linear regression analyses, adiponectin was natural-log-transformed. In order to quantify and to compare the influence on adiponectin levels of the parameters under investigation all these variables were categorized. BMI was dichotomized according to the cut-off value of overweight (25 kg/m^2) and to the cut-off value of obesity (30 kg/m^2). Waist was dichotomized according to the criteria of the NCEP defined metabolic syndrome. Intraabdominal fat, subcutaneous fat and waist/hip ratio were dichotomized according to the median in men and women separately. HOMA-IR and QUICKI were calculated only in patients without glucose lowering agents and were then dichotomized according to the median. Severity of coronary artery disease was categorized in two groups (single versus multi vessel (including two or three vessel) disease).

Subsequently, crude and, if appropriate, age, sex and for smoking habits adjusted regression coefficients were calculated for the metabolic syndrome, for insulin resistance, for the clinical estimates of obesity and for the severity of coronary artery disease with natural-log-transformed adiponectin levels. All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 10.1 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics

In table 1a baseline characteristics are outlined according to gender. Female patients were older than male patients (59 versus 57 years of age) and were less often smokers (24% versus 30%). Waist circumference (99 versus 91 cm) and intraabdominal fat (9.6 versus 8.6 cm) were higher in men than in women, whereas BMI (27 versus 29) and subcutaneous fat (2.4 versus 3.4 cm) were lower in men compared to women. Adiponectin levels were nearly twice as high in women compared to men (8.3 versus 5.1 µg/ml). Diabetes mellitus was more prevalent in female than in male patients (31 versus 20%). Multi vessel coronary artery disease was more frequent in men compared to women (45% versus 26%). Baseline characteristics are comparable between patients with and patients without abdominal fat measurements (table 1b).

Table 1a Baseline characteristics of the study population according to gender

	Male (n=188)	Female (n=39)
Age (years)[a]	57±9	59±8
Smoking[*]	30	24
Body mass index (kg/m^2)[a]	27±3	29±4
Waist circumference (cm)[a]	99±8	91±10
Waist/Hip ratio[a]	0.94±0.06	0.85±0.07
Intraabdominal fat (cm)[a]	9.6±2.3	8.6±2.8
Subcutaneous fat (cm)[b]	2.4 (1.7-3.6)	3.4 (2.8-3.7)
Adiponectin (µg/ml)[a]	5.1±3.0	8.3±3.2
hs-CRP (mg/L)[b]	2.4 (1.3-4.9)	3.1 (1.7-7.6)
Creatinine clearance (Cockcroft) (ml/min)[a]	81±17	82±16
Fasting serum Insulin[†] (mIU/L)[b]	17 (10-34)	17(9-25)
Cholesterol (mmol/L)[b]	5.1 (4.4-5.8)	5.3 (4.6-6.3)
HDL-Cholesterol (mmol/L)[b]	1.00 (0.89-1.17)	1.22 (1.08-1.52)
Triglycerides (mmol/L)[b]	1.68 (1.27-2.35)	1.76 (1.20-2.39)
Fasting serum glucose (mmol/L)[b]	5.8(5.4-6.5)	5.9 (5.1-7.1)
Blood pressure systolic (mmHg)[a]	135±19	142±24
Blood pressure diastolic (mmHg)[a]	78±9	77±10
Diabetes mellitus[‡]	20	31
Glucose lowering agents	9	18
Blood pressure lowering agents	29	45
Lipid lowering agents	53	46
Severity of coronary vessel disease[§]		
1-Vessel disease	55	74
2-Vessel disease	34	26
3-Vessel disease	11	-
Metabolic syndrome	42	62

All data in percentages, or as indicated: [a]mean ± standard deviation or [b]median with interquartiles range

[*] Still smoking or recently stopped smoking

hs-CRP: high sensitive C-Reactive Protein (plasma values > 15 mg/L excluded from analyses)

[†] Patients on glucose lowering agents excluded from analyses

[‡] Fasting serum glucose ≥ 7.0 mmol/L or self-reported diabetes

[§] According to pre-PTCA angiograms

Table 1b Baseline characteristics of the study population according to gender and presence of abdominal fat measurements

	Male Patients		Female Patients	
	Without abdominal fat measurements	*With abdominal fat measurements*	*Without abdominal fat measurements*	*With abdominal fat measurements*
	n=117	*n=71*	*n=26*	*n=13*
Age (years)[a]	57±10	58±9	59±7	60±10
Smoking[*]	33	25	27	17
Body mass index (kg/m^2)[a]	27±3	27±3	28±4	29±4
Waist circumference (cm)[a]	99±9	98±8	91±9	91±12
Waist/Hip ratio[a]	0.95±0.06	0.93±0.05	0.85±0.07	0.84±0.08
Intraabdominal fat (cm)[a]	-	9.6±2.3	-	8.6±2.8
Subcutaneous fat (cm)[b]	-	2.4 (1.7-3.6)	-	3.4 (2.8-3.7)
Adiponectin (µg/ml)[a]	5.2±3.1	4.9±2.9	8.6±3.5	7.6±2.6
hs-CRP (mg/L)[b]	2.5 (1.5-4.7)	2.3 (1.2-5.4)	3.0 (1.5-7.7)	3.5 (1.7-8.4)
Creatinine clearance (Cockcroft) ml/min[a]	80±18	83±17	85±16	76±15
Fasting serum Insulin[†] (mIU/L)[b]	14 (8-22)	26 (14-57)	17(13-24)	16(8-63)
Cholesterol (mmol/L)[b]	5.3 (4.5-5.9)	4.9 (4.4-5.5)	5.7 (5.0-6.9)	4.7 (4.5-5.5)
HDL-Cholesterol (mmol/L)[b]	1.00 (0.90-1.18)	0.99 (0.87-1.16)	1.23 (1.09-1.52)	1.22 (1.02-1.54)
Triglycerides (mmol/L)[b]	1.76 (1.29-2.44)	1.62 (1.26-2.09)	1.92 (1.46-2.48)	1.33 (1.09-1.81)
Fasting serum glucose (mmol/L)[b]	5.7(5.3-6.3)	6.0 (5.6-6.8)	5.7 (5.1-7.2)	6.3 (5.3-7.4)
Blood pressure systolic (mmHg)[a]	135±19	134±19	144±26	138±16
Blood pressure diastolic (mmHg)[a]	79±9	77±10	76±11	78±5
Diabetes mellitus[‡]	17	24	27	39
Glucose lowering agents	9	7	19	17
Blood pressure lowering agents	31	25	54	25
Lipid lowering agents	51	57	50	36
Severity of coronary vessel disease[§]				
1-Vessel disease	54	58	73	77
2-Vessel disease	36	30	27	23
3-Vessel disease	10	11	-	-
Metabolic Syndrome	44	40	65	54

All data in percentages, or as indicated: [a]mean ± standard deviation or [b]median with interquartiles range

[*] Still smoking or recently stopped smoking

hs-CRP: high sensitive C-Reactive Protein (plasma values > 15 mg/L excluded from analyses)

[†] Patients on glucose lowering agents excluded from analyses

[‡] Fasting serum glucose ≥ 7.0 mmol/L or self-reported diabetes

[§] According to pre-PTCA angiograms

Metabolic syndrome and adiponectin

The prevalence of the metabolic syndrome in this population was 45%. Patients with the metabolic syndrome had significantly lower plasma adiponectin levels (adjusted for age, sex and differences in smoking habits) than patients without the metabolic syndrome (4.8 µg/ml versus 6.4 µg/ml, p-value < 0.001). In addition, the number of components of the metabolic syndrome was associated with a significant decrease of adiponectin levels (p-value for trend 0.001) (table 2).

Table 2 Relation of the metabolic syndrome, the number of components (according to NCEP criteria) and adiponectin

	Patients	Adiponectin (µg/ml) crude	p-value	Adiponectin (µg/ml) adjusted*	p-value
	n (%)	mean ± se		mean ± se	
Metabolic syndrome					
No	124 (55)	6.2 ± 0.3		6.4 ± 0.3	
Yes	103 (45)	5.0 ± 0.3	<0.001	4.8 ± 0.3	<0.001
Number of Components					
0	14 (6)	6.2 ± 0.9		6.3 ± 0.7	
1	47 (21)	6.6 ± 0.5		7.0 ± 0.4	
2	63 (28)	5.9 ± 0.4		6.0 ± 0.4	
3	51 (22)	4.9 ± 0.5		4.9 ± 0.4	
4	31 (14)	5.2 ± 0.6		4.8 ± 0.5	
5	21 (9)	4.9 ± 0.7	0.001	4.4 ± 0.6	0.001

se standard error

* adjusted for age, gender and smoking

The metabolic syndrome, insulin resistance, obesity and severity of coronary artery disease with adiponectin

Statistically significant associations, adjusted for age, gender and smoking habits, were found for the metabolic syndrome with adiponectin (regression coefficient B (B), (95% confidence interval (95% CI)) (-0.32, (-0.46;-0.19)), for the quantitative estimates of insulin resistance with adiponectin (for HOMA-IR: (-0.23, (-0.37;-0.09)) and for QUICKI: (0.20, (0.06;0.34)) and for the severity of coronary artery disease with adiponectin (–0.17, (-0.31;-0.03)). Regarding the different estimates of obesity, significant associations were found for intraabdominal fat with adiponectin (-0.37, (-0.60;-0.14)) and for BMI (cut-off value of 25 kg/m^2) with adiponectin (-0.18, (-0.33;-0.03)). No associations were found for BMI (cut-off value of 30 kg/m^2) and waist/hip ratio with adiponectin, neither for the NCEP-defined waist criterion and subcutaneous fat (table 3).

Table 3 Relationship between the metabolic syndrome, insulin resistance, different parameters of obesity and severity of coronary artery disease with natural-log-transformed adiponectin levels expressed in regression coefficients

		Number of patients	Regression coefficient B ± se crude	95 % CI	Number of patients	Regression coefficient B ± se adjusted*	95 % CI
Metabolic syndrome							
Metabolic syndrome	(presence/absence)	227	-0.24 ± 0.08	(-0.39;-0.09)	224	-0.32 ± 0.07	(-0.46;-0.19)
Insulin resistance							
HOMA-IR	(high/low)	201	-0.22 ± 0.08	(-0.38;-0.06)	201	-0.23 ± 0.07	(-0.37;-0.09)
QUICKI	(high/low)	196	0.20 ± 0.08	(0.05; 0.36)	196	0.20 ± 0.07	(0.06; 0.34)
Obesity							
BMI†	(high/low)	226	-0.11 ± 0.09	(-0.28; 0.07)	223	-0.18 ± 0.08	(-0.33;-0.03)
BMI‡	(high/low)	226	0.11 ± 0.10	(-0.10; 0.31)	223	-0.04 ± 0.09	(-0.22; 0.14)
Waist/Hip ratio	(high/low)	169	-0.29 ± 0.50	(-1.20; 0.62)	166	-0.74 ± 0.50	(-1.63; 0.15)
Waist criterion§	(presence/absence)	169	0.10 ± 0.09	(-0.09; 0.29)	166	0.01 ± 0.09	(-0.16; 0.19)
Intraabdominal fat‖	(high/low)	85	-0.26 ± 0.13	(-0.51; -0.07)	82	-0.37 ± 0.12	(-0.60;-0.14)
Subcutaneous fat‖	(high/low)	84	-0.07 ± 0.13	(-0.33; 0.19)	81	0.04 ± 0.13	(-0.22; 0.30)
Severity coronary artery disease							
Coronary vessel disease	(multi/single)	227	-0.16 ± 0.08	(-0.32;-0.00)	224	-0.17 ± 0.07	(-0.31;-0.03)

se standard error

* Adjusted for age, gender and smoking habits

† BMI divided according to cut-off value of 25 kg/m^2

‡ BMI divided according to cut-off value of 30 kg/m^2
Waist/Hip ratio dichotomized according to the median

§ NCEP defined waist criterion (men > 102 cm and women > 88 cm)

‖ ultrasonographically determined intraabdominal fat and subcutaneous fat dichotomized separately for men and women

HOMA-IR: Homeostasis model assessment determined insulin resistance, only calculated in patients not on glucose lowering agents and dichotomized according to the median

QUICKI: Quantitative insulin sensitivity check index, only calculated in patients not on glucose lowering agents and dichotomized according to the median

DISCUSSION

In the present study in patients with coronary artery disease we demonstrated a significant inverse association between NCEP-defined metabolic syndrome, insulin resistance, several clinical estimates of obesity and severity of coronary artery disease with adiponectin levels.

The metabolic syndrome is a cluster of cardiovascular risk factors and is associated with an increased risk for the development of cardiovascular complications[26-30] and the development of type 2 diabetes.[23-25] Insulin resistance and obesity are involved in the underlying pathophysiological mechanisms. The metabolic syndrome is highly prevalent and associated with advanced vascular damage in patients with coronary artery disease.[37, 38] Adiponectin is likely to be involved in the development of insulin resistance[18, 19] and in the occurrence of cardiovascular complications.[17]

In this study, we show that in coronary artery disease patients both the metabolic syndrome as well as the quantitative estimates of insulin resistance were significantly associated with adiponectin. The strongest regression coefficient was found for the relationship between the NCEP-defined metabolic syndrome and adiponectin (B, (95 % CI): (-0.32, (–0.46;-0.19)), followed by HOMA-IR (-0.23, (-0.37;-0.09)) and QUICKI (0.20, (0.06;0.34)). However, a comparative and quantitative conclusion about the association of these different parameters with adiponectin could not be made since confidence intervals were overlapping.

An increase in the number of components of the metabolic syndrome was associated with a significant decrease in plasma adiponectin levels (adjusted for age, sex and smoking) (p-value for trend 0.001). The relation between adiponectin and insulin resistance, as measured with HOMA-IR, oral glucose tolerance test or hyperinsulinemic-euglycaemic clamp, has been described in several studies.[8-11, 13] Our data are in concordance with results of other studies showing a relation between the metabolic syndrome and adiponectin in obese and healthy subjects.[6, 39-41] To our best knowledge, there are no studies investigating the association between the NCEP-defined metabolic syndrome and quantitative estimates of insulin resistance with adiponectin in patients with coronary artery disease. Conform the results of other studies, the severity of coronary artery disease appeared significantly associated with adiponectin levels in our study population (B, (95 % CI)): (-0.17, (-0.31;-0.03))).[12, 14-17]

In conjunction with the traditional risk factors, low adiponectin levels could contribute to the increased cardiovascular risk in patients with the metabolic syndrome. Plasma adiponectin concentration can be increased by weight loss[42] and pharmacotherapy.[43] Due to the cross-sectional design of the present study, only assumptions can be made about a possible etiological relationship. Future prospective studies are needed to show whether interventions, aimed at increasing adiponectin plasma levels, beneficially influence the development of cardiovascular complications and diabetes.

More studies investigated the relationship between obesity and adiponectin in different populations including healthy subjects, patients with diabetes, obese subjects, adolescents and Pima Indians.[9, 12, 13, 44-46] By dichotomizing the parameters under investigation, the present study enables us to quantify and to compare these relationships in patients with coronary artery disease. Regarding different estimates of obesity, we demonstrated a significant inverse relationship between intraab-

dominal fat and BMI (cut-off value of 25 kg/m^2) with adiponectin levels, but not between waist circumference, waist/hip ratio, BMI (cut-off value of 30 kg/m^2) and subcutaneous fat with adiponectin. Although the regression coefficient for the association between intraabdominal fat with adiponectin was stronger than the regression coefficient for the association of BMI with adiponectin, confidence intervals were overlapping.

Our observations in patients with coronary artery disease are in agreement with other studies demonstrating a statistically significant inverse relation between BMI and intraabdominal fat with adiponectin.[12-14, 20, 44-46] In contrast to other studies,[10, 13, 41, 44] our observations did not confirm a significant association between waist circumference and waist/hip ratio with adiponectin which could probably be explained by our study population. Declining adiponectin concentrations are mostly associated with central obesity and intraabdominal fat accumulation,[44-46] and it has formerly been suggested that in patients with a high risk for the development of cardiovascular disease the amount of intraabdominal fat can be more reliable calculated ultrasonographically than with waist circumference and waist/hip ratio.[32]

We acknowledge the limited number of patients in our study population in which ultrasonographically measurements of intraabdominal and subcutaneous fat were performed. However, the population in which abdominal fat was measured was not determined by the cardiovascular risk profile, but by the date of enrolment. Besides this, baseline characteristics in patients with abdominal fat measurements and in patients without abdominal fat measurements were equally distributed.

In conclusion, in patients with coronary artery disease the NCEP-defined metabolic syndrome is significantly associated with decreased adiponectin levels. We also demonstrated that insulin resistance and obesity, both essentially involved in the pathogenesis of the metabolic syndrome, are associated with declining adiponectin levels. Low adiponectin levels, caused by adipocyte-dysfunction, may contribute to the increased cardiovascular risk associated with the metabolic syndrome.

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Chapter 4

Association of Elevated Body Iron Stores with NCEP-defined Metabolic Syndrome and Adiponectin in Patients with Manifest Vascular Disease

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ABSTRACT

Background and Aims Elevated iron stores are associated with type 2 diabetes mellitus and the metabolic syndrome, but the mechanistic link is not clear yet. Adipose tissue could be involved in the development of insulin resistance by the changed production of adipokines, so it could be hypothesized that elevated iron concentrations are involved in the development of the metabolic syndrome by interfering in adipocyte function. Aims of the present study were to investigate the relationship between ferritin with the metabolic syndrome in patients with manifest vascular disease and to explore the relationship between ferritin and adipocyte function, as measured by adiponectin levels.

Methods and Results Cross-sectional survey in 403 patients with manifest vascular disease. The metabolic syndrome was defined according to NCEP criteria. Ferritin was measured by two-site sandwich immunoassay, adiponectin with a quantitative enzyme immunoassay technique. Compared to patients in the first quartile of ferritin, patients in the fourth quartile had a 2.1 higher risk for the development of the metabolic syndrome (OR 2.1; 95% CI 1.1-3.9). A significant association was found between log-transformed ferritin (continuous variable) and the metabolic syndrome (OR 2.1; 95% CI 1.2-3.7). Log-transformed ferritin levels appeared statistically significant associated with log-transformed adiponectin levels (regression coefficient B; 95% CI -0.10; -0.17;-0.03). All results adjusted for age, sex, hs-CRP levels and adiponectin levels if appropriate.

Conclusion In patients with manifest vascular disease we demonstrated a positive association between elevated body iron stores (as measured by plasma ferritin levels) and the metabolic syndrome. In addition, an inverse relationship between ferritin and adiponectin was found, suggesting that iron could be involved in the development of the metabolic syndrome by disturbing adipocyte function.

INTRODUCTION

The metabolic syndrome is a constellation of several cardiovascular risk factors as elevated blood pressure, dyslipidemia and disturbed glucose metabolism associated with central obesity. The metabolic syndrome is highly prevalent affecting nearly 25% of non-institutionalized adult citizens in the United States[1] and is present in 45% of patients with already clinical manifestations of atherosclerosis.[2] Presence of the metabolic comprises a 2 to 3-threefold increased risk for the development of type 2 diabetes and cardiovascular disease. Although the underlying pathophysiology of this syndrome is not fully elucidated, it is generally agreed that insulin resistance plays a major role.

Results of several studies suggest that elevated body iron stores could contribute to the development of insulin resistance by demonstrating an association between ferritin with serum insulin and glucose concentrations, insulin resistance and type 2 diabetes.[3-11] It was also prospectively shown that elevated body iron stores predict the development of type 2 diabetes.[12,13] Besides this, lowering elevated body iron stores either by chelating therapy or blood letting resulted in an improvement of insulin sensitivity in patients with and patients without diabetes[12,14-16] although this could not be confirmed by others.[17,18]

The mechanistic links between elevated iron stores and the metabolic syndrome are not clear yet, but it has been suggested that excessive iron amounts could induce insulin resistance by interference of the hepatic insulin extraction[19] and by disturbance of insulin signalling due to increased oxidative stress.[20] Reactive oxygen species may play a pivotal role in cellular signalling pathways by imbalancing the cellular reduction-oxidation state. Hydrogen peroxide hereby acts as a messenger molecule directly or indirectly via its reduction product hydoxyl radical, as generated in the Haber-Weiss reaction. For this reaction, redox-active iron, or another transition metal, is a necessary component. Increased cellular oxidant concentrations induce increased nuclear factor-κB DNA binding, which influences the synthesis of inflammatory cytokines.[21]

Adipose tissue could be involved in the development of insulin resistance by the changed production of adipokines (increased production of Tumor Necrosis Factor-α and Interleukin-6 and the decreased production of adiponectin).[22] So it could be hypothesized that elevated iron concentrations are involved in the development of insulin resistance and the metabolic syndrome by interfering in adipocyte function.

Aims of the present study were to investigate the association between ferritin with the metabolic syndrome in patients with manifest vascular disease and to explore the relationship between ferritin and adipocyte function, as measured by adiponectin levels.

METHODS

Study settings, participants, and design

Patients originated from the Second Manifestations of ARTerial disease study (SMART), an ongoing prospective cohort study at the University Medical Centre Utrecht designed to establish the prevalence of concomitant arterial diseases and risk factors for atherosclerosis in a high risk population.[23] In this study participants are screened non-invasively for other manifestations of atherosclerotic diseases and risk factors than the inclusion diagnosis. The local Ethics Committee approved the study and all patients gave their written informed consent. For the present cross-sectional survey based on a nested case cohort analysis, data were used of 431 patients with clinical manifest arterial disease, including coronary artery disease, cerebrovascular disease, peripheral artery disease or abdominal aortic aneurysm. Of the 2398 patients enrolled in the SMART study between 1996 and 2003, 220 patients emerged, who suffered from a new cardiovascular event during follow-up. Second, a random sample of 10% (240 patients) was drawn as reference from the 2398 patients having a history of cardiovascular disease in the SMART population. Of these 240 patients, 16 had already been selected because of an outcome event in the initial 220 selected cases. Hence, the study population consisted of 444 patients, but because of missing blood samples of 13 patients, analyses were carried out in 423 patients. Patients with high-sensitive C-reactive protein (hs-CRP) plasma concentrations > 25 mg/L were excluded since this may indicate the presence of an active inflammatory condition, leaving 403 patients for further analyses.

Data collection

At inclusion, patients were asked to complete a standardized questionnaire requesting information about medical history, symptoms of and risk factors for cardiovascular disease, presence of vascular disease in first-degree relatives and medication use. At the time of enrolment a standardized diagnostic protocol was performed including physical examination (weight, height and waist circumference, systolic and diastolic blood pressure) and laboratory tests to determine fasting lipid, serum insulin, serum glucose, adiponectin, creatinine and hs-CRP levels. Weight and height were measured in patients without heavy clothing and shoes. Body mass index (BMI) was calculated as weight to height squared. Waist circumference was measured halfway between the lower rib and the iliac crest. Insulin was measured with an immunometric assay (Diagnostic Products Corporation, Los Angeles, USA), adiponectin and hs-CRP with a quantitative enzyme immunoassay technique (R&D Systems, USA). Ferritin was measured by two-site sandwich immunoassay (ADVIA Centaur Ferritin assay, Bayer, Germany).

Definitions

The metabolic syndrome was defined according to the definition proposed by the National Cholesterol Education program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)) (NCEP) since it is based on for daily clinical practice easily measurable components.[24] Subjects with the combination of any three or more of the following criteria met the definition for 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 or use of blood pressure lowering agents)
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 glucose ≥ 6.1 mmol/l (110 mg/dL) or use of glucose lowering agents).

If waist circumference was not available (58 patients) a BMI ≥ 30 kg/m^2 was used as determinant for obesity.[25] Homeostasis model assessment determined insulin resistance (HOMA-IR) was used as quantitative estimate of insulin resistance. HOMA-IR was calculated using the formula: HOMA-IR = (fasting serum glucose x fasting serum insulin)/22.5.[26]

Data analyses

To quantify the association between ferritin levels and the metabolic syndrome with logistic regression analyses, ferritin was divided in quartiles. Metabolic syndrome was taken as dependent variable and ferritin as independent. The first quartile was taken as reference. In addition, to explore the association between ferritin as a continuous variable with the metabolic syndrome with logistic regression analysis, ferritin was log-transformed. Subsequently, the analyses were adjusted for age, sex and hs-CRP levels. To determine the effects of adiponectin on the association between ferritin and the metabolic syndrome these analyses were also performed with adjustment for adiponectin. Dilution of the relationship would point to a role in the pathogenesis of this relationship. Ferritin and adiponectin levels were log-transformed to investigate the relationship between ferritin and adiponectin levels with linear regression analysis. Then, crude and for age, sex and hs-CRP levels adjusted regression coefficients were calculated. All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 12.01 (SPSS, Chicago, IL, USA).

RESULTS

Baseline characteristics

In table 1 the baseline characteristics of the study population are described according to quartiles of ferritin. In the highest quartile 93% of the patients is male, whereas this is 56% in the lowest quartile. In addition, in the fourth quartile patients have a higher BMI (28±4 versus 25±4 kg/m^2) and higher waist circumference (101±10 versus 93±11 cm) compared with the first quartile. The prevalence of diabetes mellitus is higher in the highest quartile compared with the lowest quartile (31% versus 17%). The prevalence of the metabolic syndrome is 63% in the fourth quartile versus 43% in the first quartile.

Ferritin and metabolic syndrome

In table 2 the associations are demonstrated between the quartiles of ferritin and the metabolic syndrome. The first quartile serves as reference. Compared to patients in the first quartile, patients in the fourth quartile had a 2.6 higher risk for the development of the metabolic syndrome (odds ratio (OR) 2.6; 95% Confidence Interval (CI) 1.4-4.7), adjusted for age, sex and hs-CRP levels. After adjustment for log-transformed adiponectin, this association weakened (OR 2.1; 95% CI 1.1-3.9). In addition, a trend for risk of development of the metabolic syndrome was observed running from the first quartile to the fourth quartile.

Table 2 Association of ferritin (quartiles) and the presence of the metabolic syndrome

	OR (95% CI) crude	OR (95% CI) adjusted for age and sex	OR (95% CI) adjusted for age, sex and hs-CRP	OR (95% CI) adjusted for age, sex, hs-CRP and adiponectin
Ferritin (quartiles)				
1	reference	reference	reference	reference
2	0.9 (0.5-1.6)	1.0 (0.6-1.7)	1.0 (0.6-1.8)	1.1 (0.6-1.9)
3	1.7 (1.0-2.9)	1.8 (1.0-3.2)	1.9 (1.0-3.3)	1.6 (0.9-3.0)
4	2.3 (1.3-4.0)	2.6 (1.4-4.8)	2.6 (1.4-4.7)	2.1 (1.1-3.9)

OR (95% CI): odds ratio (95% confidence interval)
Adiponectin: log transformed

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Table 1 Baseline characteristics of the study population according to quartiles ferritin

Serum ferritin (quartiles)	1	2	3	4
	(n=101)	(n=101)	(n=101)	(n=100)
Ferritin (µg/L)[b]	38 (20-55)	86 (75-101)	159 (140-183)	300 (245-425)
Ferritin (µg/L) (min-max)	6-64	64-120	121-203	205-1763
Age (years)[a]	62 ±12	59 ± 11	63 ± 10	64 ± 8
Male Gender	56	74	79	93
Body mass index (kg/m^2)[a]	25 ± 4	26 ± 4	27 ± 4	28 ± 4
Diabetes mellitus[‡]	17	15	18	31
Fasting serum insulin[§] (mE/L)[b]	9 (7-14)	9 (6-12)	11 (8-15)	13 (9-19)
HOMA-IR[§b]	2.2 (1.7-3.0)	2.8 (1.6-3.0)	2.8 (1.9-4.2)	3.4 (2.2-5.7)
Hs-CRP (mg/L)[b]	2.7 (1.1-5.8)	2.1 (1.1-4.4)	2.7 (1.1-5.5)	3.6 (1.9-6.3)
Adiponectin (µg/mL)[b]	6.2 (4.1-9.8)	5.7 (3.8-9.5)	4.8 (3.1-7.1)	4.2 (2.9-6.5)
LDL-cholesterol (mmol/L)[a]	3.47 ± 1.01	3.42 ± 0.93	3.66 ± 0.96	3.44 ± 1.08
Creatinine clearance[**] (ml/min)[a]	69 ± 26	73 ± 23	72 ± 24	73 ± 25
Lipid-lowering agents	39	37	32	32
Blood-pressure-lowering agents	42	41	35	44
Manifest Vascular Disease				
Cerebrovascular Disease	45	33	25	34
Peripheral Arterial Disease	34	28	29	27
Coronary Artery Disease	51	57	57	49
Abdominal Aortic Aneurysm	12	14	16	22
Metabolic Syndrome				
Metabolic Syndrome	43	41	55	63
Glucose (mmol/L)[b]	5.7 (5.2-6.4)	5.7 (5.2-6.6)	5.9 (5.4-7.2)	6.4 (5.6-8.4)
Triglycerides (mmol/L)[b]	1.94 (1.22-2.28)	1.58 (1.20-2.12)	1.77 (1.19-2.46)	1.96 (1.34-2.61)
HDL-cholesterol (mmol/L)[b]	1.19 (0.92-1.41)	1.13 (0.96-1.42)	1.06 (0.89-1.32)	0.98 (0.84-1.17)
Waist circumference (cm)[a]	93 ± 11	96 ± 11	98 ± 12	101 ± 10
Blood pressure systolic (mmHg)[a]	147 ± 25	145 ± 25	145 ± 22	148 ± 20
Blood pressure diastolic (mmHg)[a]	80 ± 12	82 ± 14	80 ± 11	82 ± 11

All data in percentages (%), or as indicated: [a]mean ± standard deviation or [b]median with interquartiles range

[‡] patients on glucose-lowering agents

[§] patients on glucose-lowering agents excluded from analyses

HOMA-IR: Homeostasis model assessment determined insulin resistance (fasting serum glucose x fasting serum insulin)/22.5

[**] according to Cockcroft

A significant association was also found between log-transformed ferritin as a continuous variable and the metabolic syndrome (OR 2.5; 95% CI 1.4-4.2), after adjustment for age, sex and hs-CRP levels. This association weakened after adjusting for adiponectin concentrations. (OR 2.1; 95% CI 1.2-3.7) (table 3).

Table 3 Association of ferritin (log transformed) and the metabolic syndrome

	Metabolic syndrome OR (95% CI) crude	Metabolic syndrome OR (95% CI) Adjusted for age, sex and hs-CRP	Metabolic syndrome OR (95% CI) Adjusted for age, sex, hs-CRP and adiponectin
Ferritin	2.2 (1.4-3.7)	2.5 (1.4-4.2)	2.1 (1.2-3.7)

OR (95% CI): odds ratio (95% confidence interval)
Adiponectin: log transformed

Ferritin and adipocyte function

Ferritin levels appeared statistically significant associated with adiponectin levels, adjusted for age, sex and hs-CRP levels (regression coefficient B (B), (95% confidence interval (95% CI)) (-0.10, (-0.17;-0.03)) (table 4).

Table 4 Relationship between ferritin (log transformed) with adiponectin levels (log transformed)

	B ± se crude	95 % CI	B ± se adjusted for age and sex	95 % CI	B ± se adjusted for age, sex and hs-CRP	95 % CI
Adiponectin	-0.12 ± 0.04	(-0.19;-0.05)	-0.09±0.04	(-0.16;-0.02)	-0.10±0.04	(-0.17;-0.03)

B ± se: Regression coefficient B ± standard error
95% CI: 95% confidence interval

DISCUSSION

In the present study we demonstrated a positive association between body iron stores, as reflected by ferritin levels, and the metabolic syndrome in patients with manifest vascular disease. In addition, we showed a significant relationship between ferritin and adiponectin levels. The relation between excessive iron amounts and insulin resistance/metabolic syndrome has previously been demonstrated mostly in healthy, non-diabetic subjects, but until now not in a cohort of patients with clinical manifest vascular disease.[3-9,12,13] Furthermore, studies investigating the relationship between ferritin and adiponectin were lacking. The mechanistic link between excessive body iron and insulin resistance is not elucidated yet. Insulin resistance could be developed by interference of iron and the associated reactive oxygen species in the hepatic insulin extraction and insulin signalling cascades, suggesting a causal relationship. On the contrary, it has also been postulated that elevated iron stores are just caused by insulin resistance.[27] However, the demonstrated predictive value of iron on the development of type 2 diabetes mellitus combined with the results of intervention studies illustrating increased insulin sensitivity after lowering body iron stores mainly indicate a causal relation.[12-16] Positive associations between several estimates of obesity and ferritin were previously demonstrated in Mexican American men.[28] However, from that study no information is available about the association between ferritin and adiponectin. In the present study, we showed a significant inverse relationship between ferritin and adiponectin. Low plasma adiponectin concentrations have been shown to be associated with the presence of insulin resistance and type 2 diabetes.[22,29-32] Infusion of adiponectin in mice improved insulin sensitivity. [33,34] Since adiponectin is adipose tissue-derived and uniquely produced by adipocytes, it could be suggested that iron is involved in the development of insulin resistance by disturbing adipocyte function. After adjustment for adiponectin the association between ferritin and the metabolic syndrome weakened but remained significant, which may suggest that ferritin is also adiponectin-independently associated with the metabolic syndrome. An explanation could be that iron directly disturbs signalling cascades and protein synthesis in skeletal muscle cells and hepatocytes inducing insulin resistance.[20]

We acknowledge some limitations of our study. Firstly, due to the cross-sectional study design only assumptions about causal relationships could be made and future (in vitro) studies further exploring the effects of ferritin on adipocyte function and adiponectin production are warranted. Secondly, we only considered adiponectin production as a reflection of adipocyte function since adiponectin is exclusively produced by adipocytes. Ideally, to confirm our results also other (derived) parameters of adipocyte function should be taken as outcome measurements. But since these outcomes, as for example HOMA-IR, are associated with adiponectin, the association between ferritin and HOMA-IR just reflects the association between ferritin and adiponectin. Lastly, elevated serum ferritin levels can be regarded as a reflection of iron overload, but ferritin is also an acute-phase reactant. The association between elevated CRP levels (as a reflection of inflammation) and the metabolic syndrome has previously been demonstrated.[35] In line with most studies investigating the relationship between elevated iron stores and insulin resistance we adjusted for inflammation in the

analyses, but it could not entirely be excluded that the demonstrated positive association between elevated ferritin levels and the metabolic syndrome is (partially) a reflection of the associated inflammatory state of the metabolic syndrome.

In conclusion, in the present study in patients with manifest vascular disease we demonstrated a positive association between elevated body iron stores (as measured by plasma ferritin levels) and the metabolic syndrome. In addition, an inverse relationship between ferritin and adiponectin was found, suggesting that iron could be involved in the development of the metabolic syndrome by disturbing adipocyte function.

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Chapter 5

The Metabolic Syndrome is associated with advanced vascular damage in patients with coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm.

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ABSTRACT

Aims The metabolic syndrome is associated with an increased risk of cardiovascular disease in patients without a cardiovascular history. We investigated whether the metabolic syndrome is related to the extent of vascular damage in patients with various manifestations of vascular disease.

Methods and Results The study population of this cross-sectional survey consisted of 502 patients recently diagnosed with coronary heart disease (CHD), 236 with stroke, 218 with peripheral arterial disease (PAD) and 89 with abdominal aortic aneurysm (AAA). Metabolic syndrome was diagnosed according to Adult Treatment Panel III criteria. Carotid Intima Media Thickness (IMT), Ankle Brachial Pressure Index (ABPI) and albuminuria were used as non-invasive markers of vascular damage and adjusted for age and sex if appropriate.

The prevalence of the metabolic syndrome in the study population was 45%. In PAD patients this was 57%; in CHD patients 40%, in stroke patients 43% and in AAA patients 45%. Patients with the metabolic syndrome had an increased mean IMT (0.98 vs. 0.92 mm, p-value < 0.01), more often a decreased ABPI (14% vs. 10%, p-value 0.06) and increased prevalence of albuminuria (20% vs. 15%, p-value 0.03) compared to patients without this syndrome. An increase in the number of components of the metabolic syndrome was associated with an increase in mean IMT (p-value for trend < 0.001), lower ABPI (p-value for trend < 0.01) and higher prevalence albuminuria (p-value for trend < 0.01).

Conclusion In patients with manifest vascular disease the presence of the metabolic syndrome is associated with advanced vascular damage.

INTRODUCTION

Several studies showed high prevalences of the metabolic syndrome in different high-risk populations,[1,2] but the magnitude of the metabolic syndrome became apparent when in an apparently healthy population a prevalence of nearly 24% was found.[3] According to Adult Treatment Panel III (ATP III) the metabolic syndrome is diagnosed when three or more metabolic abnormalities (impaired glucose metabolism, elevated blood pressure, hypertriglyceridemia, low HDL-cholesterol and central obesity) cluster in the same person.[4] This syndrome confers an increased risk for the development of diabetes mellitus and for cardiovascular morbidity and mortality.[5-8] In a population based cohort study the odds ratio (adjusted for age, sex and follow-up duration) for the development of diabetes in patients with impaired fasting glucose was 10.0 (95% confidence interval 6.1-16.5).[9] The presence of the metabolic syndrome at baseline increased the risk for the development of diabetes mellitus nearly 2 times in American Indians and in Finnish men a roughly 4 fold increase was shown.[10,11] In a study by Lakka et al. men with the metabolic syndrome had a nearly threefold increase in cardiovascular related mortality compared to subjects without the metabolic syndrome.[12] In addition, in the Botnia study the risk for coronary heart disease and stroke tripled in metabolic syndrome subjects, with an absolute 10% increase in cardiovascular mortality during 6.9 years of follow-up.[1] This increased cardiovascular risk may be explained by the individual risk factors of the metabolic syndrome in association with other not routinely measured aspects of the metabolic syndrome as impaired fibrinolysis, oxidative stress, increased small dense LDL-cholesterol, hypercoagulability, inflammation and hyperinsulinemia.[13]

From several epidemiological studies it became clear that measurement of carotid intima media thickness (IMT) can be applied as a marker for generalized atherosclerosis and as indicator of cardiovascular risk.[14-21] Similarly, microalbuminuria and decreased ankle brachial pressure index (ABPI) are markers of atherosclerosis and indicators of increased cardiovascular risk.[22-26]

It is not yet known, if among patients with already manifest atherosclerotic diseases patients with the metabolic syndrome have an increased risk of future vascular events as compared to patients without this syndrome. Aim of the current cross-sectional study is to evaluate whether patients with manifest vascular diseases and the metabolic syndrome have more vascular damage than their non-metabolic-syndrome counterparts, by means of carotid IMT, ABPI and albuminuria as non-invasive markers for vascular damage.

METHODS

Study Population

Patients originated from the SMART study (Second Manifestations of ARTerial disease), an ongoing prospective cohort study at the University Medical Centre Utrecht designed to establish the prevalence of concomitant arterial diseases and risk factors for atherosclerosis in a high risk population. Study patients were newly referred to the University Medical Centre Utrecht with a manifest atherosclerotic disease (coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm). The delay between diagnosis of the atherosclerotic disease and the time of enrolment varied from 1-40 days. The local Ethics Committee approved the study and all participants gave their written informed consent.

All patients were non-invasively screened for manifestations of atherosclerotic diseases and risk factors other than the qualifying diagnosis. Study design and definitions have been described in detail previously.[27]

Between January 1999 and July 2002 1217 patients with a qualifying diagnosis of coronary heart disease, stroke, peripheral arterial disease or abdominal aortic aneurysm entered the study. 172 patients were excluded from analyses because data were incomplete. So, in the present cross-sectional survey 1045 consecutive patients with clinically manifest atherosclerotic diseases were enrolled: 502 patients recently diagnosed with coronary heart disease (CHD), 236 with stroke, 218 with peripheral arterial disease (PAD) and 89 with an abdominal aortic aneurysm (AAA). Patients with CHD were primarily referred for percutaneous transluminal coronary angioplasty, those with stroke had had a TIA or cerebral infarction, those with PAD were symptomatic and had documented obstruction of distal arteries of the leg and those with AAA had a supra- or infrarenal aneurysm of the aorta (distal aortic anteroposterior diameter ≥ 3 cm, measured with ultrasonography).

Study Design and Methods

At the time of enrolment all patients passed a standardized protocol, including a health questionnaire on current medication use, past medical history, familial vascular history and atherosclerotic risk factors. Length, body weight, waist circumference and blood pressure were measured. Fasting blood was sampled to determine lipid, serum glucose, homocysteine and creatinine levels, and 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). Intima media thickness (IMT) 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 IMT 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.[27] The mean IMT of these six measurements was calculated only, if at least four of six measurements were available.

The resting ankle-brachial pressure index (ABPI) was measured with the subject in supine position with an 8-Mhz continuous-wave Doppler probe connected to an IMEXLAB 9000 Vascular Diagnostic System (Imex Medical Systems Inc., Golden, CO, USA). Blood pressure was taken from both arms using a semiautomatic oscillometric device (Omega 1400, Invivo Research Laboratories Inc.). The value of the highest systolic blood pressure measured at the ankle was divided to the highest blood pressure measured in both arms. The ratio (ABPI) was calculated for both legs.

Definitions

Metabolic syndrome was diagnosed according to the Adult Treatment Panel III criteria, including three or more 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 high-density lipoprotein (HDL) cholestrol (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 ≥ 6.1 mmol/L (110 mg/dL)).[4]

Patients on glucose-lowering agents or anti-hypertensive medication were regarded as having high fasting glucose and high blood pressure, respectively.

A fasting glucose ≥ 7.0 mmol/L in patients with no history of diabetes mellitus was considered as newly diagnosed diabetes mellitus. Established diabetes was defined as self-reported diabetes.

Albuminuria was calculated as the ratio of albumin to creatinine (mg albumin/mmol creatinine). Albuminuria is defined as a ratio > 3 mg albumin/mmol creatinine.[28,29]

A decreased ABPI was defined as ABPI in rest ≤ 0.90 in at least one leg.[30-32]

Outcomes of Interest

Outcomes of interest were mean IMT, percentage patients with a decreased ABPI and percentage patients with albuminuria in patients with and without the metabolic syndrome. Patients were categorized in subpopulations of cardiovascular disease.

Statistical Analyses

Differences between patients with and without metabolic syndrome were tested with chi-square (categorical variables), unpaired T-test (continuous normal distributed variables) or Mann-Whitney U (continuous skewed variables).

To adjust mean IMT for age and sex differences between patients with and without the metabolic syndrome we used analysis of covariance (ANCOVA, general linear model procedure).

We adjusted the percentage of patients with decreased ABPI and albuminuria for age and sex differences between patients with and without the metabolic syndrome.

With linear regression analysis the influence of age and sex on ABPI and albuminuria was investigated. Subsequently, in case of a significant influence, means of ABPI and albuminuria were calculated and adjusted values were calculated by adding mean value and residual for each patient. Subsequently cut off values as defined earlier (ABPI ≤ 0.90 and albuminuria as a ratio > 3 mg albumin/mmol creatinine) were applied.

Trends between the number of components of the metabolic syndrome and IMT were investigated with linear regression analysis, between number of components and ABPI and albuminuria with logistic regression analysis. If appropriate these outcomes were adjusted for age and sex.

When ABPI was the outcome of interest, patients with peripheral arterial disease were excluded from analyses.

All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 10.0 (SPSS, Chicago, IL, USA).

RESULTS

Baseline Characteristics

In Table 1 the baseline characteristics of the study population are listed, according to the presence of the metabolic syndrome: 469 patients (45%) with the metabolic syndrome, and 576 patients without (55%). Age and smoking habits were equally distributed. Patients with the metabolic syndrome had a higher creatinine clearance compared to non-metabolic syndrome patients (79 ml/min vs. 76 ml/min, p-value 0.01). As expected, all 5 diagnostic parameters of the metabolic syndrome were more prevalent in patients with the metabolic syndrome than in patients without the metabolic syndrome (p-value < 0.001). Besides this, 21% of the patients with the metabolic syndrome had a history of a vascular disease in another vascular bed compared to 16% in the non-metabolic syndrome population (p-value 0.02). From 469 patients diagnosed with the metabolic syndrome, 179 (38%) had a normal fasting glucose level and did not use glucose lowering agents.

Table 1 Baseline characteristics of the study population

	Metabolic Syndrome		
	No (n=576)	Yes (n=469)	
			p-value
Male gender	84	74	<0.001
Age (years)[1]	59±10	60±10	0.4
Body mass index (kg/m^2)[1]	25±3	28±4	<0.001
Smoking[†]	82	81	0.8
History of other vascular disease[‡]	16	21	0.02
Total Cholesterol (mmol/L)[2]	5.2 (4.5-5.9)	5.6 (4.8-6.2)	<0.001
Homocysteine (µmol/l)[1]	14±6	15±7	0.2
Serum creatinine (µmol/L)[1]	93±37	95±46	0.4
Creatinine clearance (Cockcroft) (ml/min)[1]	76±19	79±22	0.01
Diabetes mellitus[§]	7	33	<0.001
Glucose lowering agents	4	18	<0.001
Anti-hypertensive drugs	25	45	<0.001
Lipid lowering agents	38	38	0.4
Components of metabolic syndrome			
Waist circumference (cm)[1]	92±9	100±10	<0.001
Blood pressure systolic (mmHg)[1]	134±21	143±20	<0.001
Blood pressure diastolic (mmHg)[1]	78±11	81±10	<0.001
HDL-Cholesterol (mmol/L)[2]	1.21(1.04-1.42)	0.96(0.83-1.11)	<0.001
Triglycerides (mmol/L)[2]	1.33(1.05-1.65)	2.12(1.72-2.78)	<0.001
Fasting serum glucose (mmol/L)[2]	5.6(5.2-5.9)	6.2(5.6-7.2)	<0.001

All data in percentages, or as indicated: [1]mean ± standard deviation or [2]median with interquartiles range

HDL: high-density lipoprotein

† Still smoking, recently stopped smoking or previously smoking

‡ History of vascular disease other than qualifying diagnosis

§ Fasting serum glucose ≥ 7.0 mmol/L or self-reported diabetes

Outcomes of Interest

Age and sex significantly influenced the relationship between the metabolic syndrome and IMT and ABPI. The relationship between albuminuria and metabolic syndrome was influenced by age not by sex.

Patients with the metabolic syndrome had an increased mean IMT (0.98 mm vs. 0.92 mm, p-value < 0.01) and an increased prevalence of albuminuria (20% vs. 15%, p-value 0.03) compared to non-metabolic syndrome patients. A trend, albeit non significant, toward a decreased ABPI in patients with the metabolic syndrome was found (p-value 0.06) (table 2). Similar relationships were found in all subpopulations of (cardio)vascular disease, except for IMT in AAA patients (1.04 mm vs. 1.09 mm).

The prevalence of albuminuria was 28% in diabetic patients. After exclusion of patients on glucose-lowering agents, patients with the metabolic syndrome still had higher prevalences of albuminuria than patients without (16% vs. 12%). Similar observations were made when patients with anti-hypertensive drugs were excluded from analyses (18% vs. 10%).

Table 2 Non-invasive atherosclerotic markers for vascular damage in all patients and in subpopulations of cardiovascular disease in relation to the presence of the metabolic syndrome

	Metabolic syndrome	Patients	IMT (mm)	p-value	decreased ABPI*	p-value	Albuminuria	p-value
		% (n)	mean±se		%		%	
All patients	no	55(576)	0.92±0.01		10		15	
	yes	45(469)	0.98±0.01	<0.01	14	0.06	20	0.03
CHD	no	29(299)	0.85±0.01		5		13	
	yes	19(203)	0.89±0.02	0.04	7	0.3	13	0.9
Stroke	no	13(135)	1.00±0.03		18		15	
	yes	10(101)	1.06±0.03	0.2	24	0.3	27	0.02
PAD	no	9(93)	0.90±0.04		-		18	
	yes	12(125)	1.02±0.03	0.02	-		24	0.3
AAA	no	5(49)	1.09±0.07		20		16	
	yes	4(40)	1.04±0.08	0.7	28	0.4	23	0.5

se: standard error
CHD: coronary heart disease
PAD: peripheral arterial disease
AAA: abdominal aortic aneurysm
IMT: Intima Media Thickness in common carotid arteries (age-and sex adjusted)
Decreased ABPI: Ankle Brachial Pressure Index ≤ 0.9 in at least one leg (age-and sex adjusted)
Albuminuria: albumin/creatinine ratio > 3 mg/mmol (urine portion) (age-adjusted)
* Patients with PAD excluded from analyses

In table 3 it is shown that the number of single components of the metabolic syndrome is associated with an increase in mean IMT, in the prevalence of albuminuria and in the proportion of patients with decreased ABPI. Patients who had all 5 criteria constituting the metabolic syndrome had the largest IMT (1.07 mm), the highest frequency of a decreased ABPI (22%), and the highest prevalence of albuminuria (24%) compared to patients with less than 5 components.

Table 3 Components of the metabolic syndrome in relation to IMT, decreased ABPI and the prevalence of albuminuria

Metabolic syndrome components	IMT (mm)	p-value†	Decreased ABPI*	p-value†	Albuminuria	p-value†
(n)	mean±se		(%)		(%)	
0	0.85±0.04		2		13	
1	0.90±0.02		10		13	
2	0.94±0.02		12		16	
3	0.95±0.02		11		18	
4	0.97±0.03		18		21	
5	1.07±0.04	<0.001	22	<0.01	24	<0.01

IMT: Intima Media Thickness in common carotid arteries (age-and sex adjusted)
Decreased ABPI: Ankle Brachial Pressure Index ≤ 0.9 in at least one leg (age-and sex adjusted)
Albuminuria: albumin/creatinine ratio > 3 mg/mmol (urine portion) (age-adjusted)
† p-value for trend
* Patients with PAD excluded from analyses

DISCUSSION

This study detected a high prevalence of the metabolic syndrome in patients with manifest atherosclerotic arterial disease. Moreover, the presence of the metabolic syndrome was associated with more advanced atherosclerosis, measured by non-invasive techniques. Patients with the metabolic syndrome had an increased carotid IMT, more often a decreased ABPI and had a higher prevalence of albuminuria compared to patients without the metabolic syndrome. In addition, an increment in the number of components constituting the metabolic syndrome was associated with an increase in mean IMT, lower ABPI values and higher prevalence of albuminuria.

In patients with a negative history of vascular disease, IMT, ABPI and albuminuria are markers for atherosclerosis, associated with an increased risk for cardiovascular morbidity and mortality. Several studies found that these non-invasive markers could also be applied to patients with manifest vascular disease. Previously we showed that common carotid IMT appeared to be a clear marker of cardiovascular risk in patients with either manifest vascular disease or atherosclerotic risk factors.[33] Assessment of microalbuminuria in the first week after a myocardial infarction was a strong predictor for 1-year mortality.[34] In the PREVEND study the presence of microalbuminuria in patients with ST-T segment abnormalities on a resting ECG conferred an increased (cardiovascular) mortality risk.[35] In patients with peripheral arterial disease or suspected coronary artery disease the ABPI was a predictor of cardiovascular events.[36-38] Our study demonstrates that these well-established indicators of increased cardiovascular risk cluster with the metabolic syndrome. This implies that identification of the metabolic syndrome in this high-risk category of patients could indicate an even greater risk of cardiovascular events.

C-reactive protein (CRP) is regarded as a sensitive indicator of cardiovascular risk and could also be directly involved in atherogenesis.[39] Unfortunately in this study we were not able to assess CRP levels. In apparently healthy women plasma CRP concentration increases with the number of individual components of the metabolic syndrome.[7] Moreover, a significant linear relationship between CRP levels and plasma insulin concentrations was observed,[40] suggesting that in metabolic syndrome patients without cardiovascular history CRP could be used as indicator of the increased associated cardiovascular risk.

The presence of the metabolic syndrome in nearly 50% of subjects referred for treatment of an atherosclerotic arterial disorder (subjects with overt diabetes mellitus excluded) calls for a systematic approach to identification and treatment of this syndrome. The individual components that make up the syndrome should be treated coherently, with awareness of the underlying disorder: insulin resistance. Newly developed drugs such as the peroxisome proliferator-activated receptor (PPAR) agonists may help to reach targets, along with life style modifications.[41,42]

Remarkably, in our study nearly 40% of the patients diagnosed with the metabolic syndrome had normal fasting glucose and did not use glucose lowering agents. As insulin resistance is regarded as one of the major pathophysiological disturbances underlying the metabolic syndrome, normal glucose levels in these patients are most likely the result of a compensatory hyperinsulinemic state, associated with increased cardiovascular risk.[43] Although insulin has beneficial effects on endothelial function by enhancing eNOS transcription, hyperinsulinemia is associated with endothelial dysfunction by stimulating the release of the potent vasoconstrictor endothelin.[44] Since normal glucose values do not exclude insulin resistance, also in euglycemic vascular patients the metabolic syndrome should be considered.

We acknowledge some limitations of our study. Firstly, the metabolic syndrome can be diagnosed by several definitions, which implies that it may be difficult to compare outcomes of different studies. Most commonly used are the definitions proposed by The World Health Organization in 1998, and the working definition suggested by Adult Treatment Panel III.[4,45] Ford et al. compared the prevalence of the metabolic syndrome in a non-institutionalised population in the United States with the above mentioned definitions.[46] For the entire population, no difference in prevalence was found but differences in subpopulations were masked, particularly in race or ethnic groups. Because of this agreement, and also because the components according to ATP III are more easy to measure in daily clinical practice we decided to use this definition of the metabolic syndrome. Secondly, this survey is a cross sectional study, so only assumptions about possible etiological relationships can be made.

We conclude that in patients with already manifest vascular diseases, the metabolic syndrome is associated with more advanced vascular damage as measured by indicators for increased (cardio)vascular risk. Screening in a high-risk population for the metabolic syndrome identifies patients at higher cardiovascular risk which may guide pharmacological and non-pharmacological therapies in order to prevent new cardiovascular incidents.

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Chapter 6

Presence of the Metabolic Syndrome does not impair coronary collateral vessel formation in patients with documented coronary artery disease.

The metabolic syndrome and coronary collaterals

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ABSTRACT

Objective The metabolic syndrome confers an increased risk for cardiovascular morbidity and mortality. The presence of coronary collaterals may have beneficial effects during myocardial ischemia and may improve cardiovascular outcome in patients with coronary artery disease. Impaired collateral formation could be one of the reasons for the increased cardiovascular risk in patients with the metabolic syndrome. Aim of the present study was to determine the influence of the metabolic syndrome and insulin resistance on the presence of coronary collaterals.

Research Designs and Methods A cross-sectional study in 227 patients referred for elective Percutaneous Transluminal Coronary Angioplasty to the University Medical Centre Utrecht. Metabolic syndrome was diagnosed according to Adult Treatment Panel III and HOMA-IR and QUICKI were used to quantify insulin resistance. Coronary collaterals were graded with Rentrop's classification. Rentrop-grade ≥ 1 indicated the presence of collaterals. Results were adjusted for age, sex and severity of coronary artery disease.

Results 103 patients (45%) were diagnosed with the metabolic syndrome. There was no association between the metabolic syndrome and the presence of coronary collateral formation (Odds ratio (OR) 1.2; 95% confidence interval (CI) 0.7-2.0). Also, the degree of insulin resistance was not related to the presence of coronary collaterals. OR for HOMA-IR (highest versus lowest tertile) was 0.7 (95% CI 0.3-1.5) and for QUICKI (lowest versus highest tertile) 0.8 (95% CI 0.4-1.6).

Conclusions The metabolic syndrome and insulin resistance are not related to the presence of coronary collaterals in patients with documented coronary artery disease.

INTRODUCTION

The metabolic syndrome is a cluster of generally accepted cardiovascular risk factors such as impaired glucose metabolism, elevated blood pressure, dyslipidemia and central obesity.[1] Also other, often not routinely measured cardiovascular risk factors (like inflammation, increased oxidative stress, increased small dense LDL-cholesterol, impaired fibrinolysis, hypercoagulability and hyperinsulinemia), cluster in this syndrome.[2] The underlying pathophysiology is still not fully clarified, but insulin resistance is a major characteristic. Increased adipose tissue mass is involved in the development of insulin resistance by metabolic alterations such as changes in the production of cytokines.[3,4]

The prevalence of the metabolic syndrome is high, amounting to 24% in an apparently healthy westernised population.[5] In patients with manifest vascular disease the prevalence is 46%.[6] The number of subjects with the metabolic syndrome is likely to increase in the coming years due to the increased prevalence of obesity. Patients with the metabolic syndrome are at an increased risk for cardiovascular morbidity and mortality.[7-12] Several studies report a two to three fold increased risk.[13-15] This increased risk can at least partially be explained by the risk factors clustering in the metabolic syndrome.

Well developed coronary collaterals are associated with improved cardiovascular outcome in terms of limiting myocardial infarction size, prevention of ventricular aneurysm formation[16,17] and future ischemic events[18,19] in patients with coronary artery disease. Repetitive myocardial ischemia and increased shear stress are important determinants of coronary collateral development.[20,21]

It could be hypothesized that impaired coronary collateral formation contributes to the increased cardiovascular risk in metabolic syndrome patients. Since adequate collateral formation has been suggested to be critically dependent on endothelial function and nitric oxide bioavailability,[22,23] endothelial dysfunction could be one of the potential mechanisms for the decreased presence of coronary collaterals. Abaci et al. demonstrated a decreased presence of coronary collaterals in diabetic patients.[24] However, this could not be confirmed by others.[25-28] To our best knowledge, no information on coronary collaterals is available in patients with the metabolic syndrome.

Insulin resistance may be linked to endothelial dysfunction by several mechanisms including, inflammation (as reflected by elevated high sensitive C-Reactive Protein (hs-CRP) plasma levels), disruption of insulin receptor signalling cascades, increased production of cytokines and activation of the renin angiotensin system.[29,30] Adiponectin, an adipocyte-derived protein, stimulates the production of nitric oxide in vascular endothelial cells in vitro,[31] and hypoadiponectinemia is associated with insulin resistance.[32,33]

Aim of the present study is to determine the relation of the metabolic syndrome and insulin resistance with coronary collateral formation in patients referred for elective Percutaneous Transluminal Coronary Angioplasty (PTCA).

RESEARCH DESIGN AND METHODS

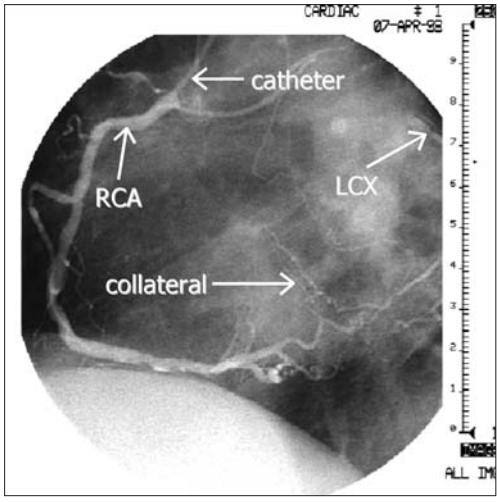
Study Population

Patients originated from the SMART study (Second Manifestations of ARTerial disease), an on going prospective cohort study at the University Medical Centre Utrecht designed to establish the prevalence of concomitant arterial diseases and risk factors for atherosclerosis in a high risk population.[34] The local Ethics Committee approved the study and all participants gave their written informed consent. For the present cross-sectional study, based on a case-cohort study investigating determinants and prognostic value of coronary collateral formation, 227 patients referred for elective Percutaneous Transluminal Coronary Angioplasty (PTCA) and included in the SMART study between January 1, 1998 and July 8, 2002 were enrolled.

Study Design and Methods

At the time of enrolment clinical information was obtained using a standardized health questionnaire for all patients. Length, body weight, waist circumference and blood pressure were measured. Fasting blood was sampled to determine lipid, serum glucose, homocysteine, creatinine, adiponectin, hs-CRP and insulin levels. Insulin was measured with an immunometric assay (Diagnostic Products Corporation, Los Angeles, USA), adiponectin with a quantitative enzyme immunoassay technique (R&D Systems, Minneapolis, USA). Two experienced observers blinded to all patient characteristics independently reviewed all pre-PTCA coronary angiograms. (figure 1) Rentrop's classification was used to determine the extent of collateralisation (grade 0 – no filling of collateral vessels; grade 1 – filling of collateral vessels without any epicardial filling of the recipient artery; grade 2 – partial epicardial filling by collateral vessels of the recipient artery; and grade 3 – complete epicardial filling by collateral vessels of the recipient artery).[35]

Figure 1. Left anterior oblique view of the right coronary arteriogram.



The left circumflex coronary artery (LCX) is proximally occluded, and fills completely by means of collateral circulation from the right coronary artery (RCA). Image courtesy of the Department of Cardiology at the Heronimus Bosch Hospital, Den Bosch, The Netherlands. From Koerselman et al.,[20] with permission.

By visual assessment of the pre-PTCA coronary angiograms severity of coronary artery disease was defined (single, two or three vessel disease) as the degree of the most severe stenosis (50-90%, 90-99% or 100% stenosis). A ≥ 50% diameter reducing stenosis was regarded as significant.[36]

Definitions

Metabolic syndrome was diagnosed according to the Adult Treatment Panel III criteria, including three or more 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 high-density lipoprotein (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 ≥ 6.1 mmol/L (110 mg/dL)).[1] Patients on glucose-lowering agents or anti-hypertensive medication were regarded as having high fasting glucose and high blood pressure, respectively. Waist circumference was not measured until January 1, 1999. If waist circumference was not available a BMI cut point of 30 kg/m^2 was used as determinant for obesity.[37] A fasting glucose ≥ 7.0 mmol/L in patients with no history of diabetes mellitus was considered as newly diagnosed diabetes mellitus. Established diabetes was defined as self-reported diabetes.

Homeostasis model assessment determined insulin resistance (HOMA-IR) and Quantitative insulin sensitivity check index (QUICKI) were used as quantitative estimates of insulin resistance. HOMA-IR was calculated using the formula: HOMA-IR = (fasting serum glucose x fasting serum insulin)/22.5,[38] and QUICKI according to the equation: (1/(log fasting serum glucose + log fasting serum insulin).[39]

The presence of coronary collaterals was defined as a Rentrop score ≥ 1. Severity of coronary artery disease was categorized in two groups (single versus multi vessel (including two or three vessel) disease). HOMA-IR and QUICKI were categorized in tertiles.

Data Analyses

Differences between patients with and without metabolic syndrome were tested with chi-square (categorical variables), unpaired T-test (continuous normal distributed variables) or Mann-Whitney U (continuous skewed variables).

Rentrop score was dichotomised (score 0 indicating the absence and score ≥ 1 indicating the presence of coronary collaterals). The relation between the presence or absence of coronary collaterals and metabolic syndrome was quantified using binary logistic regression model. Subsequently, this association was adjusted for age, sex and severity of coronary artery disease. For obvious reasons, we did not adjust for factors included in the definition of the metabolic syndrome. These analyses were also performed with the values of HOMA-IR (categorized in tertiles), QUICKI (categorized in tertiles) and the number of components of the metabolic syndrome as independent variables respectively and the presence of collaterals as dependent variable. HOMA-IR and QUICKI were only calculated in patients not on glucose lowering agents. Hs-CRP values > 15 mg/L were

excluded from analyses since they may indicate the presence of an active inflammatory disease. We also investigated the relationship between the separate continuous components of the metabolic syndrome and the presence of coronary collaterals. Regarding the association between blood pressure and coronary collateral formation and glucose levels and coronary collateral formation, patients with anti-hypertensive drugs and patients on glucose lowering agents were excluded respectively.

All statistical analyses were performed using the Statistical Package for the Social Sciences for Windows 10.1 (SPSS, Chicago, IL, USA).

RESULTS

Table 1 describes the baseline characteristics of the study population, according to the presence of the metabolic syndrome: 103 patients (45%) with the metabolic syndrome and 124 patients without (55%). In 58 patients waist circumference was not available. Substituting a BMI cut point of 30 kg/m^2 as determinant for obesity classified only two more patients with the metabolic syndrome (103 patients vs. 101 patients when BMI was not substituted). In one patient both waist circumference as BMI were missing. Age and smoking habits were equally distributed. Patients with the metabolic syndrome had a higher creatinine clearance compared to non-metabolic syndrome patients (85 ml/min vs. 79 ml/min). Metabolic syndrome patients had higher hs-CRP plasma levels (3.2 vs. 2.0 mg/L) and lower adiponectin levels (4.1 mg/L vs. 5.3 mg/L) compared to their non-metabolic syndrome counterparts. Severity of coronary artery disease was classified as single vessel disease in 53% and as multi vessel disease in 47% of the metabolic syndrome patients versus 63% and 37% in non-metabolic syndrome patients. As expected, all 5 diagnostic parameters of the metabolic syndrome were more common in patients with the metabolic syndrome than in patients without the metabolic syndrome. Rentrop-grade ≥ 1 was present in 41% of the metabolic syndrome patients and in 35% of the non-metabolic syndrome patients. Coronary collaterals were present in 36% of the patients without any components of the metabolic syndrome, in 34% of the patients with 1 component, in 37% of the patients with 2 components, in 43% of the patients with 3 components, in 42% of the patients with 4 components and in 33% of the patients with all components of the metabolic syndrome (table 2).

Table 2 Relation of the Metabolic Syndrome, the number of components (according to ATP III criteria)[1] and the presence of coronary collaterals according to Rentrop's classification[35]

	Rentrop-grade 0	Rentrop-grade ≥ 1
Metabolic syndrome		
No	65 (80)	35 (44)
Yes	59 (61)	41 (42)
Number of Components		
0	64 (9)	36 (5)
1	66 (31)	34 (16)
2	63 (40)	37 (23)
3	57 (29)	43 (22)
4	58 (18)	42 (13)
5	67 (14)	33 (7)

All data in percentages (number of patients)

Table 1 Baseline characteristics of the study population

	Metabolic Syndrome	
	No (n=124)	Yes (n=103)
Male gender	88	77
Age (years)[a]	58±10	58±8
Body mass index (kg/m^2)[a]	26±3	29±3
Smoking*	81	77
Total Cholesterol (mmol/L)[b]	5.0 (4.4-5.7)	5.4 (4.7-6.2)
Adiponectin (mg/L)[b]	5.3 (3.7-7.5)	4.1 (3.0-6.4)
hs-CRP (mg/L)[b]	2.0 (1.1-3.9)	3.2 (2.0-6.6)
Creatinine clearance (Cockcroft) ml/min[a]	79±17	85±17
Fasting serum Insulin† (mIU/L)[b]	15 (9-24)	19 (11-35)
Diabetes mellitus‡	7	40
Glucose lowering agents	3	19
Anti-hypertensive drugs	21	44
Lipid lowering agents	48	56
Parameters of coronary artery disease		
Severity of coronary vessel disease§		
1-Vessel disease	63	53
2-Vessel disease	30	36
3-Vessel-disease	7	11
Degree of most severe lesion§		
50-90% stenosis	65	59
90-99% stenosis	18	17
100% stenosis	17	24
Duration Angina Pectoris until PTCA (years)	3±5	3±5
Previous myocardial infarction	39	50
Previous PTCA and or CABG	29	31
Components of metabolic syndrome		
Waist circumference (cm)[a]	95±9	101±8
Blood pressure systolic (mmHg)[a]	132±21	140±18
Blood pressure diastolic (mmHg)[a]	76±10	80±9
HDL-Cholesterol (mmol/L)[b]	1.15(0.96-1.32)	0.93(0.82-1.10)
Triglycerides (mmol/L)[b]	1.38(1.06-1.63)	2.25(1.78-3.22)
Fasting serum glucose (mmol/L)[b]	5.6(5.2-5.9)	6.5(5.7-8.1)

All data in percentages, or as indicated: [a]mean ± standard deviation or [b]median with interquartiles range

* Still smoking, recently stopped smoking or previously smoking

† patients on glucose lowering agents excluded from analyses

‡ Fasting serum glucose ≥ 7.0 mmol/L or self-reported diabetes

§ according to pre-PTCA angiograms

Hs-CRP: high sensitive C-Reactive Protein (plasma values > 15 mg/L excluded from analyses)

PTCA: Percutaneous Transluminal Coronary Angioplasty

CABG: Coronary Artery Bypass Grafting

No difference was found in the presence of coronary collaterals between patients with and patients without the metabolic syndrome (crude odds ratio (OR) 1.3; 95% Confidence Interval (CI) 0.7-2.1). Age, sex and the severity of coronary artery disease did not influence the relationship between the metabolic syndrome and coronary collaterals (adjusted OR 1.2; 95% CI 0.7-2.0). The number of single components of the metabolic syndrome similarly showed no association with coronary collateral formation. When patients with established diabetes mellitus were excluded from analyses, results remained the same (data not shown). Also no significant associations were found between the separate continuous components of the metabolic syndrome and the presence of coronary collaterals (table 3).

Table 3 Relation of the Metabolic Syndrome, the individual components (according to the ATPIII criteria)[1] and the presence of coronary collaterals

	Crude	Adjusted for age and sex	Adjusted for age, sex and severity of coronary artery disease*
Metabolic Syndrome	1.3 (0.7-2.1)	1.3 (0.8-2.3)	1.2 (0.7-2.0)
Number of Components			
0	reference	reference	reference
1	0.9 (0.3-3.2)	0.9 (0.3-3.1)	1.1(0.3-4.1)
2	1.0 (0.3-3.5)	1.0 (0.3-3.5)	1.2 (0.3-4.1)
3	1.4 (0.4-4.7)	1.4 (0.4-4.8)	1.5 (0.4-5.3)
4	1.3 (0.4-4.8)	1.4 (0.4-5.1)	1.3 (0.3-4.9)
5	0.9(0.2-3.7)	1.0 (0.2-4.0)	1.0 (0.2-4.3)
Individual components§			
Waist circumference (cm)	1.02 (0.99-1.06)	1.02 (0.99-1.06)	1.02 (0.98-1.06)
Blood pressure systolic (mmHg) †	0.99 (0.98-1.01)	0.99 (0.97-1.01)	0.99 (0.97-1.01)
Blood pressure diastolic (mmHg) †	0.98 (0.94-1.01)	0.98 (0.94-1.01)	0.98(0.94-1.01)
HDL-Cholesterol (mmol/L)	1.05 (0.38-2.86)	1.34 (0.45-4.01)	1.53 (0.50-4.71)
Triglycerides (mmol/L)	1.09 (0.91-1.30)	1.09 (0.90-1.33)	1.06 (0.91-1.23)
Fasting serum glucose (mmol/L)‡	1.09 (0.88-1.36)	1.10 (0.88-1.36)	1.06 (0.85-1.33)

All data: Odds Ratio's (95% confidence interval)

* according to pre-PTCA angiograms (single vessel versus multi vessel disease)

† patients with anti-hypertensive drugs excluded from analyses

‡ patients on glucose lowering agents excluded from analyses

§ continuously

In table 4 it is shown that quantitative estimates of insulin resistance are not associated with the presence of coronary collaterals. Odds ratio for HOMA-IR (highest versus lowest tertile) was 0.7 (95% CI 0.3-1.5) and for QUICKI (lowest versus highest tertile) 0.8 (95% CI 0.4-1.6) after adjustment for age, sex and severity of coronary artery disease.

Additional analyses were performed after dichotomising the Rentrop score in a more functional way (Rentrop score 0-1 versus 2-3). Results essentially remained the same in comparison to analyses with Rentrop score 0 versus 1-3 (data not shown).

Table 4 Relation of quantitative estimates of insulin resistance (HOMA-IR and QUICKI) and the presence of coronary collaterals*

	Crude	Adjusted for age and sex	Adjusted for age, sex and severity of coronary artery disease†
HOMA-IR tertiles			
1	reference	reference	reference
2	1.0 (0.5-2.0)	1.0 (0.5-2.1)	0.8 (0.4-1.8)
3	0.8 (0.4-1.7)	0.8 (0.4-1.7)	0.7 (0.3-1.5)
QUICKI tertiles			
1	0.9 (0.4-1.8)	0.9 (0.4-1.8)	0.8 (0.4-1.6)
2	1.0 (0.5-2.1)	1.1 (0.5-2.2)	0.8 (0.4-1.8)
3	reference	reference	reference

* patients on glucose lowering agents excluded from analyses

All data: Odds Ratio's (95% confidence interval)

HOMA-IR: Homeostasis model assessment determined insulin resistance (fasting serum glucose x fasting serum insulin)/22.5[38]

QUICKI: Quantitative insulin sensitivity check index (1/(log fasting serum glucose + log fasting serum insulin)[39]

† according to pre-PTCA angiograms (single vessel versus multi vessel disease)

CONCLUSIONS

The metabolic syndrome is associated with an increased risk for cardiovascular morbidity and mortality.[7-15] Impaired coronary collateral formation has been reported in diabetes mellitus and may also contribute to the increased cardiovascular risk in metabolic syndrome patients. However, in the present study we could not detect a relation between the metabolic syndrome and the presence of coronary collaterals in patients referred for elective PTCA. Moreover, also no association was found between insulin resistance and coronary collaterals.

The presence of coronary collaterals can be regarded as a beneficial response given an equal level of coronary atherosclerosis. Our results were adjusted for the severity of coronary artery disease to account for the fact that repetitive myocardial ischemia is an important determinant for collateral development.

To our best knowledge this is the first clinical study examining the association between the metabolic syndrome (according to the ATP III criteria) and the presence of coronary collaterals. There are several studies with contradictory findings on coronary collateralisation in diabetic patients probably due to differences both in the used definition of coronary collateral formation and in adjustment for the severity of coronary artery disease. In their angiographic study Abaci et al. showed that diabetic patients developed a less extensive coronary collateral circulation compared to non-diabetic patients.[24] Endothelial dysfunction and blunted nitric oxide production, both associated with diabetes, were suggested to underlie this decreased collateralisation. A recently performed study found no difference in coronary collateral vessel formation between diabetic and non-diabetic patients using Rentrop's classification.[28]

In an insulin resistant state hyperinsulinemia is associated with endothelial dysfunction by the release of the potent vasoconstrictor endothelin. Also the increased production of cytokines, low-grade inflammation, defects in insulin signalling pathways, activation of the renin angiotensin system and increased oxidative stress, all associated with insulin resistance, could contribute to endothelial dysfunction.[30] However, we showed that, in patients referred for PTCA the metabolic syndrome and insulin resistance are not associated with impaired coronary collateral formation. This may be due to several reasons. Firstly, we studied patients with advanced coronary artery disease. These patients may already have an impaired endothelial function to such an extent that the influence of insulin resistance on endothelial function could be neglected. Despite the fact that patients with the metabolic syndrome have significantly higher plasma levels of hs-CRP (3.2 vs. 2.0 mg/L, p-value <0.001) and significantly lower plasma levels of adiponectin (4.1 mg/L vs. 5.3 mg/L, p-value 0.001) (hs-CRP positively[40] and adiponectin negatively[41-43] associated with endothelial dysfunction) compared to non-metabolic syndrome patients, we did not find a difference in coronary collateralisation. Secondly, vasoactive drugs, as Angiotensin Converting Enzyme (ACE) inhibitors, angiotensin receptor blockers (ARB) and statins could have positive effects on endothelial function.[44-46] Moreover, statin

use has been shown to be associated with enhanced collateralisation in patients with documented coronary artery disease.[47] Although use of lipid lowering agents was equally distributed in our study population, patients with the metabolic syndrome significantly more often use ACE inhibitors or ARB compared to patients without the metabolic syndrome (28% vs. 11%, p-value 0.001). This could have ameliorated the endothelial dysfunction in metabolic syndrome patients. However, in the present study we did not find a significant association between the use of ACE inhibitors or ARB and coronary collateralisation (data not shown).

Finally, the technique used to visualize coronary collaterals could only identify blood vessels which diameter exceeds 100 μm. With this technique, contrary to myocardial contrast echocardiography, intramural collaterals can also not be demonstrated so coronary collateral blood flow can only be semi-quantitatively assessed. It may be possible that patients with the metabolic syndrome have an impaired formation of collateral vessels with a diameter < 100 μm, or intramural situated collaterals. In addition to coronary angiography to determine coronary collateral development, several studies use intracoronary pressure and/or flow velocity assessments. Although this quantitative assessment of coronary collaterals is considered superior to the angiographic grading method used in this study,[48-50] a major limitation of this technique is that it can only be performed during angioplasty which restricts its applicability to a limited population. To investigate the influence of the metabolic syndrome on coronary collateral development in subjects without coronary artery disease non-invasive imaging techniques for coronary collateral assessment should be developed.

We conclude that there is no significant association between the metabolic syndrome or insulin resistance and the presence of coronary collaterals in patients with documented coronary artery disease.

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Chapter 7

The effects of low-dose simvastatin and ezetimibe compared with high-dose simvastatin on postprandial lipids and endothelial function in patients with the metabolic syndrome.

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ABSTRACT

Background and Aims Insulin resistance is associated with postprandial hyperlipidemia and endothelial dysfunction. Patients with the metabolic syndrome, characterized by insulin resistance, are at an increased risk of the development of cardiovascular disease. Inhibiting cholesterol absorption may influence postprandial lipid metabolism and may therefore have effects on postprandial endothelial function. Aim of the present study is to compare the effects of combination therapy of low-dose simvastatin and ezetimibe with high-dose simvastatin monotherapy on (postprandial) lipid profiles and endothelial function.

Methods Prospective, randomized, crossover, double blind trial in 19 male patients with the metabolic syndrome with high-dose simvastatin 80 mg versus combination therapy of low-dose simvastatin 10 mg with ezetimibe 10 mg. Endothelial function was ultrasonographically determined with flow-mediated dilation measurements.

Results With simvastatin monotherapy postprandial endothelial function was significantly decreased (6.9% versus 4.3%), contrary to combination therapy (7.6% versus 7.7%) despite a similar reduction in LDL-cholesterol. No differences were observed in fasting and postprandial lipid profiles between either treatment regimes.

Conclusion Combination therapy with low-dose simvastatin and ezetimibe preserved postprandial endothelial function, contrary to treatment with high-dose simvastatin monotherapy in metabolic syndrome patients, whereas no differences were observed in postprandial lipid profiles between either treatment regimes. By preserving postprandial endothelial function in the present study, combination therapy of ezetimibe with statins may additionally reduce the cardiovascular risk of the metabolic syndrome beyond LDL-cholesterol lowering.

Trial Registration Clinicaltrials.gov Identifier NCT00189085

INTRODUCTION

The metabolic syndrome is a cluster of several vascular risk factors associated with abdominal obesity (high blood pressure, hypertriglyceridemia, low HDL-cholesterol and high fasting glucose).[1] The underlying pathophysiology is still not fully clarified, but insulin resistance is a main characteristic of this syndrome.[2,3] Subjects with the metabolic syndrome are at increased risk for the development of cardiovascular morbidity and mortality and type 2 diabetes.[4-8] The metabolic syndrome is highly prevalent in patients with clinical manifestations of vascular diseases and is associated with advanced vascular damage in these patients.[9,10]

Insulin resistance is linked to endothelial dysfunction and decreased nitric oxide bioavailability by several mechanisms including, inflammation (as reflected by elevated high sensitive C Reactive Protein (hs-CRP) plasma levels), disruption of insulin receptor signalling cascades, increased production of cytokines and activation of the renin angiotensin system.[11,12] Adiponectin, an adipocyte-derived protein, stimulates the production of nitric oxide in vascular endothelial cells in vitro, and hypoadiponectinemia observed in insulin resistance is associated with endothelial dysfunction.[13-17] Endothelial function can clinically be assessed by measuring vasoreactivity of the brachial artery after an ischemic stimulus with flow-mediated dilation (FMD).[18,19]

Insulin resistance is associated with hyperlipidemia in the postprandial state.[20] Postprandial hyperlipidemia could be regarded as a cardiovascular risk factor, as indicated by the induction of postprandial endothelial dysfunction.[21-23] Chylomicron-remnants and very low density lipoprotein (VLDL) particles may impair endothelial dependent vasodilatation.[24] Statin therapy improves (postprandial) endothelial function but it is not known whether this is an indirect effect of lipid-lowering or a direct vascular effect of statins influencing the stability and bioavailability of NOS.[25-30] Inhibition of cholesterol absorption may influence postprandial lipid metabolism and may therefore have effects on postprandial endothelial function. Ezetimibe inhibits cholesterol absorption and direct vascular effects are unknown.[31] Aim of the present study is to compare the effects of the combination therapy of low-dose statin and ezetimibe with high-dose statin monotherapy on (postprandial) lipid profiles and endothelial function in male patients with the metabolic syndrome.

SUBJECTS AND METHODS

Subjects

Nineteen non-smoking obese male subjects, aged 18-70 years, were recruited by advertisement which called for subjects with waist circumference >102 cm. All subjects were screened for the presence of the metabolic syndrome according to the Adult Treatment Panel III criteria including three or more of the following metabolic abnormalities:[1]

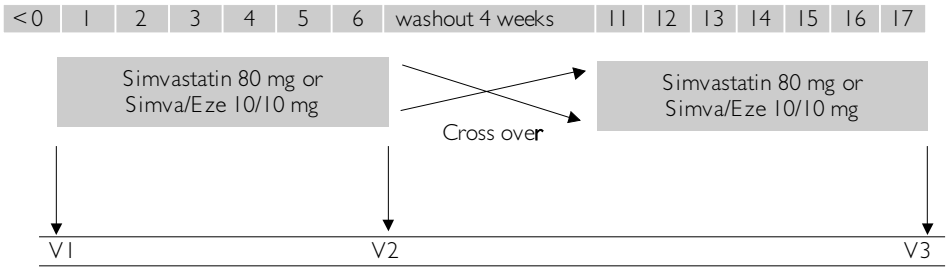
1. abdominal obesity (waist circumference > 102 cm)
2. high blood pressure (≥ 130 mmHg systolic or ≥ 85 mmHg diastolic)
3. hypertriglyceridemia (serum triglycerides ≥ 1.70 mmol/l (150 mg/dl))
4. low high-density lipoprotein (HDL) cholesterol
   (serum HDL-cholesterol < 1.04 mmol/l (40 mg/dl)
5. high fasting glucose (fasting serum glucose ≥ 6.1 mmol/l (110 mg/dl))

Glucose level ≥ 7.8 mmol/l after a standardized oral glucose tolerance test was also regarded as fulfilling the glucose criterion. Patients with thyroid- (Thyroid Stimulating Hormone (TSH) levels > 5 mU/l with clinical symptoms of hypothyroidism), hepatic- (serum aspartate transferase (ASAT) or serum alanine transferase (ALAT) > 2 times the upper limit of normal) or renal diseases (serum creatinine > 1.7 times the upper limit of normal) diseases were excluded. Other exclusion criteria were the presence of macrovascular disease, use of vasoactive medication (e.g. beta-blockers, calcium antagonists, ACE-inhibitors, angiotensin type 1 receptor blockers, statins, aspirin, non-steroidal inflammatory drugs), blood pressure ≥ 180/110 mmHg, Body Mass Index > 35, HbA1c > 6.5 % and plasma triglycerides > 8.0 mmol/l. The local Ethics Committee approved the study and all participants gave their written informed consent.

Study Design

In this prospective, randomized, crossover, double blind trial patients received once daily simvastatin 80 mg or the combination of simvastatin 10 mg and ezetimibe 10 mg (figure 1). Vascular function, as determined with flow-mediated dilation measurements, was performed after 6 weeks of treatment. Between the two treatment periods patients had a washout period of 4 weeks. Crossover of therapy occurred after washout followed by reassessment of vascular function after 6 weeks. FMD was measured before and 4 hours after an oral fat load during both treatment periods. At the beginning and at the end of each 6-week treatment period patients underwent physical examination (including height, body weight, waist circumference, body fat and blood pressure measurements) and blood sampling to determine laboratory parameters.

Figure 1 Cross-over study design



Simva: Simvastatin
Eze: Ezetimibe
V: Visit
Treatment periods lasted 6 weeks and were separated by a 4-weeks washout period
At visits 2 and 3 flow-mediated dilation measurements were performed before and after an oral fat load

METHODS

Assessment of vascular function with FMD

This is a non-invasive technique of assessing endothelial function by ultrasonography of the brachial artery. Measurements are made of the vasodilatory responses of the brachial artery to post-ischemic hyperaemia, causing endothelium-dependent dilation. The investigation was performed in a quiet, temperature-controlled room. After an overnight fast, all participants received an indwelling venous catheter in the left arm for blood withdrawal and were then asked to rest in a supine position for 1 hour before the start of the investigation.

All measurements were made with a Wall Track System (WTS) (Pie Medical, Maastricht, the Netherlands) which consists of a standard 7.5 MHz linear array transducer connected to a data acquisition system and a personal computer. In order to optimize quality of the ultrasound images of the arterial wall, ultrasound gel, a gel pad (as conductive medium) and a fixed probe holder were used. Three ECG leads were attached to the subject and a blood pressure cuff was placed 10 centimetres below the elbow of the right arm. The brachial artery in the right ante-cubital fossa was visualized using the transducer. When an optimal two-dimensional B-mode image of the brachial artery was obtained, an M-line perpendicular to the vessel was selected and the ultrasound system was switched to the M-mode. The end-diastolic vessel diameter was registered by the vessel-movement detector system repeatedly during 12 seconds. The first three measurements were averaged to provide a baseline arterial diameter. By inflation of the blood pressure cuff for 5 minutes above a pressure of 250 mmHg, ischemia was applied in the forearm distal to the location of the echo probe. Upon release of the cuff, the brachial artery will dilate through endothelial NO-release (endothelium-dependent vasodilatation). Ultrasonographic measurements were performed 4 times after cuff release at 15 seconds intervals and then 5 times after 30 seconds intervals. Maximal post-ischemic dilation was assessed by the widest lumen diameter. Then nitro-glycerine (0.4 mg) was administrated sublingually to determine endothelium-independent vasodilatation. WTS measurements are stored and analyzed off line by a blinded observer using WTS software analysis. FMD and nitro-glycerine-induced vasodilatation were expressed as percentage change relative to the baseline diameter.

Oral fat load

For the fat load, fresh cream was used which is a 40% (weight/volume) fat emulsion with a poly-unsaturated/saturated fat ratio of 0.10, containing 0.001% (w/v) cholesterol and 3% (w/v) carbohydrates representing a total energy content of 3700 kCal/l. Cream was ingested at a dose of 50 g fat and 3.75 g glucose per m^2 body surface (with a maximum of 250 ml) within 5 minutes. Participants remained supine during the day and were only allowed to drink water. Venous blood samples were obtained before and at 2, 3, 4, 5 and 8 hours after ingestion and were immediately put on ice. Plasma was isolated by centrifugation for 15 min at 3000 revolutions per minute at 4°C. Plasma samples were stored at -80°C for further analyses.

Laboratory assessment

Fasting blood was sampled to determine haemoglobin, leucocytes, thrombocytes, glucose, creatinine, creatinin kinase, TSH, homocysteine, ALAT, ASAT, HbA1c, and apoE genotyping. Total cholesterol, HDL-cholesterol, and LDL-cholesterol were analysed using commercially available assays (Wako, Osaka, Japan) using a Cobas Mira auto analyzer (Roche, Basel, Switzerland). VLDL-cholesterol was then calculated (VLDL-c = total cholesterol minus LDL-cholesterol minus HDL-cholesterol). Plasma triglycerides were analysed using an automated assay (Unimate, Roche diagnostics, Basel, Switzerland), even as plasma apolipoprotein B (Wako, Osaka, Japan). Plasma remnant-like particle cholesterol (RLP-c) was analyzed using a commercial available assay as extensively described previously.[32] Measurements of plasma adiponectin, interleukin-6 (IL-6) and hs-CRP levels were performed with a commercially available kit (ELISA; R&D Systems Inc.).

Anthropometric measurements

Weight and height were measured in patients without heavy clothing and shoes. Body mass index (BMI) was calculated as weight to height squared. Waist circumference was measured halfway between the lower rib and the iliac crest. Total body fat percentage was estimated by using Omron body fat monitor BF306 (Omron Matsusaka Co. LTD., Japan).

Statistical analyses

All values were expressed as mean ± standard deviation or as stated. FMD measurements and (postprandial) lipids were analyzed by an experienced observer blinded to all patients' characteristics and treatment. The postprandial variations of lipids were integrated as area under the curve (AUC) and were calculated by the trapezoidal rule using GraphPad Prism version 4.00 for Windows (GraphPad Software, San Diego California USA). Incremental integrated AUC (AUIC) was calculated after correction for baseline values. Differences in FMD and in AU(I)Cs for postprandial lipids between simvastatin 80 mg and combination therapy of simvastatin 10 mg with ezetimibe 10 mg were analyzed by paired t-test; statistical significance was taken at the 5% level. Carry-over and period effects were calculated with independent samples t-test.[33] Calculations were performed using SPSS for Windows version 12.1. (SPSS Inc. Chicago, IL, USA)

RESULTS

No carry-over effects or period effects between the two treatments periods were observed for FMD and postprandial lipid profiles. Demographic, clinical and laboratory characteristics of the 19 patients at baseline and after the 2 treatment periods are provided in table 1. Mean age was 54 ± 7 years. Weight, waist circumference and body fat remained stable during the study. Creatinin kinase was slightly elevated after treatment (116 ± 53 U/l versus 143 ± 67 U/l and 152 ± 134 U/l).

Fasting and post fat load lipid profiles and inflammatory parameter

Total cholesterol decreased from 5.6 ± 0.9 mmol/l to 3.7 ± 0.9 mmol/l during treatment with simvastatin 80 mg and to 3.8 ± 0.9 mmol/l during treatment with combination therapy. Plasma LDL-cholesterol concentration was similarly reduced by both treatment regimes (from 3.7 ± 0.7 mmol/l to 2.1 ± 0.5 mmol/l). In table 2 AUCs and AUICs for LDL-cholesterol, VLDL-cholesterol, RLP-cholesterol, triglycerides and apolipoprotein B were shown. With both kinds of treatment decreased AUICs for plasma levels of LDL-cholesterol, VLDL-cholesterol, and RLP-cholesterol were observed compared to the situation before treatment. No statistical significant differences were observed for the AUICs for postprandial lipids between either treatment regimes. In table 3 it was shown that before as well as 3, 4 and 8 hours after an oral fat load plasma concentrations of IL-6 were slightly higher during treatment with high-dose simvastatin monotherapy compared to treatment with low-dose simvastatin combined with ezetimibe.

Pre fat load and post fat load endothelial function

FMD was comparable during both treatment periods (6.9% versus 7.6%) (figure 2). However, during simvastatin monotherapy FMD significantly decreased after oral fat load (6.9% versus 4.3%, p-value 0.001), whereas no difference in FMD was observed after fat load during combination therapy (7.6% versus 7.7%, p-value 0.8). Nitro-glycerine-induced endothelium-independent vasodilatation of the brachial artery was comparable between the two treatment periods both at baseline, as well as after oral fat load.

Table 1 Clinical characteristics at baseline and during treatment

	Baseline[a] (n=19)	During simvastatin treatment (n=19)	During combination therapy (n=19)
Age (years)	54 ± 7	×	×
Height (m)	1.83 ± 0.6	×	×
Weight (kg)	100.3 ± 11.5	100.9 ± 11.2	100.3 ± 11.3
Body mass index (kg/m^2)	30.1 ± 2.7	30.2 ± 2.5	30.0 ± 2.6
Body fat (%)	31 ± 3	31 ± 3	30 ± 3
Laboratory parameters			
Haemoglobin (mmol/l)	9.9 ± 0.6	×	×
Haematocrite (l/l)	0.47 ± 0.03	×	×
Thrombocytes (exp^9/l)	201 ± 30	×	×
Leucocytes (exp^9/l)	4.7 ± 0.7	×	×
ASAT (U/l)	35 ± 6	37 ± 8	41 ± 15
ALAT (U/l)	45 ± 16	49 ± 17	49 ± 18
Creatinin kinase (U/l)	116 ± 53	143 ± 67	152 ± 134
Creatinine Clearance (Cockcroft) (ml/min)	77 ± 7	100 ± 17	104 ± 18
TSH (mIE/l)	1.7 ± 0.9	×	×
HbA1c (%)	5.7 ± 0.4	×	×
Total cholesterol (mmol/l)	5.6 ± 0.9	3.7 ± 0.9	3.8 ± 0.9
LDL-cholesterol (mmol/l)	3.7 ± 0.7	2.1 ± 0.5	2.1 ± 0.5
VLDL-cholesterol (mmol/l)	0.71 ± 0.25	0.46 ± 0.25	0.51 ± 0.33
Apolipoprotein B (g/l)	98 ± 16	67 ± 14	71 ± 18
homocysteine (µmol/l)	9.9 ± 1.7	9.4 ± 2.5	9.5 ± 2.5
Insulin (mU/l)	19 ± 9	17 ± 9.0	17 ± 7
HOMA-IR	5.1 ± 2.3	4.7 ± 2.4	4.6 ± 2.3
hs-CRP (mg/l)[b]	2.38 (1.8 – 4.1)	2.32 (1.8 – 4.2)	2.66 (1.5 – 5.1)
Interleukin-6 (pg/ml)	1.36 ± 0.51	1.89 ± 1.39	1.58 ± 0.74
Adiponectin (mg/l)	4.8 ± 2.4	4.8 ± 2.0	4.6 ± 2.2
ApoE genotyping			
E2/E3 % (n)	16 (3)	×	×
E3/E3 % (n)	63 (12)	×	×
E3/E4 % (n)	21 (4)	×	×
Components of the metabolic syndrome			
Triglycerides (mmol/l)	1.62 ± 0.29	1.26 ± 0.55	1.44 ± 0.56
HDL-cholesterol (mmol/l)	1.14 ± 0.26	1.14 ± 0.31	1.12 ± 0.26
Glucose (mmol/l)	6.2 ± 0.7	6.1 ± 0.6	6.1 ± 0.8
Waist circumference (cm)	110.6 ± 6.8	110.8 ± 5.5	110.4 ± 7.0
Systolic blood pressure (mmHg)	138 ± 13	135 ± 16	132 ± 8
Diastolic blood pressure (mmHg)	89 ± 6	87 ± 8	87 ± 4

[a] Values at screening or at baseline visit

All data: mean ± standard deviation or as indicated: [b]median with interquartiles range

HOMA-IR: Homeostasis model assessment determined insulin resistance (fasting serum glucose x fasting serum insulin)/22.5

Table 2 Postprandial lipids (area under the curve, baseline not corrected (AUC) and baseline corrected (AUIC))

	Before treatment	After 6 weeks simva 80 mg	After 6 weeks simva/eze 10/10 mg	p-value*
AUC				
LDL-c[a] (mmol h/l)	29.4 ± 1.3	16.3 ± 0.9	16.7 ± 0.9	0.9
VLDL-c[a] (mmol h/l)	7.4 ± 0.6	4.5 ± 0.4	5.3 ± 0.7	0.2
RLP-c[b] (mmol h/l)	268 ± 27	139 ± 11	135 ± 9	0.5
Triglycerides[a] (mmol h/l)	17.2 ± 0.9	14.3 ± 1.1	17.2 ± 1.6	0.1
ApoB[a] (g h/l)	783 ± 31	530 ± 23	568 ± 35	0.4
AUIC				
LDL-c[a] (mmol h/l)	1.2 ± 0.2	0.6 ± 0.1	0.8 ± 0.1	0.5
VLDL-c[a] (mmol h/l)	1.8 ± 0.3	0.9 ± 0.1	1.3 ± 0.2	0.3
RLP-c[b] (mmol h/l)	52 ± 7	31 ± 4	31 ± 3	0.6
Triglycerides[a] (mmol h/l)	4.3 ± 0.4	4.5 ± 0.5	5.8 ± 0.7	0.1
ApoB[a] (g h/l)	23 ± 4	22 ± 6	20 ± 3	0.9

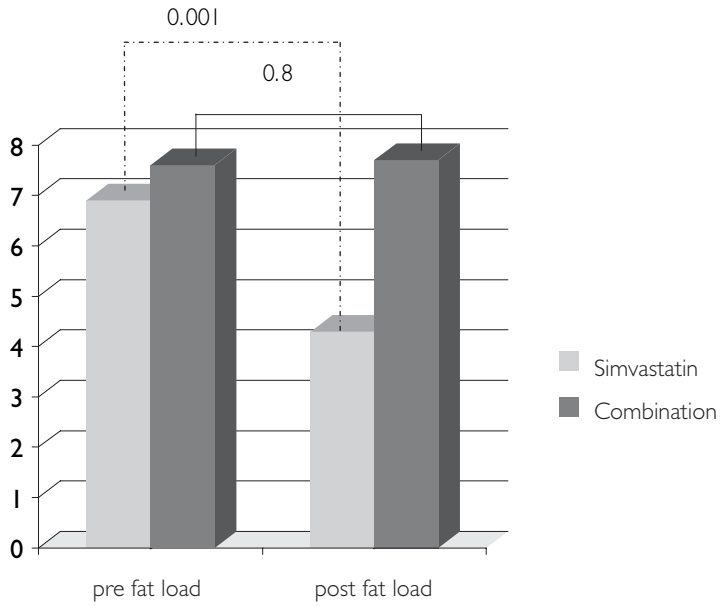
All data: mean ± se
simva: simvastatin
eze: ezetimibe
[a] measurements before and 2, 3, 4, 5 and 8 hours after oral fat load
[b] measurements before and 3, 4 and 8 hours after oral fat load
* p-value for differences between treatment with simva 80 mg and simva/eze 10/10 mg
RLP-c: Plasma remnant-like particle cholesterol

Table 3 Fasting and postprandial Interleukin-6 plasma levels

	Before treatment	After 6 weeks simva 80 mg	After 6 weeks simva/eze 10/10 mg
Interleukin-6 (pg/ml)			
fasting	1.36 ± 0.12	1.89 ± 0.32	1.58 ± 0.17
3 hours after fat load	2.34 ± 0.30	3.04 ± 0.70	2.52± 0.45
4 hours after fat load	2.96 ± 0.44	3.41 ± 0.67	2.87 ± 0.44
8 hours after fat load	4.37 ± 0.69	3.79 ± 0.46	3.74 ± 0.49

All data: mean ± se
simva: simvastatin
eze: ezetimibe

Figure 2 (Postprandial) endothelial function according to both treatment regimes



	Simvastatin	Combination therapy
Baseline		
Baseline diameter (mm)[a]	5.54 ± 0.15	5.30 ± 0.18
Post-ischemic maximum diameter (mm)[a]	5.91 ± 0.16	5.71 ± 0.19
Nitro-glycerine-induced maximum diameter (mm)[a]	6.22 ± 0.17	5.97 ± 0.15
Nitro-glycerine-induced vasodilatation	12.1 ± 1.1	13.4 ± 1.9
After oral fat load		
Baseline diameter (mm)[a]	5.65 ± 0.17	5.37 ± 0.13
Post-ischemic diameter (mm)[a]	5.88 ± 0.16	5.76 ± 0.12
Nitro-glycerine- induced maximum diameter (mm)[a]	6.20 ± 0.18	6.05 ± 0.12
Nitro-glycerine-induced vasodilatation	10.0 ± 1.4	13.1 ± 1.1

Data in percentages, or as indicated: [a] mean ± se

DISCUSSION

In the present randomized, double blind, crossover trial in male patients with the metabolic syndrome combination therapy of low-dose simvastatin and ezetimibe preserved postprandial endothelial function contrary to high dose simvastatin monotherapy, whereas the same reduction in (fasting) plasma LDL-cholesterol was obtained after 6 weeks treatment. No significant differences in postprandial lipid profiles were observed with either treatment regimes.

The metabolic syndrome confers a two to threefold increased risk for the development of cardiovascular morbidity and mortality.[4-6] This increased risk could at least be explained by the individual risk factors comprising the metabolic syndrome. However, also other not routinely measured risk factors associated with insulin resistance as decreased fibrinolysis, increased amount of small dense LDL-cholesterol, hypercoagulability, inflammation, hyperinsulinemia and endothelial dysfunction could contribute to the observed increased cardiovascular risk.[34-36] Insulin resistance is associated with postprandial hyperlipidemia.[20] Since endothelial dysfunction can be regarded as a surrogate endpoint for cardiovascular morbidity, blunted (postprandial) endothelial function could contribute to the increased cardiovascular risk in the metabolic syndrome. On top of lowering plasma LDL-cholesterol, preserving postprandial endothelial function by the combination therapy of low-dose simvastatin with ezetimibe may confer additional cardiovascular risk reduction in patients with the metabolic syndrome.

Ezetimibe decreases LDL-cholesterol by inhibition of uptake of dietary and biliary cholesterol.[31] Ezetimibe binds to the Niemann-Pick C1 Like 1 protein at the brush border membrane of enterocytes, a receptor involved in intestinal cholesterol uptake, thereby preventing dietary cholestol uptake.[37] We initially hypothesized that postprandial lipid metabolism would benefit from treatment with a cholesterol uptake inhibitor like ezetimibe in combination with simvastatin compared to high-dose simvastatin. However, in the present study we did not observe differences in postprandial lipid profiles during both treatment regimes. In a recent report, no significant effects of ezetimibe on the postprandial kinetics of intestinally derived apoB 48-containing triglyceride-rich lipoprotein particles were observed. It was shown that ezetimibe treatment led to a reduction of plasma LDL-cholesterol by increasing the catabolism of hepatic derived apoB-100 containing lipoproteins without reducing chylomicron particle number.[38]

Post fat load inflammation may be influenced differently by both treatments resulting in differential effects on post fat load endothelial function in our study. Low-grade inflammation (reflected by elevated concentrations of CRP) is associated with endothelial dysfunction[12] and it was previously shown that at each statin dose level, coadministration of ezetimibe induced significantly more hs-CRP reduction compared to monotherapy, not explained by changes in fasting lipids.[39,40] In the present study plasma levels of IL-6 were marginally higher during treatment with high-dose simvastatin monotherapy compared to treatment with low-dose simvastin combined with ezetimibe, also at

the time of assesment of endothelial function. Since hepatic CRP release is under influence of plasma levels of IL-6, increased levels of hs-CRP during treatment with simvastatin 80 mg monotherapy can also be expected. [41]

In patients with chronic heart failure it has recently been shown, that 4 weeks of treatment with simvastatin10 mg improved endothelial function contrary to treatment with ezetimibe 10 mg monotherapy, despite a similar decrease in plasma LDL-cholesterol concentration.[42] It was suggested that pleiotropic, LDL-cholesterol-independent effects of statins were involved (i.e. increased vascular nitric oxide bioavailability, reduced oxidant stress, improved endothelial progenitor cell function). Positive effects of LDL-cholesterol lowering on endothelial function have already been described in various studies, as well as endothelial dysfunction after an oral fat load.[21-23,43-45] In the present study we did not observe a difference in pre fat load endothelial function between treatment with simvastatin 80 mg and simvastatin 10 mg combined with ezetimibe 10 mg. We therefore could not confirm the existence of pleiotropic effects of high-dose statins in our study cohort. In addition, combination therapy of low-dose simvastatin with ezetimibe preserved endothelial function after an oral fat load, contrary to high dose simvastatin monotherapy. Though, the existence of pleiotropic effects of statins can not completely be ruled out by these findings, since it is possible that beyond 10 mg simvastatin the maximal pleiotropic effects are already reached. The effects of different lipid-lowering regimes on endothelial function in patients with the metabolic syndrome have been investigated in an open-label non randomized comparison.[46] In a small number of patients combination therapy of atorvastatin 40 mg and ezetimibe 10 mg resulted in more reduction in serum total cholesterol and triglycerides concentrations and better endothelial function compared to atorvastatin 40 mg alone. In contrast to our study, plasma LDL-cholesterol reductions were not equal which could contribute to the differences in endothelial function. Besides this, lipid profiles and endothelial function were not assessed postprandial.

We acknowledge some limitations of our study. We only included male patients with the metabolic syndrome. Therefore, caution should be taken to generalize these results to female patients. Carry-over and cross-over effects were not observed and are therefore unlikely to have influenced our results but could not be completely ruled out. Considering the elimination half-life of statins and ezetimibe and a washout period of 4 weeks plus 6 weeks treatment carry-over effects are unlikely to have occurred. To assess postprandial endothelial function and lipid profiles a standardized, but non-physiological, high fat meal was used.

In conclusion, in male patients with the metabolic syndrome, 6 weeks treatment of low-dose simvastatin combined with ezetimibe preserved postprandial endothelial function contrary to high-dose simvastatin monotherapy, whereas no differences were observed in postprandial lipid profiles between either treatment regimes. By preserving postprandial endothelial function in the present study, combination therapy of ezetimibe with statins may additionally reduce the cardiovascular risk of the metabolic syndrome beyond LDL-cholesterol lowering.

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Chapter 8

Defining the metabolic syndrome: resolving unresolved issues?

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ABSTRACT

Objectives Several definitions exist for the metabolic syndrome. In concert with the blood pressure- and glucose criteria of the NCEP definition, it is now suggested to incorporate the use of fibrates and nicotinic acid in the dyslipidemia criteria. However, statins are the most widely prescribed drugs to lower LDL-cholesterol, but also affect triglycerides and HDL-cholesterol levels. Aims of the present study were to investigate the influence of allowing for lipid-lowering therapy on the prevalence of the NCEP-defined metabolic syndrome and to compare the characteristics of identified patients according to the newly proposed IDF definition with the NCEP definition.

Design Cross-sectional study

Setting University Medical Centre Utrecht

Subjects 2373 patients with manifest vascular disease

Interventions Allowing for the influence of lipid-lowering therapy took place by modifying the NCEP definition (NCEP-rev1: use of lipid-lowering agents qualified for the hypertriglyceridemia criterion; NCEP-rev2: triglycerides and HDL-cholesterol concentrations were recalculated for lipid-lowering agents).

Results Prevalence of the metabolic syndrome was 41% (NCEP definition), 50% (NCEP-rev1), 44% (NCEP-rev2) and 52% according to the IDF definition. Patients only identified with the NCEP definition showed lower HDL-cholesterol, higher triglycerides and higher fasting glucose levels compared with patients only diagnosed with the IDF definition.

Conclusions Allowing for the use of lipid-lowering drugs in the NCEP-definition may identify an additional group of patients at elevated risk for cardiovascular diseases and diabetes. The NCEP definition of metabolic syndrome identifies patients with a worse cardiovascular risk profile compared to patients qualifying for the metabolic syndrome with the IDF definition in a cohort of patients with clinical manifestations of vascular disease.

INTRODUCTION

The metabolic syndrome is a cluster of cardiovascular risk factors associated with abdominal obesity: dyslipidemia, elevated blood pressure and hyperglycaemia.[1] Patients with the metabolic syndrome have a 2-3 fold increased risk for the development of type 2 diabetes and cardiovascular morbidity and mortality.[2-5] In nearly 20-25% of apparently healthy individuals and 45% of patients with clinical manifestations of atherosclerosis the metabolic syndrome is present.[6,7] In the coming years the prevalence will increase due to an increased prevalence of overweight and obesity. According to current guidelines for cardiovascular risk management the presence of the metabolic syndrome may give direction to treatment.[8] Identification of the metabolic syndrome not only has (therapeutic) consequences for the individual patient but can also have major effects for public health care in terms of (future) costs and use of health care resources. Therefore it is of great importance to have a diagnosis based on well established diagnostic criteria that allows reliable estimation of risk.

Presently there are, as a result of different visions regarding pathophysiology and practical clinical use, various definitions for metabolic syndrome based on different criteria (table 1). Very recently, a new definition for the metabolic syndrome was introduced by the International Diabetes Federation, based on the obligatory presence of an increased waist circumference.[9] Regarding these different definitions several issues need to be clarified. In the WHO and original NCEP definitions the use of blood pressure- and glucose-lowering agents was already considered as fulfilling the criteria for high blood pressure and glucose, respectively. Only in the most recent issued definitions by NCEP[10] and IDF, the use of specific treatment (as fibrates and nicotinic acid) for elevated triglycerides and low HDL-cholesterol levels were taken into account. However, statins are the most widely prescribed drugs for cardiovascular risk reduction because of their LDL-cholesterol lowering properties, but statins also affect plasma triglycerides (-15%) and HDL-cholesterol (+ 5%).[11,12] Therefore, in patients on statin therapy it would be reasonable to take these effects into account in judging whether an individual qualifies for the dyslipidemia criteria of the metabolic syndrome.

Table 1 Definitions of the metabolic syndrome

NCEP (ATP III) 2001	IDF 2005
Combination of 3 or more of the following parameters:	Central obesity # + 2 of the following:
1. Fasting glucose ≥ 6.1 mmol/l and/or medication	1. Fasting glucose ≥ 5.6 mmol/l and/or medication
2. Waist > 102 cm (♂), > 88 cm (♀)	2. Triglycerides ≥ 1.7 mmol/l or specific treatment
3. Triglycerides ≥ 1.7 mmol/l	3. HDL < 1.03 mmol/l (♂), < 1.29 mmol/l (♀) or specific treatment
4. HDL < 1.03 mmol/l (♂), < 1.29 mmol/l (♀)	4. Blood pressure ≥ 130/85 mmHg and/or medication
5. Blood pressure ≥ 130/85 mmHg and/or medication	

Waist > 94 cm (♂), > 80 cm (♀)

Studies investigating the prevalence and cardiovascular risk profile of identified patients with the IDF definition compared to patients diagnosed by the widely used NCEP definition are hardly available in patients with manifest vascular disease. Aims of the present study were to (1) investigate the influence of allowing for lipid-lowering therapy on the prevalence of the NCEP-defined metabolic syndrome and (2) to compare the prevalence and characteristics of identified patients according to the newly proposed IDF definition with the NCEP definition for metabolic syndrome in a cohort of patients with clinical manifestations of vascular disease.

MATERIALS AND METHODS

The prevalence estimates of the metabolic syndrome were calculated in patients originating from the SMART study (Second Manifestations of ARTerial disease), an ongoing prospective cohort study at the University Medical Centre Utrecht designed to establish the prevalence of concomitant arterial diseases and risk factors for atherosclerosis in a high risk population.[13] Included were 2373 patients with (a history of) manifest vascular disease (coronary heart disease, TIA or ischemic stroke, peripheral arterial disease or abdominal aortic aneurysm).

In the present cohort, the distribution of classes of lipid-lowering drugs among patients treated with lipid-lowering agents (50 %) was as follows: 99% statins, 2% fibrates, 0.5% bile acid binding agents and/or 0.4% cholesterol absorption inhibitor. We allowed for the influence of lipid-lowering therapy by modifying the NCEP definition in two different ways. In the first revised NCEP-definition (NCEP-rev1), the use of lipid-lowering agents qualified for the hypertriglyceridemia criterion. In the second revised NCEP definition (NCEP-rev2) triglycerides and HDL-cholesterol plasma concentrations were recalculated if lipid-lowering agents were used; triglycerides were computed by multiplying with 1.18 (100/85), HDL-cholesterol by multiplying with 0.95 (100/105). Subsequently, the same cut-off values of the traditional NCEP definition were applied.

The SMART study is based on a research protocol that is consistent with the principles of the Declaration of Helsinki. The local Ethics Committee approved the study and all participants gave their written informed consent.

RESULTS

According to the currently used NCEP definition the metabolic syndrome was present in 41% of the study population, whereas this prevalence was 50% according to NCEP-rev1 definition and 44% according to NCEP-rev2 definition (table 2). The characteristics of the metabolic syndrome patients identified with NCEP, NCEP-rev1 and NCEP-rev2 were comparable with respect to age, gender and cardiovascular risk profile. Incorporating the use of lipid-lowering agents in the triglycerides criterion (NCEP-rev1) revealed 214 more patients (22%) with the metabolic syndrome, whereas recalculation of triglycerides and HDL-cholesterol (NCEP-rev2) diagnosed only 64 patients more (7%).

With the IDF definition, 52% of the patients in our cohort were diagnosed with metabolic syndrome (table 3). Remarkably, from the 974 patients diagnosed according to the NCEP definition, only 838 were also diagnosed using the IDF definition. An additional 402 patients were diagnosed according to the IDF definition but not by the NCEP definition. As expected, these patients (NCEP-/IDF+) had lower glucose plasma levels since the cuff-of value according to this IDF definition had been strengthened. Besides this, these patients showed higher HDL-cholesterol and lower triglycerides concentrations compared with patients diagnosed with the NCEP definition but not by the IDF definition (NCEP+/IDF-). Due to the obligatory presence of abdominal obesity, waist circumference and BMI were higher in these patients.

Table 2 Prevalence and characteristics of metabolic syndrome patients according to NCEP and revised definitions

Metabolic syndrome (n)	NCEP 974 (41%)	NCEP-rev1 1188 (50%)	NCEP-rev2 1038 (44%)
Male Gender	72	73	72
Age (years)[a]	60±10	60±10	60±10
Smoking[*]	32	31	31
Body mass index (kg/m^2)[a]	29±4	29±4	29±4
Waist circumference (cm)[a]	102±11	101±11	101±11
Cholesterol (mmol/l)[b]	5.3 (4.5-6.0)	5.1 (4.4-5.9)	5.2 (4.5-6.0)
LDL-Cholesterol (mmol/l)[b]	3.2 (2.5-3.9)	3.1 (2.5-3.8)	3.2 (2.5-3.9)
HDL-Cholesterol (mmol/l)[b]	1.01 (0.87-1.20)	1.03 (0.88-1.27)	1.02 (0.87-1.21)
Triglycerides (mmol/l)[b]	2.1 (1.7-2.8)	1.9 (1.4-2.6)	2.0 (1.6-2.8)
Fasting serum glucose (mmol/l)[b]	6.3 (5.7-7.8)	6.3 (5.6-7.6)	6.3 (5.7-7.8)
Blood pressure systolic (mmHg)[a]	145±21	145±21	145±21
Blood pressure diastolic (mmHg)[a]	83±11	83±11	83±11
Fasting serum glucose≥ 7.0 mmol/l[‡]	16	14	15
Glucose lowering agents	24	23	23
Blood pressure lowering agents	53	54	54
Lipid lowering agents	50	59	53

NCEP-rev1 definition: NCEP-defined metabolic syndrome with patients on lipid-lowering agents regarded as having elevated triglycerides
NCEP-rev2 definition: NCEP-defined metabolic syndrome with in patients on lipid-lowering agents triglycerides multiplied by 100/85 and HDL-cholesterol multiplied by 100/105
All data in percentages, or as indicated: [a]mean ± standard deviation or [b]median with interquartiles range
[*] Still smoking or recently stopped smoking
[‡] Patients on glucose lowering agents excluded from analyses

Table 3 Prevalence and characteristics of metabolic syndrome patients according to NCEP and/or IDF definition

Metabolic syndrome n (%)	NCEP 974 (41%)	IDF 1240 (52%)	NCEP-/IDF+ 402 (17%)	NCEP+/IDF- 136 (6%)	NCEP+/IDF+ 838 (35%)
Male Gender	72	73	82	88	69
Age (years)[a]	60±10	60±10	61±10	58±10	60±10
Smoking[*]	32	29	25	42	31
Body mass index (kg/m^2)[a]	29±4	29±4	27±2	24±2	29±4
Waist circumference (cm)[a]	102±11	102±9	97±7	88±6	104±10
Cholesterol (mmol/l)[b]	5.3 (4.5-6.0)	5.2 (4.5-6.0)	5.1 (4.5-5.8)	5.1 (4.5-5.7)	5.3 (4.5-6.1)
LDL-Cholesterol (mmol/l)[b]	3.2 (2.5-3.9)	3.2 (2.6-3.9)	3.1 (2.6-3.8)	3.2 (2.5-3.8)	3.2 (2.5-3.9)
HDL-Cholesterol (mmol/l)[b]	1.01 (0.87-1.20)	1.09 (0.91-1.30)	1.23 (1.05-1.44)	0.92 (0.80-1.03)	1.02 (0.88-1.21)
Triglycerides (mmol/l)[b]	2.1 (1.7-2.8)	1.9 (1.4-2.6)	1.5 (1.1-2.0)	2.2 (1.8-2.7)	2.1 (1.6-2.8)
Fasting serum glucose (mmol/l)[b]	6.3 (5.7-7.8)	6.1 (5.6-7.2)	5.7 (5.4-6.0)	6.3 (5.6-7.4)	6.3 (5.7-7.8)
Blood pressure systolic (mmHg)[a]	145±21	145±22	144±23	143±23	145±21
Blood pressure diastolic (mmHg)[a]	83±11	83±11	83±12	82±11	83±11
Fasting serum glucose≥ 7.0 mmol/l[‡]	16	12	4	13	16
Glucose lowering agents	24	19	9	23	24
Blood pressure lowering agents	53	51	46	52	54
Lipid lowering agents	50	51	55	50	50

All data in percentages, or as indicated: [a]mean ± standard deviation or [b]median with interquartiles range
[*] Still smoking or recently stopped smoking
[‡] Patients on glucose lowering agents excluded from analyses

DISCUSSION

The present study shows definition-dependent differences in the prevalence of the metabolic syndrome and differences in cardiovascular risk profile of identified patients. Given the effects of statin therapy on plasma triglycerides and HDL-cholesterol concentrations, it seems plausible to allow for these effects when judging if a patient on lipid-lowering therapy qualifies for the HDL-cholesterol or elevated triglycerides criteria of the metabolic syndrome. Recalculation of the triglycerides and HDL-cholesterol concentrations in patients on lipid-lowering therapy would be the most elegant, but also the most laborious method. We do not feel that this additional effort is feasible in clinical practice and does not counterbalance the marginal yield of additionally diagnosed metabolic syndrome patients (7%) with the same cardiovascular risk profile. The other method, in which the use of lipid-lowering therapy qualified for the triglycerides criterion of the metabolic syndrome, yielded 214 (22%) more patients. Patients identified with metabolic syndrome by this revised definition show similar baseline characteristics and cardiovascular risk factors as those patients diagnosed with the currently used NCEP definition. Now it is clear that patients on lipid-lowering therapy with the metabolic syndrome, identified with the NCEP-rev1 definition, are identical in terms of cardiovascular risk profile, it is plausible that they are at similar elevated risk. Of course, the best way to compare the actual cardiovascular risk in both groups would be with a follow-up study design. But the influence of lipid-lowering therapy on both the prevalence of the metabolic syndrome as well on the cardiovascular prognosis would hinder this analysis.[11,14]

With the IDF definition 52% (1240 patients) of the population under study was diagnosed with the metabolic syndrome compared with 41% (974 patients) defined with the NCEP definition. Strikingly, 402 patients were classified with the IDF definition, but not with the NCEP definition (and 136 patients vice versa). Patients diagnosed with the IDF definition but not with the NCEP definition showed a higher waist circumference and BMI, but had a more favourable lipid profile and lower plasma glucose levels compared with patients classified with the NCEP definition but not with the IDF definition. This indicates that metabolic syndrome diagnosed by the IDF criteria, identifies patients at lower cardiovascular risk compared with the NCEP criteria. In concert with current knowledge of the pathophysiological pathways underlying the metabolic syndrome, the IDF attaches a great value to abdominal obesity by the obligation of the presence of an increased waist circumference in their definition. However, this probably resulted in the identification of obese patients not (yet) being insulin resistant or not suffering from metabolic consequences due to insulin resistance. More than 95% of metabolic syndrome patients identified by the NCEP definition are insulin resistant as measured by the gold standard of hyperinsulinemic-euglycaemic clamping.[15] It has been shown that there is no difference in diabetes prediction between the IDF and NCEP metabolic syndrome definitions.[16] Studies in white men and in healthy women showed that both the NCEP definition as well as the IDF definition of the metabolic syndrome significantly predicted cardiovascular disease, although the hazard ratio's were marginally higher for the NCEP definition compared to the IDF definition.[17,18] However, in patients referred for coronary angiography NCEP defined metabolic

syndrome but not IDF defined metabolic syndrome predicted clinical cardiovascular events.[19] In the present study in patients with different manifestations of vascular disease (coronary heart disease, TIA or ischemic stroke, peripheral arterial disease or abdominal aortic aneurysm) patients with different cardiovascular risk profiles were identified by both definitions. Future follow-up studies are warranted to determine whether the IDF definition or the NCEP definition of metabolic syndrome better predicts increased risk for development of cardiovascular complications.

Although suggested in the IDF definition, we did not incorporate the use of specific treatment for elevated triglycerides levels or low HDL-cholesterol levels in our analyses for 2 reasons; firstly, a well described definition of specific treatment is lacking and secondly, presuming that fibrates and bile acid binding agents were mentioned, statins are with 99% the most used lipid-lowering drugs in our study cohort.

In conclusion, in a cohort of patients with clinical manifest vascular disease the prevalence of the metabolic syndrome varies depending on the definition used. Allowing for the use of lipid-lowering drugs may identify an additional group of patients at increased cardiovascular risk and at elevated risk for developing type 2 diabetes mellitus. Although the newly proposed IDF definition elegantly pays attention to the elementary role of abdominal obesity in the development of the metabolic syndrome, this results in the identification of patients with a more favourable cardiovascular risk profile compared to NCEP-defined metabolic syndrome patients.

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Chapter 9

Summary and Conclusions

General Discussion

Appendix

SUMMARY AND CONCLUSIONS

In the Western population cardiovascular diseases are the most common cause of mortality and morbidity. There are several important risk factors for cardiovascular diseases, among them hypertension, hypercholesterolemia, diabetes and obesity. The clustering of cardiovascular risk factors associated with central obesity is often referred to as the metabolic syndrome. Due to different visions regarding the definitions, the underlying pathophysiology and the clinical usefulness, the metabolic syndrome attracts much attention of clinical research and clinicians.

It has been shown that in apparently healthy individuals the prevalence of the metabolic syndrome is 20-25%, and the coming years the prevalence is expected to increase due to an increased prevalence of obesity. In addition, several cross-sectional and prospective studies demonstrated a 2 to 3 fold increased risk for the development of cardiovascular disease and type 2 diabetes in patients with the metabolic syndrome. This implies, despite critical concerns regarding the clinical relevance of metabolic syndrome, the existence of a huge amount of (asymptomatic) subjects at elevated risk for cardiovascular events and type 2 diabetes. Patients with clinical manifestations of arterial atherosclerosis (as peripheral arterial disease, cerebrovascular disease, coronary artery disease and abdominal aneurysm) are at elevated risk for recurrent vascular events. It is not known whether the presence of the metabolic syndrome puts patients with already manifest atherosclerotic disease at even higher cardiovascular risk. In **chapter 2** we demonstrated that in 46% of the patients with manifest vascular diseases the metabolic syndrome is prevalent. From **chapter 5** can be concluded that in patients with already clinical manifestations of a vascular disease, the metabolic syndrome is associated with even more vascular damage as measured by indicators for increased (cardio)vascular risk. Patients with the metabolic syndrome had an increased mean Intima Media Thickness (IMT) (0.98 mm vs. 0.92 mm) and an increased prevalence of albuminuria (20% vs. 15%) compared to non-metabolic syndrome patients. A trend, albeit non significant, toward a decreased Ankle Brachial Pressure Index (ABPI) in patients with the metabolic syndrome was found.

The underlying pathophysiology of the metabolic syndrome is not fully understood, but insulin resistance plays a fundamental role in the development and maintenance of the metabolic syndrome. However, also abdominal obesity, inflammation, endothelial dysfunction and an imbalanced autonomic nerve system have been suggested to be involved whereby cause and consequence are often not easily to distinguish. Genetic susceptibility for the development of the metabolic syndrome seems to contribute as well. By the production of several substances (adipokines), among them adiponectin, adipose tissue possesses important endocrinological functions. Insulin resistance is driven by the changed production of adipokines, observed in adipocyte dysfunction. Adiponectin is present at high concentrations in the human circulation and although it is adipose tissue-derived, plasma concentrations are higher in lean compared to obese subjects. Low plasma adiponectin levels have prospectively been shown to be associated with coronary artery disease. In **chapter 3** we showed that in patients with coronary artery disease the NCEP-defined metabolic syndrome is significantly

associated with decreased adiponectin levels. We also demonstrated that insulin resistance and obesity, both essentially involved in the pathogenesis of the metabolic syndrome, are associated with declining adiponectin concentrations. So it could be postulated that low plasma adiponectin levels, caused by adipocyte-dysfunction, may contribute to both the development of the metabolic syndrome as well as to the increased cardiovascular risk associated with the metabolic syndrome. In **chapter 4** we demonstrated a positive association between body iron stores, as reflected by ferritin levels, and the metabolic syndrome in patients with manifest vascular disease. In addition, we showed a significant inverse relation between ferritin and adiponectin. The mechanistic link between excessive body iron and insulin resistance is not elucidated yet. Since adiponectin production is decreased in adipocyte dysfunction, it could be suggested that iron is involved in the development of insulin resistance by disturbing adipocyte function.

Several studies investigated whether the increased cardiovascular risk of the metabolic syndrome is higher than expected from its individual components. Due to conflicting results this is still a topic of debate. If the observed risk indeed exceeds the sum of risk caused by the individual components, this could be explained by other not routinely measured aspects of the metabolic syndrome associated with insulin resistance as impaired fibrinolysis, oxidative stress, increased amount of small dense LDL-cholesterol, hypercoagulability, inflammation, hyperinsulinemia and decreased adiponectin levels. Impaired coronary collateral formation could be among them, since well developed coronary collaterals are associated with improved cardiovascular outcome in terms of limiting myocardial infarction size, prevention of ventricular aneurysm formation and future ischemic events in patients with coronary artery disease. In **chapter 6** we examined whether the metabolic syndrome is associated with impaired coronary collateral vessel formation in patients with documented coronary artery disease. We were not able to demonstrate a significant (inverse) relationship between the metabolic syndrome and coronary collateral formation, suggesting that impaired collateral formation does not contribute to the increased cardiovascular risk in patients with coronary artery disease.

Criticism of the metabolic syndrome is partially caused by conflicting visions regarding the kind of treatment. Studies investigating the effects of treatment of the individual risk factors versus treatment of the (main) underlying disorder, insulin resistance, on clinical relevant outcomes are lacking. But, from a pathophysiological point of view it could be suggested that by improving insulin sensitivity both the individual components as well as the not routinely measured risk factors associated with insulin resistance improve and thereby decreasing the risk for cardiovascular complications and the development of type 2 diabetes. It has recently been suggested in a report of the NCEP (National Cholesterol Education program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults) that in patients with manifest vascular disease and the metabolic syndrome the target for treatment of LDL-cholesterol could further be lowered from 2.6 to below 1.8 mmol/l. This could be achieved by treatment with high-dose simvastatin or with low-dose simvastatin combined with ezetimibe, a cholesterol absorption inhibitor.

Insulin resistance is associated with hyperlipidemia in the postprandial state. Postprandial hyperlipidemia could be regarded as a cardiovascular risk factor, as indicated by postprandial endothelial dysfunction. Inhibiting cholesterol absorption may influence postprandial lipid metabolism and may therefore have effects on postprandial endothelial function. In **chapter 7** we compared the effects of low-dose simvastatin combined with ezetimibe versus high-dose simvastatin monotherapy on postprandial endothelial function in male patients with the metabolic syndrome. From this study it can be concluded that combination therapy of low-dose simvastatin with ezetimibe preserved postprandial endothelial function contrary to high-dose simvastatin monotherapy, whereas no differences were observed in postprandial lipid profiles between either treatment regimes. By preserving postprandial endothelial function in the present study, combination therapy of ezetimibe with statins may additionally reduce the cardiovascular risk of the metabolic syndrome beyond LDL-cholesterol lowering.

Due to different visions regarding pathophysiology and practical clinical use, various definitions for the metabolic syndrome based on different criteria are presently used. Among them the definition proposed by the World Health Organisation (WHO), the NCEP-definition and the definition of the International Diabetes Federation (IDF). In concert with the blood pressure- and glucose criteria of the NCEP and WHO definition, it is now suggested to incorporate the use of specific treatment (fibrates and nicotinic acid) in the dyslipidemia criteria. However, statins are the most widely prescribed drugs to lower LDL-cholesterol and cardiovascular risk, but statins also affect triglycerides and HDL-cholesterol levels. In **chapter 8** we demonstrated that allowance for the use of lipid-lowering drugs may identify an additional group of patients with the metabolic syndrome. Patients identified with metabolic syndrome by this revised definition show similar baseline characteristics and cardiovascular risk factors as those patients diagnosed with the currently used NCEP definition. Furthermore, we showed that, although the newly proposed IDF definition elegantly pays attention to the elementary role of abdominal obesity in the development of the metabolic syndrome, use of this definition results in the identification of patients with a more favourable cardiovascular risk profile compared with NCEP-defined metabolic syndrome patients.

GENERAL DISCUSSION

The relevance of the metabolic syndrome for clinical practice can be considered from an etiological, a diagnostic, a prognostic and a therapeutic point of view.

Etiological point of view

The observation that cardiovascular risk factors tend to cluster in individual patients is intriguing and has therefore elicited basic and clinical research trying to understand and unravel the underlying pathophysiological mechanisms behind this clustering. It is now generally accepted that insulin resistance plays an important role. The abdominal adipose tissue can be regarded as an organ with important endocrinological functions, by the production of adipokines and cytokines, involved in energy homeostasis, inflammation and fibrinolysis. In case of adiposity the production of several inflammatory cytokines as TNF-α, leptin and interleukin-6 is increased, whereas the production of adiponectin is diminished, as a cause or a consequence of insulin resistance. We think that not only the excess of adipose tissue alters the production of adipokines by itself, but that adipocyte dysfunction also contributes to the metabolic changes. We showed that increased body iron stores could influence adipocyte function by the observed inverse relationship between plasma levels of ferritin and adiponectin. This calls for future (in vitro) studies, investigating the mechanisms in which iron effects adipocyte function. Knowledge of the underlying pathophysiology of a disease directs the development of logical and coherent treatment.

The concept of the metabolic syndrome could also help to understand the presence of the individual risk factors. For example, in many patients with "essential" hypertension, the elevated blood pressure is present in the context of the metabolic syndrome. In an insulin resistant state, activation of the sympathetic nerve system and increased renal sodium retention together with endothelial dysfunction could contribute to an increased blood pressure. Therefore hypertension is likely to be often the consequence of insulin resistance. Knowledge of the pathophysiology of insulin resistance-associated hypertension has consequences for medical treatment.

Diagnostic point of view

The propositions of several definitions to diagnose the metabolic syndrome feed the criticism surrounding the metabolic syndrome as not being a single disease entity. However, the main difference, caused by different visions regarding pathophysiology, between the WHO and NCEP definitions regards whether or not to demonstrate insulin resistance. The NCEP-definition is based on, for daily clinical practice, easily measurable components, and it has been shown that, although this definition does not require the proven demonstration of insulin resistance, 95% of the metabolic syndrome patients are indeed insulin resistant according to the gold standard for insulin resistance: hyperinsulinemic-euglycaemic clamp. According to both definitions, use of glucose or blood pressure lowering agents qualifies for the glucose or blood pressure criterion respectively. Only in the most recently issued NCEP-definition the use of specific treatment is incorporated in the dyslipidemia

criteria, but neither definition accounts for statin use. Based on results presented in this thesis, we prefer the use of the NCEP definition due to its practical suitability, but with the addition that patients on statin therapy, in concert with patients using fibrates or nicotinic acid, qualify for the dyslipidemia criteria.

The term syndrome comes from the Greek word 'sundromos' which means 'running together' (sun ('with') and dromos ('running, course')). The understanding of the co-occurrence of cardiovascular risk factors in stead of diagnosing single risk factors, make clinicians aware of the underlying disorder, insulin resistance. This means not only an increased risk for the development of cardiovascular disease but also for the development of type 2 diabetes.

Recently the IDF introduced a new definition in which the presence of abdominal obesity is required. In addition, the thresholds for increased waist circumference and glucose levels were lowered. In concept, this new definition has advantages because it elegantly puts forward the central role of abdominal obesity in the metabolic syndrome. However, we showed that by the IDF definition of the metabolic syndrome more patients were identified with a more favourable cardiovascular risk profile compared to the NCEP definition. In the meantime 2 prospective analyses (1 study in white men and 1 study in older women) were performed, demonstrating nearly comparable mortality predictions for the NCEP and IDF definition. However, in patients referred for coronary angiography IDF defined metabolic syndrome did not predict vascular events contrary to NCEP defined metabolic syndrome. Before embracing another new definition, further follow-up studies in other populations, including patients with manifest vascular disease, should be performed.

Prognostic point of view

It is important to be informed about the metabolic syndrome status in a patient, since it has been demonstrated that the presence of the NCEP-defined metabolic syndrome gives additional prognostic information for total and cardiovascular mortality on top of established cardiovascular risk algorithms as the Framingham risk equation. In addition, even when the metabolic syndrome is equally reliable as established risk algorithms in identifying patients at high cardiovascular risk, the NCEP-defined metabolic syndrome is more easy to use in daily clinical practice.

The question whether the cardiovascular risk associated with the metabolic syndrome is more than the sum of the risk caused by its parts is not answered yet and is likely to be answered never. The only way to resolve this issue would be with a follow-up study investigating the hazard ratio on cardiovascular morbidity and mortality for each individual component whereby during the follow-up period no other risk factors develop and no treatment occurs. The risk for cardiovascular disease should also be measured in another cohort of untreated patients with three or more risk factors comprising the metabolic syndrome. Than, in these patients, a comparison should be made between the actually observed risk and the expected risk quantified by hazard ratio's from the individual components and if the observed risk indeed exceeds the calculated risk, this pleads for the presence

of other risk factors than the individual components clustering in the metabolic syndrome. It should obviously be clear that for practical, logistic and ethic reasons this study would never be performed. However, based on the results of this thesis it should be advocated to screen for the metabolic syndrome in patients with already clinical manifestations of vascular disease. Firstly, in nearly 50% of these patients the metabolic syndrome can be expected and secondly, patients with vascular disease and the metabolic syndrome are at even higher cardiovascular risk than patients with vascular disease without the metabolic syndrome.

Therapeutic point of view

Of course, cardiovascular risk factors in patients with manifest vascular disease should be treated according to the current guidelines for patients at high vascular risk whether or not the metabolic syndrome is present. However, identification of the metabolic syndrome could increase the awareness of both physicians as well as patients for the important role of life style interventions (weight reduction and increase in physical activity) to improve insulin resistance, the most important pathophysiological mechanism behind the clustering of the individual risk factors. Also medical treatment of central obesity may be an option in the (near) future. Treatment of the underlying disorder rather than symptomatic treatment of the individual risk factors seems logical for two reasons. Firstly, by improvement of insulin sensitivity the individual risk factors could improve and no longer require (aggressive) treatment. Secondly, assuming that the not routinely measured factors associated with insulin resistance indeed contribute to the cardiovascular risk associated with the metabolic syndrome, improvement of insulin resistance could also have favourable effects on these risk factors.

This thesis also showed that in patients with the metabolic syndrome combination therapy of ezetimibe with low-dose simvastatin preserved postprandial endothelial function contrary to high-dose simvastatin monotherapy. Due to life style habits the Western human being is more postprandial than fasting, which implies a long time of endothelial dysfunction. Since endothelial dysfunction can be considered as a surrogate endpoint of cardiovascular disease, modulation of postprandial endothelial function by treatment of ezetimibe combined with low-dose simvastatin could contribute to the prevention of cardiovascular complications in patients with the metabolic syndrome beyond its effects on fasting LDL-cholesterol.

Therapeutic modulation of low adiponectin levels in patients with the metabolic syndrome by exogenous administration of adiponectin could not only decrease insulin resistance, but also has favourable effects on cardiovascular risk. Unfortunately, although adiponectin infusion in mice improved insulin sensitivity, studies in humans are lacking.

In conclusion, this thesis showed that:

- in patients with manifest vascular disease the prevalence of the metabolic syndrome is 46%
- patients with already manifest vascular disease and the metabolic syndrome have advanced vascular damage compared to patients without the metabolic syndrome
- in patients with coronary artery disease, the NCEP-defined metabolic syndrome, insulin resistance and obesity are all significantly associated with plasma adiponectin levels
- in patients with manifest vascular disease body iron stores, as reflected by ferritin levels, are significantly associated with the metabolic syndrome and adiponectin plasma levels
- the metabolic syndrome and insulin resistance are not related to the presence of coronary collaterals in patients with documented coronary artery disease
- in patients with the metabolic syndrome combination therapy of low-dose simvastatin and ezetimibe preserved postprandial endothelial function contrary to high-dose simvastatin monotherapy, whereas the same reduction in (fasting) plasma LDL-cholesterol was obtained after 6 weeks treatment
- in a cohort of patients with manifest vascular disease allowance for the use of lipid-lowering drugs in the NCEP-definition may identify an additional group of patients at elevated risk for cardiovascular disease and diabetes
- in a cohort of patients with manifest vascular disease the NCEP and IDF definitions identify metabolic syndrome patients with different cardiovascular risk profiles

APPENDIX

Oral glucose tolerance test or Metabolic Syndrome criteria to predict risk in patients with coronary heart disease?

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With great interest we read the article by Bartnik et al. investigating the predictive value of newly detected abnormal glucose tolerance, as assessed by oral glucose tolerance test (OGTT), on long-term outcome after myocardial infarction.[1] This is a remarkable finding, given the poor reproducibility of the OGTT.[2] This test should be performed under standardized conditions which require a diet containing more than 150 g of carbohydrate daily during the three days before the test, a reasonable (30-50g) carbohydrate containing meal the evening before the test, and one needs to allow for factors that could influence the glucometabolic state e.g. certain medications (diuretics, salicylates and sympaticomimetics), inactivity and infection. We would think that, identification of the Metabolic Syndrome, as estimate of abnormal glucose metabolism, is a simpler method to estimate increased cardiovascular risk. The Metabolic Syndrome is a cluster of cardiovascular risk factors and is associated with an increased risk for cardiovascular morbidity and mortality and for the development of type 2 diabetes. According to the definition proposed by the NCEP (National Cholesterol Education Program),[3] the metabolic syndrome can be identified if three or more of the following metabolic abnormalities are present: waist circumference > 102 cm in men and > 88 cm in women, blood pressure ≥ 130 mmHg systolic or ≥ 85 mmHg diastolic, serum triglycerides ≥ 1.70 mmol/l, serum HDL-cholesterol < 1.04 mmol/l in men and < 1.29 mmol/l in women and fasting serum glucose ≥ 6.1 mmol/l. Since waist circumference is not always available, a widely accepted method is to substitute body mass index.

In patients with coronary heart disease the metabolic syndrome is indeed highly prevalent (41%)[4] and associated with increased cardiovascular risk.[5] Besides this, according to a recently published report of the NCEP, the presence of the metabolic syndrome indicates *very* high risk for patients with coronary artery disease. For these patients it should be considered to lower the therapeutic goal of LDL-lowering therapy to 1.8 mmol/l in stead of 2.6 mmol/l.[6] It would be valuable to the scientific community to be informed about the presence and predictive value of the Metabolic Syndrome in the study population described by Bartnik et al., as they have near complete data including blood pressure, glucose, HDL-cholesterol and triglyceride levels to do so.[1]

Impaired glucose tolerance seems an important predictor of long-term outcome in patients with coronary heart disease. To detect abnormal glucose metabolism and predict cardiovascular risk for many patients both in-hospital and in the outpatient clinic setting, an easy to use and practical test is needed. Identifying the Metabolic Syndrome according to NCEP criteria is such a simple method.

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Chapter 10

Nederlandse Samenvatting

Dankwoord

Curriculum Vitae

Publications

NEDERLANDSE SAMENVATTING

In de Westerse wereld vormen hart- en vaatziekten de belangrijkste oorzaken van ziekte en overlijden. Bij het ontstaan van hart- en vaatziekten speelt atherosclerose (slagaderverkalking) een zeer belangrijke rol. Belangrijke risicofactoren voor het ontwikkelen van atherosclerose zijn hypertensie (verhoogde bloeddruk), dyslipidemie (afwijkend vet- en cholesterolgehalte), diabetes (suikerziekte) en overgewicht. Deze risicofactoren komen vaak tegelijkertijd voor en er wordt in dat geval gesproken van het metabool syndroom. De term syndroom komt van het Griekse woord "sundromos" (sun ('met') en dromos ('gaan')) en betekent letterlijk "samengaan". Het metabool syndroom betekent dan ook "het samengaan van metabole risicofactoren". Volgens een eenvoudig in de praktijk te gebruiken definitie is er sprake van het metabool syndroom bij een (willekeurige) combinatie van 3 of meer van de volgende afwijkingen:

1. Teveel buikvet: tailleomvang: > 102 cm (mannen) of > 88 cm (vrouwen)
2. Hoge bloeddruk: ≥ 130 mmHg systolisch of ≥ 85 mmHg diastolisch (of het gebruik van bloeddrukverlagende medicijnen)
3. Verhoogde concentratie triglyceriden (vetten): triglyceriden ≥ 1.70 mmol/l
4. Verlaagde concentratie HDL-cholesterol ("goed" cholesterol): HDL-cholesterol < 1.04 mmol/l (mannen) of < 1.29 mmol/l (vrouwen)
5. Verhoogde concentratie glucose (suikergehalte): glucose ≥ 6.1 mmol/l (of het gebruik van glucoseverlagende medicijnen)

Het is nog niet helemaal duidelijk waardoor het metabool syndroom wordt veroorzaakt, maar een verminderde gevoeligheid voor insuline en teveel buikvet spelen een belangrijke rol. Insuline is een hormoon dat een belangrijke rol speelt bij de regulatie van het glucosegehalte in het bloed door onder andere de opname van glucose in spier- en vetweefsel te bevorderen en de glucoseproductie door de lever te remmen. Verder speelt insuline een rol in de vet- en eiwithuishouding in het lichaam. Wanneer het lichaam minder gevoelig is voor insuline, wordt er gesproken van insuline resistentie. In geval van insuline resistentie zal de alvleesklier ter compensatie meer insuline gaan produceren. Wanneer dit compensatiemechanisme niet meer voldoende is, zal de glucose concentratie in het bloed stijgen. Wanneer er sprake is van insuline resistentie, zijn de vetcellen minder gevoelig voor de remmende werking van insuline op de uitscheiding van vrije vetzuren. Hierdoor neemt de concentratie vrije vetzuren in het bloed toe, waardoor de lever meer triglyceriden gaat maken. Ondermeer door verminderde beschikbaarheid van bouwstenen voor het vervaardigen van HDL-cholesterol en door versnelde afbraak van HDL-cholesterol daalt de concentratie van HDL-cholesterol in het bloed. Bovendien houden de nieren in een insuline resistente situatie meer zout vast, vindt er activatie plaats van het sympathische zenuwstelsel en is er sprake van disfunctioneren van de bloedvaten wat allemaal kan bijdragen aan een verhoogde bloeddruk.

Vetcellen blijken actieve cellen te zijn die diverse stoffen, adipokines genaamd, produceren. Een veranderde productie van adipokines, ten gevolge van disfunctioneren van de vetcel, kan bijdragen aan het ontstaan van insuline resistentie. Door toename in inzichten in de oorzaak, de prognose, de diagnostiek en nieuwe therapeutische mogelijkheden staat het metabool syndroom de laatste tijd volop in de belangstelling.

In een ogenschijnlijk gezonde populatie heeft 20-25% van de mensen het metabool syndroom en de verwachting is dat het aantal mensen met het metabool syndroom de komende jaren, gezien de toename van overgewicht, verder zal stijgen. Verschillende studies hebben laten zien dat patiënten met het metabool syndroom een 2 tot 3 keer verhoogde kans hebben op het ontwikkelen van hart- en vaatziekten en type 2 diabetes mellitus. Een groot aantal mensen, vaak nu nog zonder klinische verschijnselen van hart- en vaatziekten of diabetes, hebben zo een verhoogd risico op het ontwikkelen van hart- en vaatziekten en type 2 diabetes mellitus. Patiënten met klinisch vaatlijden (zoals perifeer vaatlijden, cerebrovasculaire aandoeningen, coronaire hartziekten en abdominale aneurysmata) hebben een verhoogd risico op het ontwikkelen van nieuwe vaataandoeningen. In **hoofdstuk 2** tonen wij aan dat het metabool syndroom bij 46% van de patiënten met klinisch vaatlijden aanwezig is. Uit **hoofdstuk 5** kan worden geconcludeerd dat bij patiënten die al symptomen van vaatlijden hebben, het metabool syndroom gepaard gaat met nog meer vaatschade. De uitgebreidheid van het vaatlijden is hierbij vastgesteld door de dikte van de halsslagader te meten, (de zogenaamde intima media dikte (IMT)), de eiwituitscheiding door de nieren en de enkel-arm index te meten. Zo hadden patiënten met het metabool syndroom een dikkere IMT (0.98 mm versus 0.92 mm), vaker eiwit in de urine (albuminurie) (20% versus 15%) en vaker een verlaagde enkel-arm index vergeleken met patiënten zonder het metabool syndroom.

Adiponectine, een adipokine, wordt geproduceerd door vetcellen en heeft een beschermend effect ten aanzien van het ontstaan van atherosclerose en insuline resistentie en diabetes. Mensen met overgewicht blijken vaak lagere plasma concentraties adiponectine te hebben dan mensen zonder overgewicht. Er bestaat ook een omgekeerde relatie tussen adiponectine en insuline resistentie. Bovendien voorspellen lage plasma concentraties adiponectine het ontstaan van cardiovasculaire ziekten. In **hoofdstuk 3** wordt aangetoond dat het metabool syndroom geassocieerd is met verlaagde plasma concentraties adiponectine bij patiënten met coronaire hartziekten (slagaderverkalking van de kransslagaders). Ook verschillende maten van overgewicht en insuline resistentie blijken geassocieerd met verlaagde adiponectine spiegels. Lage plasma adiponectine concentraties, als gevolg van disfunctioneren van de vetcel, zouden dus zowel bij het ontstaan van het metabool syndroom als bij het verhoogde cardiovasculaire risico samenhangend met het metabool syndroom, betrokken kunnen zijn. In **hoofdstuk 4** wordt een relatie aangetoond tussen verhoogde plasma concentraties ijzer (gemeten door middel van ferritine) en het metabool syndroom bij patiënten met klinisch vaatlijden. Daarnaast wordt er een omgekeerde relatie tussen ferritine en adiponectine beschreven, wat suggereert dat ijzer mogelijk betrokken is bij het ontstaan van het metabool syndroom door verstoring van de vetcel functie.

In een aantal studies is onderzocht of de kans op cardiovasculaire aandoeningen bij patiënten met het metabool syndroom hoger is dan de optelsom van het cardiovasculaire risico van de individuele componenten. De resultaten van deze studies zijn niet eenduidig. Als het risico inderdaad hoger is dan op grond van de individuele componenten verwacht mag worden, dan zou dit veroorzaakt kunnen worden doordat andere risicofactoren een rol spelen. Er bestaan namelijk risicofactoren die samenhangen met insuline resistentie, zoals onder andere een verhoogde stollingsneiging, een verminderde functie van de bloedvaten, kleinere cholesterol deeltjes in het bloed, ontsteking en verminderde adiponectine concentraties, maar die niet routinematig gemeten worden. Wanneer er sprake is van atherosclerose van de kransslagaders bestaat de mogelijkheid dat er nieuwe bloedvatbruggetjes (collateralen) worden gevormd om ook het hartweefsel achter de vernauwing van bloed te kunnen voorzien. Verminderde collateraal vorming van de kransslagaders zou ook kunnen bijdragen aan het verhoogde risico op cardiovasculaire aandoeningen van het metabool syndroom. In **hoofdstuk 6** wordt deze hypothese getoetst, waarbij echter geen associatie wordt gevonden tussen het metabool syndroom en coronaire collateraalvorming.

Er bestaan verschillende strategieën om het metabool syndroom te behandelen. Direct vergelijkend onderzoek met verschillende specifieke interventies gericht op behandeling van het metabool syndroom en behandeling van de individuele risicofactoren ontbreekt nog. Toch lijkt het logisch om behandeling te richten op verbetering van de insuline gevoeligheid, waarmee niet alleen de individuele componenten van het metabool syndroom als wel de niet routinematig gemeten risicofactoren, geassocieerd met verminderde insuline gevoeligheid, zouden kunnen verbeteren. Wanneer bij patiënten met manifest vaatlijden het metabool syndroom wordt gediagnosticeerd kan dit, volgens een recente Amerikaanse publicatie, aanleiding zijn een nog lagere waarde na te streven van het LDL-cholesterol ("slechte cholesterol") (<1,8 mmol/l) dan gebruikelijk is bij patiënten met een hoog risico op cardiovasculaire aandoeningen. Dit kan worden bereikt door behandeling met een hoge dosering cholesterolsynthese remmer (statine), dan wel door een lage dosering statine gecombineerd met een cholesterol absorptie remmer (ezetimibe). Postprandiale hyperlipidemie (verhoogd vetgehalte na de maaltijd) kan worden gezien als een cardiovasculaire risicofactor doordat het de bloedvatfunctie na de maaltijd negatief beïnvloedt (endotheeldysfunctie). Remming van cholesterolopname kan het postprandiale lipidenprofiel beïnvloeden en daarmee de postprandiale endotheelfunctie. In **hoofdstuk 7** worden de effecten vergeleken van de combinatietherapie van lage dosering simvastatine (10 mg) en ezetimibe (10 mg) met de behandeling van hoge dosering simvastatine (80 mg) op postprandiale endotheelfunctie bij mannelijke patiënten met het metabool syndroom. Op grond van deze studie kan worden geconcludeerd dat een gecombineerde behandeling van simvastatine 10 mg/ ezetimibe 10 mg geen verlaging van de postprandiale endotheelfunctie geeft, in tegenstelling tot simvastatine 80 mg, bij een vergelijkbare LDL-cholesterol verlaging. Door het positief beïnvloeden van de bloedvatfunctie na de maaltijd, kan combinatietherapie van ezetimibe met simvastatine mogelijk extra bijdragen aan verlaging van het cardiovasculaire risico bovenop verlaging van het risico door verlaging van de plasma LDL-cholesterol concentratie bij patiënten met het metabool syndroom.

Door verschillende inzichten in de oorzaak van het metabool syndroom en de relevantie voor de dagelijkse praktijk wordt er een aantal verschillende definities gehanteerd, die gebaseerd zijn op verschillende criteria, zoals de definitie van de World Health Organisation (WHO), de NCEP (National Cholesterol Education program Expert Panel on Detection, Evaluation and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III)) definitie en de definitie van de Internationale Diabetes Federatie (IDF). Zowel bij de NCEP definitie als bij de IDF definitie wordt, net zoals bij het gebruik van bloeddruk- en glucoseverlagende medicamenten, het gebruik van medicatie specifiek gericht op dyslipidemie (zoals fibraten en nicotinezuur analogen) meegenomen in het criterium voor gestoorde vetstofwisseling. Echter, statines zijn de meest voorgeschreven medicamenten om het LDL-cholesterol gehalte en het cardiovasculaire risico te verlagen. Statines hebben echter ook effect op de plasma triglyceriden en HDL-cholesterol concentraties. In **hoofdstuk 8** wordt aangetoond dat wanneer rekening wordt gehouden met het gebruik van statines er een extra groep patiënten wordt gediagnosticeerd met het metabool syndroom. Deze patiënten blijken een vergelijkbaar cardiovasculair risicoprofiel te hebben als de patiënten die worden gediagnosticeerd met het metabool syndroom wanneer geen rekening wordt gehouden met het gebruik van statines. Daarnaast blijkt de IDF definitie, die het bestaan van teveel abdominaal vet verplicht stelt, patiënten te identificeren met een gunstiger cardiovasculair risico profiel dan de NCEP definitie.

Het herkennen van het metabool syndroom is belangrijk in de dagelijkse klinische praktijk omdat het concept van het metabool syndroom een rol speelt bij de oorzaak van aandoeningen, maar ook bij de diagnostiek, de prognose en therapie. Het feit dat meerdere risicofactoren vaak samen voorkomen heeft geleid tot veel wetenschappelijke studies naar de oorzaak van deze samenhang. Insuline resistentie is de belangrijkste onderliggende oorzaak van het metabool syndroom. Behandelstrategieën zijn dan logischerwijs gericht op verbetering van de insuline gevoeligheid. Bovendien kan ook verklaard worden waarom bepaalde risicofactoren bij een patiënt voorkomen. Zo wordt bij veel patiënten duidelijk wat de oorzaak is van verhoogde bloeddruk; namelijk hoge bloeddruk als onderdeel van het metabool syndroom. Hetzelfde geldt voor stoornissen in het vetstofwisseling. Wanneer een arts bij een patiënt de samenhang van meerdere cardiovasculaire risicofactoren herkent als het metabool syndroom, betekent dit voor deze patiënt niet alleen een verhoogd risico op hart- en vaatziekten maar ook op diabetes mellitus. In plaats van het behandelen van iedere risicofactor afzonderlijk kan nu ook geprobeerd worden om de oorzaak van het metabool syndroom te behandelen door de gevoeligheid van het lichaam voor insuline te vergroten door maatregelen gericht op toename van lichamelijke activiteit en gewichtsafname. Wanneer voor zowel arts als patiënt het belang van deze maatregelen duidelijk is, wordt de kans op succes wellicht groter. Ook medicamenteuze therapie gericht op verbetering van de insuline gevoeligheid zou vroegtijdig kunnen worden ingezet. De resultaten van grote studies zullen eerst moeten worden afgewacht.

Concluderend, in dit proefschrift wordt aangetoond dat:

- bij patiënten met manifest vaatlijden de prevalentie van het metabool syndroom 46% bedraagt
- patiënten met manifest vaatlijden en het metabool syndroom meer vaatschade hebben dan patiënten met manifest vaatlijden zonder het metabool syndroom
- bij patiënten met coronaire hartziekten het metabool syndroom, insuline resistentie en overgewicht allemaal geassocieerd zijn met de adiponectine concentratie in het bloed
- bij patiënten met manifest vaatlijden de hoeveelheid ijzer in het lichaam (ferritine) geassocieerd is met het metabool syndroom en met de adiponectine concentratie in het bloed
- het metabool syndroom en insuline resistentie niet geassocieerd zijn met de aanwezigheid van collateraal vorming van de kransslagaders bij patiënten met coronaire hartziekten
- bij patiënten met het metabool syndroom combinatie therapie van lage dosering statine met ezetimibe behoud geeft van endotheelfunctie na de maaltijd in tegenstelling tot hoge dosering statine, bij dezelfde LDL-cholesterol daling, na 6 weken behandeling
- wanneer bij patiënten met manifest vaatlijden bij de NCEP definitie voor metabool syndroom rekening wordt gehouden met het gebruik van statines een additionele groep patiënten geïdentificeerd wordt met een verhoogd risico op de ontwikkeling van cardiovasculaire aandoeningen en type 2 diabetes mellitus
- in een groep patiënten met manifest vaatlijden door de NCEP en IDF definities metabool syndroom patiënten met een verschillend cardiovasculair risicoprofiel worden geïdentificeerd

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CURRICULUM VITAE

Jobien Karen Olijhoek was born on the 15th of December, 1974, in Veldhoven, the Netherlands. After graduation from the Gymnasium at the "Van Maerlant Lyceum" in Eindhoven in 1993, she started Medical School at the University of Utrecht. She obtained her medical degree in November 1999. During this period she performed a research project about growth hormone deficiency after transsphenoidal surgery for clinically non-functioning pituitary adenoma at the department of Internal Medicine at the University Utrecht. She also participated in a project investigating the role of oxygen radical stress in patients with hemochromatosis and she was involved in a study concerning the role of sympathetic activity on endothelial function.

She started her clinical career as a resident at the department of Internal Medicine at "Het Rode Kruis Ziekenhuis" in Beverwijk in January 2000, where she started her training in Internal Medicine in September 2000 under supervision of dr. M.W. Kunst.

In September 2002 she started with the work described in this thesis at the department of Vascular Medicine, University Medical Centre Utrecht, under supervision of dr. F.L.J. Visseren and prof.dr.Y. van der Graaf. In May 2005 she continued her training in Internal Medicine at the University Medical Centre Utrecht under supervision of prof.dr. E. van der Wall.

Jobien Olijhoek lives together with Arjen Pronk and since July 2005 they have a son Thijs Pronk.

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