DAVID E. LAAKSONEN

Role of Physical Exercise, Fitness and Aerobic Training in Type 1 Diabetic and Healthy Men in Relation to the Lipid Profile, Lipid Peroxidation and the Metabolic Syndrome

Doctoral dissertation

To be presented by permission of the Faculty of Medicine of the University of Kuopio for public examination in Auditorium L1, Cantia building, University of Kuopio, on Wednesday the 18th of December 2002, at 12 noon

Department of Physiology
Department of Medicine
Department of Clinical Nutrition
Research Institute of Public Health
University of Kuopio and Kuopio University Hospital
Laaksonen, David E. Role of Physical Exercise, Fitness and Aerobic Training in Type 1 Diabetic and Healthy Men in Relation to the Lipid Profile, Lipid Peroxidation and the Metabolic Syndrome
ISSN 1235-0303

ABSTRACT
Dyslipidemia and possibly lipid peroxidation play important roles in the development of macro- and microvascular disease in type 1 diabetes mellitus. Little is known, however, of the role of aerobic exercise in dyslipidemia and resting and exercise-induced lipid peroxidation in type 1 diabetes. Despite the well-known effect of leisure-time physical activity (LTPA) on components of the metabolic syndrome, little is known of the association of LTPA and cardiorespiratory fitness (maximal oxygen consumption, VO_{2max}) with development of the metabolic syndrome itself. A randomized controlled trial assessing the effect of a 12-16 week aerobic exercise program on VO_{2max} and the lipid profile was carried out in otherwise healthy young men with type 1 diabetes. The effect of acute physical exercise on oxidative stress and antioxidant defenses and the relation to VO_{2max} in men with type 1 diabetes was also evaluated. To test four recently proposed definitions by the World Health Organization (WHO) and National Cholesterol Education Program (NCEP) of the metabolic syndrome, the sensitivity and specificity of the definitions for prevalent and incident diabetes were assessed in a population-based cohort of middle-aged men. We also studied the associations of LTPA and cardiorespiratory fitness with prevalent and incident cases of the metabolic syndrome. A 12-16 week endurance exercise program produced antiatherogenic changes in lipid, lipoprotein and apolipoprotein levels in 20 type 1 diabetic men who for the most part were already physically active at baseline. The most favorable training-induced changes in the high-density lipoprotein cholesterol (HDL)/low-density lipoprotein cholesterol (LDL) and apolipoprotein A-I/apolipoprotein B ratios were in patients with low baseline HDL/LDL levels, likely the group with the most benefit to be gained by such changes. Plasma thiobarbituric acid reactive substances (TBARS), a measure of lipid peroxidation, was higher in nine healthy young men with type 1 diabetes than in control men both at rest and after exercise, suggesting increased oxidative stress. An inverse correlation between resting plasma TBARS and VO_{2max} was found in the diabetic men, which could imply a protective effect of physical fitness against lipid peroxidation. The nine young diabetic men also had lower erythrocyte Cu/Zn-superoxide dismutase and catalase activity, but higher glutathione reductase activity. Coupled with increased plasma TBARS and blood total glutathione levels in the diabetic men, these changes may reflect increased susceptibility to oxidative stress and compensatory adaptations of glutathione homeostasis in response to increased oxidative stress. The WHO and NCEP definitions of the metabolic syndrome appear valid, identifying individuals of a population-based cohort of middle-aged men (n=1005) with a 5-9 -fold increased likelihood of developing diabetes during follow up. The modified WHO definition based on waist-hip ratio >0.9 was the most sensitive in detecting prevalent and incident diabetes and had good specificity. The NCEP definition of the metabolic syndrome with adiposity defined as waist girth >102 cm was the most specific, but did not detect most cases of incident diabetes. In a subset of men without diabetes or the metabolic syndrome at baseline, those who engaged in more LTPA, especially vigorous, or who were more fit were less likely to develop the metabolic syndrome during the four-year follow up. These findings support promotion of moderate and vigorous leisure-time physical activity in otherwise healthy type 1 diabetic men to improve dyslipidemia and cardiorespiratory fitness and possibly decrease lipid peroxidation, and in middle-aged non-diabetic men, to decrease the risk for development of the metabolic syndrome and thereby chronic and progressive diseases such as diabetes and atherosclerosis.

National Library of Medicine Classification: WK 810, QU 85, QT 255
Medical Subject Headings: diabetes, insulin-dependent; diabetes, non-insulin-dependent; metabolic syndrome X; exercise; physical fitness; oxidative stress; lipid peroxidation; antioxidants; glutathione; obesity; hyperinsulinemia; apolipoproteins, lipoproteins; triglycerides; hypertension; randomized controlled trials; prospective studies; risk factors; male.
ACKNOWLEDGMENTS

This work was carried out in the Department of Physiology and the Department of Medicine, University of Kuopio, in 1994-2001. These studies were realized with the help of a large number of individuals, to whom I would like to express my gratitude:

Docent Chandan K. Sen, PhD, head of the Laboratory of Molecular Medicine, Department of Surgery and Molecular and Cellular Biochemistry, Davis Heart and Lung Research Institute, the Ohio State University Medical Center, is my main supervisor. Chandan has an infectious enthusiasm for science and an intellect, drive and other personal qualities that have led to a mastery of a wide range of techniques in microbiology and genetics that make my head spin. It was in discussions with Chandan in 1994 that the idea to study oxidative stress and exercise in type I diabetes arose.

Professor Leo Niskanen, MD, PhD, Department of Medicine, Kuopio University Hospital, has provided the closest supervision and collaboration throughout these studies. Leo’s enthusiasm and energy for research on wide-ranging topics and study designs and ability to combine research and clinical work have been an inspiration to me. Through Leo I have been able to become involved in a major clinical trial and in projects in epidemiology and nutrition, with the metabolic syndrome or insulin and glucose metabolism as the thread tying them all together.

Professor Matti Uusitupa, Rector of the University of Kuopio, has also played a critical supervisory role in this thesis. Matti’s clear common-sense approach to problems in science has been important in many phases of this thesis. Matti insisted on maintaining a randomized controlled design in Publication 1, even though I was ready to shift to an uncontrolled design because of worries about statistical power. This insistence was an important lesson in methodology and study design.

Professor Osmo Hänninen, head of the Department of Physiology, University of Kuopio, has a sometimes idiosyncratic enthusiasm for an impressively wide range of topics in science. Osmo gave me the opportunity to become involved in research. Without his sometimes idiosyncratic and always unprejudiced qualities, I would probably never have gotten that chance. The founding of the TULES Graduate School was for the most part conceived and realized by Osmo.

Docent Timo Lakka, visiting scientist at the Pennington Biomedical Research Center, Louisiana State University, has also played a critical role in supervision and collaboration. Our early discussions about physical activity and fitness at the cafeteria table eventually lead to concrete collaboration in the Kuopio Ischaemic Heart Disease Risk Factor Study. Timo and Hanna Lakkas’ collaboration and supervision have allowed the epidemiologist in me to express itself and develop. Their collaboration and friendship have lead to exciting projects beyond this thesis.
Professor Scott Powers, PhD, head of the Department of Exercise and Sport Sciences and Physiology, University of Florida, and Docent Sari Mäkimmäki, MD, PhD, Department of Endocrinology, Malmö University Hospital, Sweden, made insightful and constructive comments and important suggestions for improvement while undertaking the time-consuming task of reviewing this thesis.

Professor Jukka T. Salonen, MD, PhD, MScPh, head of the Research Institute of Public Health, has kindly allowed me to participate in projects in the Kuopio Ischaemic Heart Disease Risk Factor Study, and has also played an important role as co-author in several of my papers.

Mustafa Atalay, MD, PhD, MPH, of the Department of Physiology, played a critical role in Studies 1-3. Without his laboratory and organizational skills, these studies could never have been carried out. I appreciate the sacrifices both he and Savita Khanna, PhD, made in Study 1, which also involved weekends of work in Joensuu.

I would like to acknowledge my other co-authors for their contributions, including Professor Rainer Rauramaa, MD, PhD, head of the Kuopio Research Institute of Exercise Medicine; Juha Mustonen, MD, PhD, head of the Department of Medicine, North Karelia Central Hospital; and George A. Kaplan, PhD, of the Department of Epidemiology, School of Public Health, University of Michigan.

The staff and colleagues at the Department of Physiology have provided important support throughout this study. Ms. Eeva-Liisa Palkispää and Ms. Riitta Venäläinen provided important assistance to Mustafa in the laboratory. Ms. Kaija Kettunen skillfully carried out the lipid, lipoprotein and apolipoprotein measurements of Study 1. Ms. Raija Holopainen has also given important assistance.

This study was supported by the TULES Graduate School, the Finnish Ministry of Education, the Academy of Finland, the National Heart, Lung and Blood Institute, the City of Kuopio, the Ida Montin Foundation, the Yrjö Jahnsson Foundation, the North Savo Cultural Foundation and the Juho Vainio Foundation.

Special thanks go to Riitta Paajanen, for her understanding and support over all these years. Also special thanks to my parents, G. Donald (Laaksonen) Larson, MD, and Alisa (Haponen) Larson, for their fostering of a respect for learning without ever applying pressure, and for their support, financial and otherwise, over my many years of higher education (16 in all - I’m a slow learner).

Kuopio, November 2002

David Laaksonen
# Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ACSM</td>
<td>American College of Sports Medicine</td>
</tr>
<tr>
<td>AGE</td>
<td>advanced glycation endproducts</td>
</tr>
<tr>
<td>apo A-I</td>
<td>apolipoprotein A-I</td>
</tr>
<tr>
<td>apo B</td>
<td>apolipoprotein B</td>
</tr>
<tr>
<td>BMI</td>
<td>body mass index</td>
</tr>
<tr>
<td>CDC</td>
<td>Center for Disease Control</td>
</tr>
<tr>
<td>CHD</td>
<td>coronary heart disease</td>
</tr>
<tr>
<td>CVD</td>
<td>cardiovascular disease</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>DM</td>
<td>diabetes mellitus</td>
</tr>
<tr>
<td>E%</td>
<td>energy percent</td>
</tr>
<tr>
<td>EC</td>
<td>extracellular</td>
</tr>
<tr>
<td>EGIR</td>
<td>European Group for the Study of Insulin Resistance</td>
</tr>
<tr>
<td>GADA</td>
<td>glutamic acid decarboxylase antibodies</td>
</tr>
<tr>
<td>GLUT</td>
<td>glucose transporter protein</td>
</tr>
<tr>
<td>GPX</td>
<td>glutathione peroxidase</td>
</tr>
<tr>
<td>GRD</td>
<td>glutathione reductase</td>
</tr>
<tr>
<td>GSH</td>
<td>reduced glutathione</td>
</tr>
<tr>
<td>GSSG</td>
<td>glutathione disulfide</td>
</tr>
<tr>
<td>GST</td>
<td>glutathione-S-transferase</td>
</tr>
<tr>
<td>HbA1c</td>
<td>hemoglobin A1c</td>
</tr>
<tr>
<td>HDL</td>
<td>high-density lipoprotein</td>
</tr>
<tr>
<td>HOMA</td>
<td>homeostasis model assessment</td>
</tr>
<tr>
<td>ICA</td>
<td>islet cell antibodies</td>
</tr>
<tr>
<td>IFG</td>
<td>impaired fasting glycemia</td>
</tr>
<tr>
<td>IGT</td>
<td>impaired glucose tolerance</td>
</tr>
<tr>
<td>ISH</td>
<td>International Society of Hypertension</td>
</tr>
<tr>
<td>KIHD</td>
<td>Kuopio Ischaemic Heart Disease Risk Factor Study</td>
</tr>
<tr>
<td>LADA</td>
<td>latent autoimmune diabetes in adults</td>
</tr>
<tr>
<td>LDL</td>
<td>low-density lipoprotein</td>
</tr>
<tr>
<td>LTPA</td>
<td>leisure-time physical activity</td>
</tr>
<tr>
<td>MDA</td>
<td>malondialdehyde</td>
</tr>
<tr>
<td>METs</td>
<td>metabolic equivalents</td>
</tr>
<tr>
<td>MODY</td>
<td>maturity-onset diabetes of the young</td>
</tr>
<tr>
<td>MSy</td>
<td>metabolic syndrome</td>
</tr>
<tr>
<td>N/A</td>
<td>not applicable</td>
</tr>
<tr>
<td>NCEP</td>
<td>National Cholesterol Education Program</td>
</tr>
<tr>
<td>OR</td>
<td>odds ratio</td>
</tr>
<tr>
<td>QUICKI</td>
<td>quantitative insulin sensitivity check index</td>
</tr>
</tbody>
</table>
ROS  reactive oxygen species
SOD  superoxide dismutase
TBARS  thiobarbituric acid reactive substances
TGH  total glutathione
TRAP  total peroxyl radical trapping potential
VLDL  very-low-density lipoprotein
VO_{2\text{max}}  maximal oxygen consumption
W_{\text{max}}  maximal exercise capacity (in Watts per kg)
WHO  World Health Organization
WHR  waist-hip ratio
LIST OF PUBLICATIONS

This dissertation is based on the following original publications, which will be referred to in the text as Studies 1-5.


6 DISCUSSION

6.1 Study design and methods 57
6.2 Main findings 60
  6.2.1 Study 1. Randomized controlled study assessing the effect of regular aerobic exercise on the lipid profile in men with type 1 diabetes mellitus 60
  6.2.2 Studies 2 and 3. Altered antioxidant enzyme defenses and increased resting and exercise induced oxidative stress in young, insulin-dependent diabetic men 62
  6.2.3 Study 4. The metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study 65
  6.2.4 Study 5. Low levels of leisure-time physical activity and cardiorespiratory fitness predict development of the metabolic syndrome 67

7 SUMMARY AND CONCLUSIONS 70

8 FUTURE DIRECTIONS 72

9 REFERENCES 73
1 INTRODUCTION

Type 1 and type 2 diabetes mellitus are major worldwide health problems predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to development of nephropathy, neuropathy and retinopathy (1). The metabolic syndrome, a concurrence of disturbed glucose and insulin metabolism, overweight and abdominal fat distribution, mild dyslipidemia and hypertension, is from a clinical and public health standpoint most important because of its association with subsequent development of type 2 diabetes mellitus and cardiovascular disease (CVD) (2-9). Roughly one third of middle-aged Americans may have the metabolic syndrome as defined by the National Cholesterol Education Program (NCEP) (10). Using a different definition, 17% of men and 7% of women were estimated to have the metabolic syndrome based on a community study in Peksämäki, Finland (11).

Physical exercise is a cornerstone of therapy for type 1 and type 2 diabetes mellitus (DM). Observational studies suggest that physical activity and physical fitness may decrease the risk for CVD in both non-diabetic persons (12-17) and those with type 1 (18) and type 2 diabetes (19). This protective effect may be mediated in part through components of the metabolic syndrome. In non-diabetic persons, intervention studies, physical exercise has in variable degrees and at least in the short term decreased weight and visceral fat accumulation (20-23), increased high-density lipoprotein (HDL) cholesterol and decreased triglyceride levels (23-25), decreased blood pressure (23, 26) and improved insulin sensitivity (20-22). Physical exercise may also decrease serum low-density lipoprotein (LDL) cholesterol levels (e.g., (27)). Results from mainly small and uncontrolled studies testing the effects of regular aerobic exercise on the lipid profile in type 1 DM individuals have, however, been variable (28-31).

Oxidative stress has been increasingly implicated in the accelerated atherosclerosis and microvascular complications of diabetes mellitus (32-35). Oxidative stress can result in widespread lipid, protein and DNA damage (36), including oxidative modification of LDL cholesterol, believed to be central in the pathogenesis of atherosclerosis, and endothelial dysfunction (32, 35, 37, 38).

Many recent studies suggest that even moderate exercise increases free radical production beyond the capacity of antioxidant defenses, resulting in oxidative stress (27-30). On the other hand, regular exercise may strengthen antioxidant defenses and decrease resting and acute exercise-induced oxidative stress (39-41). Little is known about exercise-induced oxidative stress in diabetes mellitus.

The mechanisms underlying the apparent increased oxidative stress in diabetes are not entirely clear. Accumulating evidence points to many, often interrelated mechanisms (32-35), increasing production of reactive oxygen species such as superoxide (42-45) or hydrogen peroxide (46, 47), or decreasing antioxidant defenses (48-51). These mechanisms include glucose autoxidation (44, 52) and formation of advanced glycation endproducts (AGE) (32, 53), activation of the polyol pathway (33, 34, 54-57) and altered cell and glutathione redox status (54-57) and ascorbate metabolism (58), antioxidant enzyme inactivation (59-61),
perturbations in nitric oxide and prostaglandin metabolism (35) and insulin resistance (62-64). No consensus has been reached as to the relative importance of these mechanisms. Despite strong evidence indicating a pathogenic role of oxidative stress in the development of atherosclerosis and microvascular complications in DM, controversy exists about whether the increased oxidative stress is merely associative rather than causal, or even whether oxidative stress is increased at all in DM.

In prospective cohort studies, higher levels of physical activity have quite consistently protected against development of both CVD and type 2 diabetes mellitus (16, 17, 65-67), both of which are commonly associated with the metabolic syndrome. Although the pathogenesis of the metabolic syndrome remains unclear, the metabolic syndrome is in its early stages characterized by mild and varying degrees of abnormalities of insulin, glucose and lipid metabolism, hypertension and overweight, which if unchecked may progress over years to overt diseases such as diabetes and atherosclerosis in its various manifestations (6). Because of the current epidemic of overweight and sedentary lifestyle worldwide, the metabolic syndrome poses a serious and growing problem for clinicians and public health officials alike (68).

Although physical exercise favorably affects individual components of the metabolic syndrome, little evidence exists showing that physical activity prevents the metabolic syndrome itself. Such information is necessary for healthcare providers and public health policy makers seeking to prevent the consequences of the metabolic syndrome already at an early phase.

Previously, clinical and epidemiological research on the metabolic syndrome was hampered by the lack of standard definitions. To address this problem, the World Health Organization (WHO) (69) and the NCEP (70) have recently published definitions of the metabolic syndrome.

The purpose of this series of studies was to assess 1) the effect of aerobic exercise training on lipid and lipoprotein levels in type 1 diabetes, 2) resting and exercise induced oxidative stress and 3) the association of leisure-time physical activity and cardiorespiratory fitness with development of the metabolic syndrome.
2 REVIEW OF THE LITERATURE

2.1. Classification of diabetes mellitus

Diabetes mellitus is a major worldwide health problem predisposing to markedly increased cardiovascular mortality and serious morbidity and mortality related to development of nephropathy, neuropathy and retinopathy (1). Diabetes mellitus is characterized by derangements in carbohydrate and lipid metabolism, and is diagnosed by the presence of hyperglycemia. Diabetes has been traditionally divided mainly into type 1 and type 2 DM, with other less common forms.

Type 1 DM make up about 15% of the cases of DM in Finland, is marked by deficient or absent insulin secretion by the pancreas and tends to occur before middle age (71). The presence of islet cell antibodies (ICA) or glutamic acid decarboxylase antibodies (GADA), markers of autoimmune β-cell destruction, are usually detected at onset. Especially in younger patients, development of symptomatic hyperglycemia is rapid, and ketoacidosis common. Features of the metabolic syndrome are not usually present. Like the general population, however, many type 1 diabetic patients develop insulin resistance and features of the metabolic syndrome, which may have adverse consequences with respect to microvascular complications and CVD (72-75). Insulin resistance may alternatively develop as a consequence of hyperglycemia (glucose toxicity) (76). A subgroup of adult-onset diabetes with ICA or GADA and slow-onset insulin deficiency are now classified according to the most recent WHO classification as a subgroup of type 1 DM (69, 77, 78). In Finland, up to 10% of all diabetic patients have this form of diabetes, also called latent autoimmune diabetes in adults (LADA) (77, 79).

Type 2 diabetes is the most common form of diabetes, about 85% in Finland (71). Due to dietary habits and increasing obesity and sedentariness in both Western and developing countries, the prevalence of type 2 DM is growing at an exponential rate (80-82). Type 2 DM is characterized by insulin resistance coupled with an inability of the pancreas to sufficiently compensate by increasing insulin secretion, with onset generally in middle or old age. Onset is insidious, and ketoacidosis is rare. The prevalence of type 2 DM among adults varies from less than 5% to over 40% depending on the population in question (1). The pathogenesis of type 2 diabetes is still unclear, although multiple genetic and environmental factors clearly interplay to produce the disease. Although the pathophysiology is still unclear, variable defects of metabolism in skeletal muscle, fat, liver and pancreas contribute to increased insulin resistance and abnormal insulin secretion. In the Botnia study, roughly 85% of type 2 diabetic patients had the metabolic syndrome as defined by the WHO (69).

The current WHO criteria for type 2 diabetes mellitus use a fasting plasma glucose level of ≥7.0 or a two-hour post-load level of 11.1 mmol/l in a 75-g oral glucose tolerance test as cutoffs for type 2 diabetes (69). These criteria are similar to the American Diabetes Association criteria (83). The American criteria differ especially from previous criteria in that
an oral glucose tolerance test is recommended only when the fasting glucose level is below 7.0 mmol/l but the suspicion of diabetes is high (84).

Maturity-onset diabetes of the young (MODY) is a genetically, metabolically, and clinically heterogeneous type of type 2 diabetes mellitus that appears to account for less than 5% of diabetes (85, 86). Gestational diabetes mellitus is carbohydrate intolerance with onset or first recognition during pregnancy (87). Women with gestational diabetes also are at greater risk for developing type 2 diabetes themselves (88). There are numerous other uncommon forms of diabetes that are not included in the above classifications. Insulin-deficient diabetes can result from destruction of islet cells through acute, recurrent, or chronic pancreatitis (89). Rare mitochondrial mutations have been described in which diabetes is a manifestation (90). Uncommon lipodystrophy syndromes are frequently associated with hyperinsulinemia and subsequent diabetes (91).

2.2 The lipid profile in type 1 diabetes

The lipid profile is quantitatively normal in type 1 diabetic patients in good glycemic control and without microvascular complications, with only subtle adverse changes in e.g. VLDL and LDL size and HDL and LDL cholesterol triglyceride content (92, 93). Despite a relatively normal lipid profile on average, it should be noted that similarly high proportions of type 1 diabetic patients as non-diabetic individuals have elevated LDL lipoprotein concentrations (92, 93). In patients in poor glycemic control or who have nephropathy, elevated LDL cholesterol, apolipoprotein B and triglyceride levels are more often present (92-94). Adverse levels of HDL cholesterol and triglycerides are also associated with manifestations of the metabolic syndrome in type 1 diabetes (74).

2.2.1 Lipoproteins, apolipoproteins and lipids as risk factors in type 1 diabetes

Decreased HDL and high LDL cholesterol and triglyceride levels are established cardiovascular risk factors in non-diabetic (95) and type 2 (non-insulin-dependent) DM individuals (96). The role of the HDL subfractions HDL_{2} and HDL_{3} are more controversial, with some (97), but not all (98) studies suggesting that HDL_{2} cholesterol may be more important in reducing cardiovascular risk because of its role in reverse cholesterol transport (99). Low apolipoprotein (apo) A-I and high apo B levels are also associated with increased risk for cardiovascular death (98). Much less, however, is known of the role of lipoprotein and apolipoprotein levels in the pathogenesis of the accelerated atherosclerosis (100) in type 1 DM. Even so, results from cross-sectional studies suggest that lipoprotein and apolipoprotein levels are also important cardiovascular risk factors in type 1 DM (73, 101, 102).

2.2.2 Aerobic exercise and the lipid profile in type 1 diabetes mellitus

Regular exercise in non-diabetic subjects is best known to increase HDL cholesterol and the HDL/total cholesterol ratio (e.g., (103, 104); reviewed in (23, 105)). Many studies have also shown that endurance training decreases LDL cholesterol and less frequently triglyceride
levels (e.g., (27)). The role of weight loss or body composition changes in these lipid changes is still controversial (106, 107), although many studies have shown favorable effects of regular exercise on the lipid profile independent of weight loss (108). Antiatherogenic effects of physical exercise on apolipoproteins B (apo B) and A-I (apo A-I) in non-diabetic individuals have been less consistently observed, but appear to have been related mainly to weight loss (109-113).

Results from mainly small and uncontrolled studies testing the effects of regular aerobic exercise on the lipid profile in type 1 DM individuals have been variable. In a small controlled but not randomized study Yki-Jarvinen et al. (29) also found increases in the HDL/total cholesterol ratio, without significant changes in HDL- or total cholesterol, body mass index (BMI) or glycemic control after six weeks of ergometer cycling exercise for 60 min 4 days a week. The relative change did not differ significantly between the training and control groups, however. In an uncontrolled study investigating the effect of three months of regular exercise in 20 type 1 DM men and women 22-48 years old, LDL decreased by 14% and HDL increased by 10%, with concomitant weight loss and decreased percent body fat (31). Corresponding changes in apo B and apo A-I were also found. A 16-week program of 60 min mixed and aerobic exercise three times a week decreased total cholesterol without effects on HDL, triglycerides, body weight or glycemic control in nine 25-46 year old men with type 1 DM in an uncontrolled study (28). Twenty minutes of daily bicycle exercise had no effect on major lipid profile indices after five months in 25-45 year old women with type 1 DM, although a small improvement in maximal oxygen consumption (VO_{2max}) was noted (30). Reasons for conflicting results may be differences in the number, age and gender of the subjects, the type of training protocol, glycemic status and baseline lipid status or seasonal variation in lipids.

2.3 Oxidative stress and antioxidant defenses

Oxidative stress has been defined as the imbalance of pro-oxidant and antioxidant forces in favor of the former (114, 115). Oxidative stress can result in widespread lipid, protein and DNA damage (36), including oxidative modification of LDL cholesterol, believed to be central in the pathogenesis of atherosclerosis, and endothelial dysfunction (32, 35, 37, 38, 114). Oxidized LDL cholesterol is found in high concentrations in atherosclerotic lesions, and at least in vitro, uptake of LDL by mononuclear cells and macrophages does not occur without oxidation of the LDL (32, 35, 37, 38, 114, 116). The apparent increased oxidative stress in diabetes mellitus has been implicated in the accelerated atherosclerosis and microvascular complications of diabetes (32-35).

The mechanisms underlying the increased oxidative stress in diabetes are not entirely clear. Accumulating evidence points to many, often interrelated mechanisms (32-35), increasing production of reactive oxygen species (ROS) such as superoxide (42-45) or hydrogen peroxide (46, 47), or decreasing antioxidant defenses (Figure 1, (48-51)). Glucose autooxidation and formation of advanced glycation endproducts (AGE) not only generate ROS,
but also may activate nuclear factor-κB and adhesion molecules and induce lipid peroxidation (32, 53, 117). Activation of the polyol pathway may decrease the NADPH/NADP⁺ ratio, resulting in reductive stress and possibly adversely affecting NADPH-dependent antioxidant enzyme activity (33, 34, 54-57). Increased reductive and oxidative stress may also alter cell and glutathione redox status (54-57) and ascorbate metabolism (58, 118-120), although plasma vitamin E levels are not decreased (121). Glycation may also inactivate antioxidant enzymes like glutathione reductase and superoxide dismutase (59-61). Endothelial dysfunction and injury may occur as a result of increased oxidative stress (35, 122). Nitric oxide, itself an ROS, may react with superoxide to form the highly toxic peroxynitrite radical (122). Increased prostaglandin synthesis and alterations in the balance of opposing prostaglandins may also contribute to endothelial dysfunction and platelet activation (35). High insulin and insulin-like growth factor-1 concentrations may increase superoxide production in mononuclear cells (63). Insulin resistance has also been linked to lipid peroxidation and impaired antioxidant defenses (62-64). No consensus has been reached as to the relative importance of these mechanisms.

2.3.1 Alterations in glutathione metabolism in type 1 diabetes

Tissue glutathione plays a central role in antioxidant defenses (115, 123). Reduced glutathione (GSH) detoxifies reactive oxygen species such as hydrogen peroxide and lipid peroxides directly or in a glutathione peroxidase (GPX)-catalyzed mechanism. Glutathione also regenerates the major aqueous and lipid phase antioxidants ascorbate and α-tocopherol. Glutathione reductase (GRD) catalyzes the NADPH-dependent reduction of oxidized glutathione, serving to maintain intracellular glutathione stores and a favorable redox status. Glutathione-S-transferase (GST) catalyzes the reaction between the -SH group and potential alkylating agents, rendering them more water soluble and suitable for transport out of the cell. GST can also use peroxides as a substrate (124).
Platelet GSH content were ten-fold lower in type 1 DM patients with glycated Hb greater than 7%, but no further decrease was found when glycated Hb was greater than 11% (125). Di Simplicio et al. (126) found normal GSH levels, increased GRD activity and decreased thiol transferase activity in platelets of 46 type 1 DM patients. Platelets from the DM patients also had a lower level of threshold for aggregation induced by arachidonic acid. Children with type 1 DM also had lower erythrocyte GSH than control subjects (127). Hemoglobin A\textsubscript{1c} (Hb\textsubscript{A1c}) was inversely correlated with red cell GSH content. Thornalley et al. (128) found an inverse correlation between erythrocyte GSH levels and the presence of DM complications in type 1 patients. Normal blood GSH levels were found in 43 patients with type 1 DM compared to 21 non-diabetic subjects (129).

Most studies have found decreased blood or red cell glutathione levels in type 2 DM patients (56, 130-133). Less firm conclusions can be drawn in type 1 DM patients. Further information is also needed about whether levels are decreased in patients without complications and whether patients with complications have even lower levels, although some studies do suggest this. The pathophysiological significance of decreased glutathione levels in diabetes remains to be shown.

2.3.2 Glutathione-dependent enzymes in type 1 diabetes

Blood GRD activity was lower in 11 children with type 1 DM compared to 49 healthy children (134). On the other hand, normal red cell GRD activity has been found (125, 135) In type 1 DM red cell selenium content and GPX activity were decreased (136). Walter et al. (135) found no difference in whole blood GPX activity in 57 type 1 and type 2 DM patients compared to 28 non-diabetic control patients, a finding supported by Leonard et al. (137). Normal red cell GST enzyme kinetics have also been found in type 1 DM patients (125).

Changes in glutathione-dependent enzymes in diabetic patients are inconsistent. Differences in results cannot be completely explained by study methodology.

2.3.3 Impairment of superoxide dismutase and catalase activity in type 1 diabetes

Superoxide dismutase and catalase are major antioxidant enzymes (138). SOD exists in three different isofoms. Cu,Zn-SOD is mostly in the cytosol and dismutates superoxide to hydrogen peroxide. Extracellular (EC) SOD is found in the plasma and extracellular space. Mn-SOD is located in mitochondria. Catalase is a hydrogen peroxide decomposing enzyme mainly localized to peroxisomes or microperoxisomes. Decreased Cu,Zn-SOD activity coupled with the increased superoxide or H\textsubscript{2}O\textsubscript{2} production that may occur in DM (43, 44) could predispose to increased oxidative stress, especially if not compensated with increased catalase or Se-GPX activity. Superoxide may react with other reactive oxygen species such as nitric oxide to form highly toxic species such as peroxynitrite, in addition to having direct toxic effects (35). Alternatively, superoxide can be dismutated to much more reactive hydrogen peroxide, which through the Fenton reaction can then lead to highly toxic hydroxyl radical formation (44).
Red cell Cu,Zn/SOD activity has also been found to be decreased in type 1 DM patients (61, 139). Red cell glycosylated Cu,Zn-SOD levels were elevated in type 1 DM patients (61). Glycation appears to decrease Cu,Zn-SOD activity, which could predispose to oxidative damage (61). Decreased red cell Cu,Zn-SOD activity has been found in type 1 DM patients with retinopathy compared to type 1 DM patients without microvascular complications (140, 141), although no difference was found between patients without retinopathy and healthy individuals (140). Yaqub et al. (142) reported increased red cell superoxide dismutase and serum malondialdehyde (MDA) in patients with type 1 DM and normo- and microalbuminuria compared to healthy subjects. There was no difference, however, between DM patients with normo- or microalbuminuria, in agreement with another study (137). In contrast, red cell Cu,Zn-SOD activity has been found to be similar in Type 1 DM patients and healthy individuals, irrespective of microvascular complications (135). EC-SOD can also be glycated, although glycation does not affect enzyme activity (143). EC-SOD activity was found to be similar in 23 children with type 1 DM of varying duration and healthy children (144).

The wide variability among studies does not allow conclusions to be drawn as to whether SOD isoform activity is abnormal in diabetic patients. Again, differences in methodology or study design do not completely explain the conflicting findings among studies. Less information is available about catalase activity in type 1 DM. Normal red blood cell catalase activity has been reported (145).

2.3.4 Lipid peroxidation in type 1 diabetes

Use of thiobarbituric acid reactive substances (TBARS) as an index of lipid peroxidation was pioneered by Yagi et al. (146), whose group also showed increased plasma TBARS levels in diabetes (147). Walter et al. (135) found increased plasma peroxide concentrations in 57 Type 1 and Type 2 DM patients compared to 28 non-diabetic control patients. Higher plasma MDA levels were found in 67 middle aged diabetic patients (20 type 1, 47 type 2) than in 40 healthy subjects (148). MDA levels showed a significant correlation with glycosylated Hb. Women with well controlled type 1 DM had higher levels of lipid peroxidation during pregnancy than healthy women (149).

Plasma TBARS levels were higher in 117 type 1 and 2 DM patients than in 53 control subjects, independently of metabolic control (150). There were no differences between type 1 and type 2 patients. Plasma MDA and lipid hydroperoxide levels were elevated in hospitalized ketotic type 1 DM patients (151). One week after achieving glycemic control with insulin treatment, MDA levels approached reference values. Plasma TBARS were elevated in women but not men in a study investigating lipid peroxidation in 56 young adult type 1 DM and 56 matched non-diabetic control subjects (152). TBARS levels were elevated in 158 DM patients compared to control subjects (153). TBARS levels were increased in 18 type 1 DM patients with no or mild retinopathy compared to previously established reference values (154). The initial plasma H2O2 and MDA levels in 15 patients with Type 1 and 15 with
Type 2 diabetes before and after 2 weeks of intensive treatment were higher than in control subjects (46). After 2 weeks of treatment, the values for both parameters were lower; although still higher than in the control group. Lipid hydroperoxides and conjugated dienes were elevated and total antioxidant capacity decreased in 72 patients with well-controlled type 1 DM and without complications, independently of metabolic control or diabetes duration (51). In a later study by the same group, these basic findings were repeated in 37 patients with uncomplicated type 1 diabetes and 29 non-diabetic men and women. Compared with the diabetic men, diabetic women had even higher levels of lipid hydroperoxides and lower antioxidant capacity (155).

On the other hand, serum levels of a conjugated diene isomer of linoleic acid was lower in type 1 DM patients than control subjects (156). No difference in serum conjugated diene levels between otherwise healthy diabetic patients and healthy control subjects were noted, although conjugated diene levels were increased in 26 diabetic patients with microangiopathy compared to 36 diabetic patients without microangiopathy and 36 control subjects (140). Plasma TBARS levels were similar in 17-40 year old type 1 DM patients as in control subjects, and were also similar in smokers (137). Zoppini et al. (157) also found similar plasma TBARS levels in 56 type 1 DM patients as in 32 age- and sex-matched control subjects, but TBARS were higher in type 1 DM smokers. No differences in plasma MDA and 8-iso-prostaglandin F2α levels were found between 38 type 1 diabetic patients and 41 control subjects, despite a lower total antioxidant capacity (121).

Whether lipid peroxidation is increased in DM even before development of micro- and macrovascular disease is unclear. Many published studies have found increased lipid peroxidation in type 1 DM patients, but conflicting results have also been found. Inconsistent evidence also suggests that increased lipid peroxidation and impaired antioxidant defenses may be more pronounced in women with type 1 DM. The differing findings cannot be explained simply based on study design or methodology. A causal role for lipid peroxidation in the development of diabetic macro- and microvascular complications is far from established.

### 2.3.5 Lipid peroxidation and type 1 diabetic complications

Jennings et al. (158) reported increased serum conjugated diene levels in 26 diabetic patients with microangiopathy compared to 36 diabetic patients without microangiopathy. Lipid peroxides were also significantly elevated in 15 type 1 patients with retinopathy compared to type 1 DM patients without microvascular complications (140). Plasma TBARS levels correlated with albumin excretion in 64 type 1 and type 2 DM patients (159). Twenty-one normotensive type 1 diabetic patients without microalbuminuria but with evidence of endothelial injury (elevated levels of plasma von Willebrand factor, soluble thrombomodulin content and angiotensin converting enzyme activity) had elevated levels of serum MDA compared to patients without evidence of endothelial injury (160). Type 1 and 2 DM patients in poor metabolic control or with angiopathy had higher levels of TBARS than those in good
control or without angiopathy, independently of lipid levels (153). In type 1 DM patients with microangiopathy, the oxidized LDL/normal LDL antibody ratio was paradoxically lower than in patients without complications, most likely due to oxidized LDL specific immune complexes found exclusively in antibody-negative patients (161).

In contrast, levels of serum MDA were similar between 33 type 1 DM patients with microalbuminuria and 49 patients without microalbuminuria (142). TBARS levels were similar in 16 patients with micro- or macroalbuminuria compared to 69 normoalbuminuric patients in young type 1 DM patients (137).

There seems to be no clear consensus as to whether patients who have developed diabetic complications have increased lipid peroxidation compared to patients without complications, although more studies have reported higher levels of lipid peroxidation in DM patients with complications than in patients without complications. Further studies are needed to clarify this issue and also whether such increased oxidative stress is pathologically important or merely a marker of micro- or macrovascular damage.

2.3.6 Susceptibility of LDL cholesterol to oxidation in type 1 diabetes

Susceptibility of LDL to oxidation was strongly correlated with degree of LDL glycosylation. LDL and red blood cell (RBC) membranes in 11 normolipidemic type 1 and 18 type 2 DM patients were more susceptible to oxidation than in normal subjects (162). The susceptibility of LDL to copper-catalyzed oxidation was greatest in 22 familial hypertriglyceridemic patients while intermediate values were found in 24 type 1, 16 type 2 and 14 abdominally obese patients compared to gluteal-femoral obese subjects and controls (163). The different susceptibility to oxidation found in the different groups of patients was only partially explained by plasma triglyceride values. Plasma TRAP (total peroxyl radical trapping potential) was less and susceptibility of LDL to oxidation as measured by the lag phase of conjugated diene formation after initiation of LDL oxidation by the addition of copper was greater in poorly controlled type 1 diabetic subjects than in normal control subjects (49). This could not be attributed to the presence of oxidation-susceptible, small, dense LDL particles in the diabetic subjects, whose lipoprotein particle distribution did not differ from the control subjects. LDL from both type 1 (n=20) and type 2 (n=20) diabetic patients exhibited a shorter lag phase duration for conjugated diene formation, regardless of the presence of vascular complications (164). LDL exhibited a shorter lagtime and a lower $\alpha$-tocopherol/LDL ratio for 10 type 1 and 53 type 2 diabetic patients than for sex and age-matched control subjects (165). The lagtime was positively correlated to the LDL $\alpha$-tocopherol/LDL and inversely correlated to HbA$_{1c}$. Recently diagnosed type 1 DM patients (n=25) with poor glycemic control showed higher electronegative LDL (suggesting a higher degree of oxidaton), similar LDL subtraction phenotype and lower susceptibility to oxidation compared to 25 matched healthy control subjects (166). After three months of intensive insulin therapy, HbA$_{1c}$ and LDL electronegativity decreased, but no changes in LDL susceptibility to oxidation or LDL subtraction phenotype were observed.
In contrast, there was no difference between 20 type 1 diabetic patients in moderate glycemic control and non-diabetic subjects in the susceptibility of LDL cholesterol to either copper-dependent or non-transition metal-dependent oxidation (167). Furthermore, there was no difference between the groups for LDL vitamin E content, LDL fatty acid composition in cholesterol esters or triglycerides, but LDL glycation was elevated in the type 1 DM subjects. There was no difference between 34 type 1 DM patients without clinical signs of vascular disease and 22 healthy control patients in the oxidizability of LDL and very-low-density lipoprotein (VLDL) (168). There was no difference in the susceptibility to in vitro oxidation of LDL isolated from 15 type 1 DM patients in good glycemic control and with no evidence of macrovascular disease or proteinuria compared with control subjects (169). The particle size, lipid composition, fatty acid content, antioxidant content, and glycation were similar for LDL isolated from both groups. LDL size was smaller in 31 type 1 diabetic patients than in 45 control subjects, but susceptibility of LDL cholesterol to oxidation was similar (170).

Most studies have found increased susceptibility of LDL cholesterol to oxidation in DM patients, although some studies have had conflicting results. Studies carried out to date do not allow firm conclusions to be drawn about whether LDL is more susceptible to oxidation in DM patients without complications than in healthy subjects, or about what effect complications and glycemic control have on the susceptibility of LDL to oxidation.

2.3.7 Autoantibodies to oxidized cholesterol in type 1 diabetes

Levels of anti-oxidized LDL antibodies and anti-MDA-modified LDL antibodies were similar in 16 type 1 diabetes mellitus patients free of macrovascular complications and 16 control subjects (171). In 101 type 1 DM normo- and macroalbuminuric patients with a long duration of diabetes and 54 healthy subjects, antibodies against MDA-modified LDL did not differ among normoalbuminuric DM, albuminuric DM and control subjects (172). In contrast, antibodies to oxidized LDL cholesterol were 1.5-fold higher in 38 type 1 diabetic patients free of macrovascular disease than in 33 normal subjects (173). Antibodies to oxidized LDL were correlated with age in normal subjects, but not with age, duration of disease, LDL-cholesterol, HbA1c or degree of microvascular complications in patients with type 1 diabetes.

Relatively few studies have examined the association of type 1 DM with autoantibodies to oxidized LDL, but no clear consensus suggesting increased oxidized LDL antibodies in type 1 diabetes has been found. The fact that type 1 diabetes has an autoimmune basis could explain some of the variation in results.

2.3.8 Oxidative stress and antioxidant defenses in physical exercise

Many recent studies have shown that even moderate exercise may increase free radical production beyond the capacity of antioxidant defenses, resulting in oxidative stress (174-179). In animals, exercise training may strengthen antioxidant defenses and may reduce resting and acute exercise-induced oxidative stress (178, 180-183). Several theses and reviews on the topic have been published by members of our research team (115, 178, 184-186).
Therefore review of oxidative stress and antioxidant defenses in physical exercise here will be limited briefly to exercise intervention studies and oxidative stress in humans.

Relatively few studies on the effect of exercise training on indices of oxidative stress or antioxidant defenses in humans have been published. A 10-month exercise program that increased VO$_{2 \text{max}}$ by 19% also decreased LDL oxidation and other lipid risk factors in an uncontrolled study in 34 sedentary men and 70 women (39). On the other hand, three months of relatively intense running training in nine fit men decreased circulating antioxidants (uric acid, SH-groups, α-tocopherol, beta-carotene, retinol) except ascorbate, without affecting the lag time for the susceptibility of plasma LDL to oxidation in vitro (187). Normoxic and especially intermittent hypoxic training attenuated the increases in lipid hydroperoxides and MDA induced by acute normoxic exercise after four weeks of aerobic training in a trial in which the normoxic training group served as the control group (40). Twelve weeks of high-intensity endurance training increased erythrocyte SOD and GPX antioxidant enzyme activities and decreased neutrophil superoxide production in response to exhausting exercise in an uncontrolled study (41). A reduction in exercise-induced lipid peroxidation in erythrocyte membrane was also observed. Reduced glutathione levels increased in five age-matched control subjects with high-intensity aerobic training, whereas only oxidized glutathione levels increased in 17 patients with COPD (188). Immediately after acute treadmill exercise, 46 claudicants developed significant neutrophil activation and degranulation with free radical damage, an effect that decreased after three months of exercise training. No effect was seen in 22 control subjects (189).

No randomized controlled trials of aerobic training on indices of oxidative stress or antioxidant defenses have yet been published. Nonetheless, some evidence suggests that exercise training may favorably affect indices of oxidative stress and antioxidant protection in some diseases and in healthy persons, although contradictory findings exist (187).

2.4 The metabolic syndrome

The concurrence of disturbed glucose and insulin metabolism, overweight and abdominal fat distribution, mild dyslipidemia and hypertension, has given rise to the concept of the metabolic syndrome, also known as Syndrome X, the Deadly Quartet, and the insulin resistance syndrome (2-6). Although the metabolic syndrome has been in the scientific limelight only since being re-introduced as Syndrome X in 1988 (2, 6), clustering of hypertension, hyperglycemia and gout was described already in 1923 (190). Insulin resistance has been considered to be the underlying abnormality of this syndrome. The pathogenesis of this syndrome has multiple origins, but obesity and sedentary lifestyle coupled with diet and still largely unknown genetic factors clearly interact to produce it (2-6, 191). The metabolic syndrome is from a clinical and public health standpoint most important because of subsequent high morbidity and mortality from diseases such as type 2 diabetes and CVD (2-9). Patients with type 1 diabetes are also not immune from the metabolic syndrome and its
consequences, including CVD and microvascular disease (72-75). Overweight and physical inactivity also appear to bring about the metabolic syndrome in type 1 diabetes (74).

As the epidemic of obesity and sedentary lifestyle continues worldwide, the metabolic syndrome and its consequences, especially diabetes, can be expected to become increasingly common at younger ages. In the US type 2 diabetes is indeed becoming alarmingly common in particularly Hispanic, black and American Indian children (82). Although the metabolic syndrome has been less closely associated with coronary heart disease than with type 2 diabetes, the obesity epidemic and its associated metabolic syndrome may explain the plateauing in the decline in the incidence of myocardial infarction over the past ten years in the United States (192).

2.4.1 Pathophysiology of the metabolic syndrome

The pathogenesis of the metabolic syndrome is poorly understood, and will be discussed here only briefly. An abdominal distribution of fat appears to be particularly deleterious (Figure 2; (193-196)). Abdominal fat can also be divided into subcutaneous and visceral compartments that can be assessed with computed tomography or magnetic resonance imaging. Mainly experimental evidence suggests that abdominal obesity may mediate its deleterious effects on carbohydrate and lipid metabolism through the increased lipolytic activity of especially omental fat, which drains directly into the portal-venous system (197). This in turn results in higher non-esterified fatty acid concentrations, with consequent insulin resistance in the liver and skeletal muscle, and dyslipidemia. According to this “portal hypothesis”, because of the higher lipolytic activity of visceral than subcutaneous abdominal fat, visceral fat should be more closely associated with insulin resistance and its associated metabolic derangements (197). The pathophysiological significance of these subdivisions are unclear, however (22, 198-207).

More recently, the concept of ectopic fat deposition has been developed (208-211). In addition to the quantity of abdominal subcutaneous and visceral fat, the degree of lipid storage in skeletal muscle and liver has also been shown to be powerful determinants of insulin sensitivity. Peripheral adipocytes have limited reserves for storing fat. Those reserves in turn depend in part on genetic and environmental factors. As the ability of the peripheral adipocyte to store fat is exceeded, the fat cells become insulin resistant, resulting in increased lipolysis and release of fatty acids into the blood stream, and decreased uptake of fatty acids. This in turn results in not only abdominal subcutaneous and visceral fat deposition, but also storage of lipids in liver and skeletal muscle.

Triglyceride accumulation in the liver results in decreased hepatic insulin sensitivity and increased VLDL production, which results in increased transfer of cholesterol esters from HDL and LDL cholesterol to VLDL cholesterol in exchange for triglyceride (99, 208, 209). This in turn impairs reverse cholesterol transport and results in a decrease HDL levels, a shift in balance to HDL₃ cholesterol, and a shift from large buoyant LDL particles to small dense
LDL particles. Increased hepatic insulin resistance also results in inappropriate gluconeogenesis postprandially.

Skeletal muscle is a major determinant of whole-body glucose disposal (209, 210). More recent evidence suggests that intramuscular lipid deposits play a major role in decreasing glucose uptake in skeletal muscle (208-211). Intramuscular lipids appear to decrease glycogen synthesis and impair glucose transport by activating protein kinase C0, which results in a cascade that phosphorylates insulin substrates 1 and 2, impairing the insulin receptor's ability to activate phosphatidylinositol kinase 3 and ultimately impairing glucose transport into the cell. Paradoxically, the ability to utilize fatty acids as an energy source in the resting state is impaired in insulin resistance, whereas in insulin-stimulated states, glucose oxidation is impaired (210).

As the metabolic syndrome becomes more severe, interplay between genetic susceptibility, insulin resistance and diet may lead to progressive β-cell failure and impaired insulin secretory capacity (212-216). As β-cell secretory capacity declines, impaired glucose tolerance (IGT) develops. IGT is common in older persons, up to 25% of individuals of European descent. Roughly 5-10% of persons with IGT convert to frank diabetes yearly, again with weight gain, diet, genetic susceptibility and insulin resistance contributing to the progressive β-cell failure. The manifestations of cardiovascular risk factors such as dyslipidemia, hypertension, endothelial dysfunction, inflammation, hypercoagulability and impaired fibrinolysis, obesity and abnormal insulin and glucose metabolism predispose persons with the metabolic syndrome to development of another important end-stage consequence of the metabolic syndrome, cardiovascular disease (2-9).

Disturbances in the adrenal-pituitary axis (217), inflammation (218) and abnormal sex steroid metabolism (219) have all been proposed to contribute to or exacerbate the development of the metabolic syndrome, but evidence for these abnormalities as the primary mechanism for the pathogenesis of the metabolic syndrome is insufficient. Adipose tissue also produces hormones, cytokines and other peptides such as angiotensinogen, adipin, acylation-stimulating protein, adiponectin, retinol-binding protein, leptin, resistin, tumor necrosis factor α, interleukin 6, plasminogen activator inhibitor-1 that may play a role in insulin resistance, inflammation and the development of diabetes and CVD (216, 218, 220).

The pathophysiology behind the association of obesity and insulin resistance with hypertension is also poorly understood. Contributing mechanisms include resistance to insulin-mediated vasodilation and endothelial dysfunction (221, 222), hyperinsulinemia-mediated increased sodium and water absorption (221, 223, 224) and activation of the sympathetic nervous system (221, 223, 224).

Environmental and genetic (225, 226) factors contribute to both the development of overweight and the propensity for insulin resistance and ectopic fat deposition and other manifestations of the metabolic syndrome (Figure 2). Environmental factors include sedentary lifestyle and poor physical fitness (23, 68, 227), diet (214, 227, 228), low childhood and adult socioeconomic status (229-231) and low birthweight and rapid childhood growth (232-234).
2.4.2 Definitions of the metabolic syndrome

Despite the abundant epidemiological and experimental research that has been published on the metabolic syndrome, definitions of the metabolic syndrome and the various cut-offs for its components have varied widely (6). The World Health Organization (WHO) consultation for the classification of diabetes and its complications (69) and the National Cholesterol Education Program (NCEP) Expert Panel have recently published definitions of the metabolic syndrome (70).

The WHO published a working definition of the metabolic syndrome meant to facilitate research on the metabolic syndrome and aid comparability between studies, rather than serve as a strict definition (69). The metabolic syndrome was defined (without assumptions of causality) for men as: insulin resistance in the top 25% of the population as measured by the euglycemic hyperinsulinemic clamp or presence of impaired glucose tolerance (IGT) or type 2 diabetes and the presence of at least two of the following: abdominal obesity (waist-hip ratio >0.90 or BMI ≥30 kg/m²), dyslipidemia (serum triglycerides ≥1.70 mmol/l or HDL cholesterol <0.9 mmol/l), hypertension (≥160/90), or microalbuminuria. These core components were considered most suitable for a general definition (6), although many other disturbances, e.g. disorders of coagulation and endothelial function, hyperuricemia and elevated leptin levels, have been associated with the metabolic syndrome (Figure 2).
This working definition has not been without criticism. Inclusion of microalbuminuria as a core component is controversial, and microalbuminuria in non-diabetic individuals is uncommon (235-238). The most appropriate measure of abdominal obesity is also in dispute. Although waist-hip ratio may carry information relevant to disease endpoints independently of waist girth or BMI (196), waist circumference correlates better with visceral fat deposits as measured by computerized tomography (239). Defining adiposity as waist girth ≥94 cm has been proposed by the European Group for the Study of Insulin Resistance (EGIR) (238). Furthermore, the euglycemic hyperinsulinemic clamp is not practical for epidemiological research. The EGIR recommended use of fasting insulin levels to estimate insulin resistance and IFG as a substitute for IGT in epidemiological studies (238). The EGIR also proposed lower cut-offs for hypertension (≥140/90) (238, 240, 241) that are in accordance with current WHO-ISH (International Society of Hypertension) and Sixth Joint National Committee recommendations (238, 240, 241).

The NCEP Expert Panel has also recently published a definition of the metabolic syndrome for clinical use (70). The metabolic syndrome was defined as three or more of the following: fasting plasma glucose levels ≥6.1 mmol/l, serum triglycerides ≥1.7 mmol/l, serum HDL <1.0 mmol/l, blood pressure ≥130/85 mmHg, waist girth >102 cm. Use of waist circumference >94 cm was suggested for some men who may be genetically susceptible to insulin resistance (70). Over 30% of middle-aged persons in the US have been reported to have the metabolic syndrome as defined by the NCEP (10).

2.4.3 Components of the metabolic syndrome

2.4.3.1 Hyperinsulinemia and insulin resistance

Hyperinsulinemia and insulin resistance have consistently predicted type 2 diabetes, even when adjusted for other components of the metabolic syndrome (242-245). Hyperinsulinemia has also predicted hypertension independently of obesity (246-248), although in some studies only in subgroups, such as non-diabetic non-Hispanic whites (249) and lean normoglycemic individuals (250). Hyperinsulinemia has also predicted dyslipidemia independently of obesity in some (248, 250), but not all (251) studies. These findings suggest that insulin resistance may precede development of hypertension and dyslipidemia in the early stages of the metabolic syndrome. Hyperinsulinemia has also predicted CVD incidence or mortality (252-258), although often not independently of other cardiovascular risk factors (252, 253, 255, 257).

The gold standard for measuring whole-body insulin resistance is the euglycemic hyperinsulinemic clamp (259). The procedure is time- and labor-intensive, however, and not practical for especially epidemiological studies or routine clinical use. As a substitute, use of fasting insulin levels has been recommended (238). Indeed, fasting insulin levels have a correlation of at least 0.6 in non-diabetic individuals (260). Although no internationally agreed cut-offs are available, the top quarter of insulin resistance as measured by the clamp
(69) or as estimated by fasting insulin levels (238) has been recommended. The homeostasis model assessment (HOMA) (261) is a common method of estimating insulin resistance based on fasting insulin and glucose levels. The recently validated quantitative insulin sensitivity check index (QUICKI) is also based on fasting insulin and glucose concentrations and is closely (inversely) correlated with HOMA, differing mainly in being normally distributed (262). The correlation of insulin sensitivity as estimated by QUICKI and the euglycemic clamp was 0.75, better than the minimal model intravenous glucose tolerance test (262). Some controversy still exists, however, about whether these measures predict insulin resistance better than fasting insulin levels (263).

Factor analysis has been used to reduce intercorrelated variables into a smaller set of underlying uncorrelated factors that can be used to explain complex underlying physiological phenomena, and is particularly well suited for analysis with components of or related to the metabolic syndrome (8, 264, 265). Although previous studies sometimes have generated separate lipid (7, 8, 266) or blood pressure factors (7, 266-270), with differences at least in part related to the variables entered into the analyses, the factor explaining the greatest variance has consistently had heavy loadings by measures of adiposity and fat distribution, insulin and glucose (7, 8, 264, 266-272), all components of the metabolic syndrome.

2.4.3.2 Hyperglycemia

In epidemiological studies employing factor analysis, fasting glucose and two-hour post-load glucose levels have also consistently associated with the factor explaining the greatest variance and having heavy loadings by measures of adiposity and fat distribution and insulin (7, 8, 264, 266-272). Both fasting and two-hour post-load glucose levels can therefore be considered a core component of the metabolic syndrome.

Type 1 and type 2 diabetes mellitus have a well-characterized 2-4-fold increased risk for CVD that is independent of known cardiovascular risk factors (100, 273, 274). IFG and IGT also predict cardiovascular mortality (275-277). There is a graded increase in the cardiovascular risk of fasting and two-hour post-load glucose levels even in the normal range (278). Both IFG and IGT are strong predictors of future diabetes (279-281).

2.4.3.3 Overweight and an abdominal fat distribution

The most widely used measure of adiposity is the BMI (kg/m²), which is independent of height. Despite its crudeness, BMI provides a good index of overall adiposity at the population level (68). Somewhat more accurate calculations of percent body fat may be obtained from skinfold measures and bioelectrical impedance, but these measures require sex- and age-dependent norms that may vary from population to population (282, 283). The most accurate and widely used measurements of adiposity are currently obtained through underwater weighing and dual-energy X-ray absorptiometry (282, 283), although these methods are not practical for most epidemiological studies. The WHO and the National
Institute of Health have defined overweight as BMI $\geq 25$ kg/m$^2$, and obesity as BMI $\geq 30$ kg/m$^2$ (68, 284).

An abdominal distribution of fat appears to be particularly deleterious (193-196). Waist and the waist-hip ratio are the most common anthropometric measures of abdominal fat distribution. Waist girth and even BMI correlate better than the waist-hip ratio with CT or MRI measures of abdominal obesity (285). It has been suggested that the use of waist circumference should be preferred over waist-hip ratio (68, 284), although the waist-hip ratio may offer additional information affecting health outcomes not related to abdominal fat distribution (81). It should be noted, however, that as obesity increases, abdominal obesity also generally increases. Even BMI alone correlates nearly as well as waist circumference with abdominal fat as measured by computed tomography (285). Cut-offs of 94 cm and 102 cm for waist circumference have been suggested as action levels for intervention in men. These cut-offs are based on a large cross-sectional population-based study in the Netherlands, in which those cut-offs corresponded to BMIs of $\geq 25$ and 30 kg/m$^2$, and were associated with increased prevalence of chronic diseases and cardiovascular risk factors (286).

Visceral abdominal fat has been reported to be associated with insulin resistance independently of total body fat or subcutaneous abdominal fat (22, 198-202), but many other studies have found that subcutaneous abdominal adipose tissue is as strong or stronger correlate of insulin resistance (203-207). Subcutaneous fat tissue can be further divided into deep and superficial compartments, and visceral fat tissue can be divided into retroperitoneal and intraperitoneal compartments (205, 207, 287), although the pathophysiological significance of these subdivisions are unclear (202).

Adiposity and an abdominal fat distribution have also consistently loaded onto the factor explaining the greatest variance and having heavy loadings by measures of insulin and glucose metabolism in epidemiological studies employing factor analysis (7, 8, 264, 266-272). Although insulin resistance has been considered to be the underlying abnormality of the metabolic syndrome, overweight and obesity are clearly the main triggering factors (6).

An abdominal distribution of fat as measured by waist girth or waist-hip ratio has predicted cardiovascular endpoints even after adjustment for BMI (193-196). Interestingly, the independent contribution of waist circumference or waist-hip ratio over BMI to the development of diabetes is not as clear (288-290).

2.4.3.4 Dyslipidemia

Low fasting serum HDL cholesterol levels and hypertriglyceridemia are consistently associated with the other components of the metabolic syndrome (2-6, 251, 291). Other lipid subfractions such as apolipoprotein A1 and B levels, small dense LDL cholesterol and HDL subfractions are associated with the metabolic syndrome as well (6, 251, 291).

Dyslipidemia has predicted the incidence of type 2 diabetes mellitus in several studies (292-295). Low HDL cholesterol levels are a well-established risk factor for CVD (296). The independent role of triglycerides as a cardiovascular risk factor is more controversial,
although a meta-analysis suggests that triglycerides are an independent risk factor (297). The Veterans Affairs High-Density Lipoprotein Cholesterol Intervention Trial showed a decrease in cardiovascular events in men with low HDL cholesterol levels but normal LDL cholesterol levels who treated with gemfibrozil. Because gemfibrozil is an HDL-elevating and triglyceride-lowering drug, this study offers additional support for the importance of triglyceride and HDL levels as cardiovascular risk factors (298).

2.4.3.5 Blood pressure

Hyperinsulinemia was associated with the incidence of hypertension and dyslipidemia in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) cohort of middle-aged men (248). Obesity and abdominal fat distribution also have a well-described association with hypertension (299-306).

Hypertension is a classic cardiovascular risk factor, as has been demonstrated by both longitudinal cohort studies and blood pressure medication trials (307, 308). The magnitude of the decrease in coronary morbidity and mortality is less than what would be predicted by epidemiological studies, however. This has been speculated to be due in part to adverse effects of (high-dose) diuretics and (non-selective) beta-blockers on insulin resistance, dyslipidemia and other factors related to the metabolic syndrome, or alternatively, that only part of the mortality associated with hypertension is due to blood pressure itself (309-312). Hypertension is also an independent risk factor for type 2 diabetes (292, 293, 295, 313).

2.4.3.6 Physical activity and cardiorespiratory fitness

In intervention studies in non-diabetic persons, aerobic physical exercise has in variable degrees and at least in the short term decreased weight and visceral fat accumulation (20-23), improved insulin sensitivity (20-23) increased HDL cholesterol and decreased triglyceride levels (23-25), and decreased blood pressure (23, 26) in addition to increasing cardiorespiratory fitness. These changes have often occurred independently of weight loss, although it is not completely clear how much of these favorable effects are independent of weight loss and changes in body composition (314).

The mechanisms by which exercise may increase insulin sensitivity independently of weight loss are only partly understood. Exercise appears to acutely increase glucose uptake in part through the mechanistic action of contraction, perhaps partially mediated by increased translocation of glucose transporter protein (GLUT) 4 to the plasma membrane (315). Tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 is also increased (315), which may explain in part increased insulin-stimulated glucose transport in skeletal muscle. The acute effects of exercise mostly disappear within 24 hours. More chronically, regular exercise appears to increase GLUT 4 protein expression and translocation (315), insulin receptor autophosphorylation (316), insulin-stimulated glucose transport in skeletal muscle (315), whole body glucose disposal and glucose tolerance (317, 318). Other factors contributing to the mechanisms by which regular exercise may increase insulin
sensitivity include effects on the interplay between skeletal muscle fiber type, oxidative capacity and intramuscular lipid content (319) and blood flow and endothelial function (320). Mechanisms by which physical exercise may produce favorable changes more specifically in lipoprotein and lipid metabolism even in the absence of weight loss include decreasing hepatic triglyceride lipase activity and increasing skeletal muscle lipoprotein lipase activity (108, 321). Hepatic lipase activity seems to be inversely related to insulin sensitivity (322), whereas skeletal muscle lipoprotein lipase activity may be positively related to insulin sensitivity (323).

No trials or observational studies regarding physical fitness or aerobic exercise and the development of the metabolic syndrome using standard definitions have been published. In a cross-sectional study, Whaley and coworkers (324) found a strong inverse dose-response relationship between total time on a maximal treadmill exercise test and the number of metabolic abnormalities in a large cohort of men and women attending the Cooper Clinic. Carroll et al. (325) observed an inverse dose-response relationship between indirectly predicted VO_{2max} (in ml/kg per minute), and the likelihood of the clustering of metabolic factors in 711 working men who presented for preventive assessment at a private hospital. In the study by Carroll and coworkers (325), the age-adjusted association between physical activity and the likelihood of the clustering of metabolic factors increased with the intensity of physical activity.

Recently, lifestyle interventions including regular physical activity have been shown to more than half the incidence of diabetes in persons with IGT (326, 327). Whether this would also apply to persons with the metabolic syndrome in general, or whether exercise alone would have a therapeutic effect has not been tested.

Observational studies suggest that physical activity and cardiorespiratory fitness may decrease the risk for CVD both in non-diabetic persons (12-17) and those with type 1 (18) and type 2 diabetes (19). Longitudinal cohort studies also show a decreased incidence of diabetes mellitus in persons who are fit or who engage in moderate or vigorous levels of physical activity compared to sedentary or unfit individuals (67, 328, 329).

The relative benefit of vigorous physical activity compared with moderate-intensity physical activity has been debated. Although the trial and epidemiological data are not completely consistent, vigorous physical activity and high cardiorespiratory fitness seem to offer greater benefit against most cardiovascular and metabolic risk factors than moderate physical activity or fitness (23). The shape of the dose-response relationship for the intensity of physical activity or cardiorespiratory fitness with respect to cardiovascular mortality has also been debated. Some have argued that the relationship is curvilinear (330), with the most benefit gained in the low-fit or sedentary groups, whereas others have argued that at least for physical activity the relationship is linear (331), or even that a minimum threshold level in intensity around 6 METs is necessary for physical activity to be cardioprotective (332). Low-intensity leisure-time physical activity (LTPA) has consistently been less strongly associated
with most chronic disease endpoints than moderate or vigorous exercise (23, 65, 67), but may have other important functions, e.g. in weight control after weight loss in the obese (333).

Based on the intervention and epidemiological evidence, the Center for Disease Control (CDC) and the American College of Sports Medicine (ACSM) have jointly published recommendations that adults engage in at least 30 min of moderate physical activity on most, and preferably all, days of the week (23). It is nonetheless acknowledged that further benefit may be gained by engaging in regular vigorous physical activity.

2.4.3.7 Other factors related to the metabolic syndrome

Many other factors have also been found to be associated with the metabolic syndrome (6), including other lipid, lipoprotein and apolipoprotein abnormalities such as increased small dense LDL lipoprotein (334), elevated apolipoprotein B and decreased apolipoprotein A-2 concentrations, hemostatic factors including fibrinogen (335, 336), inflammatory factors including C-reactive protein (CRP) (336-339), hyperuricemia (340), hyperleptinemia (341), endothelial dysfunction (342), sleep apnea (343) and alterations in sex hormones including decreased testosterone levels in men, increased androgen levels in women and decreased sex hormone binding globulins in both sexes (341, 344, 345). Microalbuminuria has also been proposed to be related to the metabolic syndrome (235, 346).

For the purpose of a general definition, however, abdominal obesity, disturbances in insulin and glucose metabolism, dyslipidemia and hypertension as core components have been considered most appropriate (69). Microalbuminuria was originally proposed by the WHO as a core component of the metabolic syndrome. Microalbuminuria in non-diabetic individuals is uncommon (347), however, and inclusion of microalbuminuria as a core component is controversial (235-238). The NCEP considered similar core components in their clinically oriented definition of the metabolic syndrome, but did not include a measure of insulin resistance or hyperinsulinemia, nor did they include microalbuminuria (70).
3 AIMS OF THE STUDY

The purpose of this series of studies was to assess the effect of aerobic exercise training on lipid and lipoprotein levels and resting and exercise induced oxidative stress in type 1 diabetes and to assess the association of leisure-time physical activity and cardiorespiratory fitness with development of the metabolic syndrome.

The specific aims of the study were:
1. To test the hypothesis that a 12-16 week aerobic exercise program would induce antitherogenic changes in lipid, lipoprotein and apolipoprotein levels and to assess the role that changes in body fat, body mass or glycemic control may play in mediating the effect of regular exercise on those changes in men with type 1 DM. (Study 1).
2. To investigate oxidative stress at rest and in response to acute physical exercise using widely used indices of lipid peroxidation and glutathione redox status and the relation of oxidative stress to cardiorespiratory fitness in otherwise healthy young men with type 1 diabetes mellitus (Study 2).
3. To compare selected major antioxidant enzyme activities at rest and in response to acute physical exercise in otherwise healthy young men with type 1 diabetes mellitus (Study 3).
4. To compare the sensitivity, specificity and risk for prevalent and incident diabetes of definitions of the metabolic syndrome based on the WHO consultation and NCEP recommendations in a cohort of middle-aged non-diabetic men who were followed for four years (Study 4).
5. To investigate the associations of leisure-time physical activity of various intensities and cardiorespiratory fitness with development of the metabolic syndrome as defined by the WHO and the NCEP over four years in middle-aged non-diabetic men from Eastern Finland without the metabolic syndrome at baseline (Study 5).
4 METHODS

4.1 Subjects.

The Ethics Committee of the University of Kuopio approved all studies. All subjects gave their written informed consent. The design and size of the studies are summarized below.

Table 1. The design, size, type of intervention or length of follow-up and endpoints of studies 1-5.

<table>
<thead>
<tr>
<th>Study</th>
<th>Design</th>
<th>Size</th>
<th>Intervention/Follow up</th>
<th>Endpoints</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>randomized controlled trial</td>
<td>20 trained and 22 control type 1 diabetic men</td>
<td>12-16 weeks of running exercise</td>
<td>lipid profile</td>
</tr>
<tr>
<td>2</td>
<td>case-control study</td>
<td>9 type 1 diabetic and 13 control men</td>
<td>40 min exercise at 60% VO2max</td>
<td>lipid peroxidation</td>
</tr>
<tr>
<td>3</td>
<td>case-control study</td>
<td>9 type 1 diabetic and 13 control men</td>
<td>40 min exercise at 60% VO2max</td>
<td>endogenous antioxidants</td>
</tr>
<tr>
<td>4</td>
<td>prospective cohort study</td>
<td>1005 non-diabetic middle-aged men</td>
<td>four-year follow up</td>
<td>association of the metabolic syndrome with diabetes</td>
</tr>
<tr>
<td>5</td>
<td>prospective cohort study</td>
<td>612 (77%) non-diabetic men without the metabolic syndrome</td>
<td>four-year follow up</td>
<td>association of physical activity and fitness with the metabolic syndrome</td>
</tr>
</tbody>
</table>

Study 1. Otherwise healthy men (n=56) aged 20-40 years with type 1 DM were recruited into study 1 after giving written informed consent (Table 4, Results section). Diabetic subjects were chosen from patients diagnosed with type 1 diabetes who were followed at the Kuopio University Hospital, the North Karelian Central Hospital and the Joensuu Health Center in Eastern Finland. Subjects were in moderate glycemic control (mean HbA1c = 8.3% ± 1.2; normal range 4.0 - 6.0%), without clinically evident atherosclerotic disease or microalbuminuria (no record of overnight urinary albumin excretion ≥20 μg/min and normal serum creatinine). None had more than mild background retinopathy as determined by an ophthalmologist based on ophthalmoscopy or fundus photographs. Achilles reflexes were missing in three subjects. Patients were otherwise without signs or symptoms of peripheral or autonomic neuropathy. Ten patients entering the study were current smokers. All underwent clinical examination, routine laboratory tests and electrocardiogram to rule out significant diseases. Reasons for exclusion included any cardiovascular (based on symptoms or electrocardiogram changes suggestive of ischemia during a maximal exercise test) or pulmonary disease, and chronic medication other than insulin.

Randomization (Study 1). The men were divided into two groups based on median age. Subjects within the two groups were paired based on VO2max and randomized into trained and control (untrained) groups. The trained group underwent a 12-16 week program of moderate intensity endurance training. The untrained group was instructed to continue their normal level of physical activity. Of the 56 men participating in baseline measurements, 42 (training
group n=20, age 31.7 ± 5.8 y [mean ± SD], duration of diabetes 13.8 ± 9.2 y; control group n=22, age 29.8 ± 6.4 y, duration of diabetes 10.8 ± 5.8 y) completed the study. All subjects were encouraged to finish the study regardless of adherence to the exercise goals of the group to which they were assigned. Reasons given by those not completing the study included time constraints imposed by work, major home repairs and moving out of the area. Of the six smokers finishing the study, two belonged to the training group and four to the control group.

Training (Study 1). The training program consisted of 12-16 weeks of moderate intensity, sustained running. The first week consisted of 20-30 min running at about 50-60% VO2max mixed with walking as necessary three times a week. Training was gradually increased on an individualized basis, with a goal of 30-60 min running at 60-80% VO2max 4-5 times a week, although in practice only about one fourth of the training group participants achieved this level. Regular contact was maintained by telephone at 1-3 week intervals or as necessary. Insulin dosage was adjusted as needed throughout the study based on regular home blood glucose monitoring and symptoms. All subjects were instructed to follow the American Diabetes Association recommendations for glycemic control during exercise for type 1 diabetic patients (348).

Studies 2 and 3. Young otherwise healthy men with type 1 diabetes (n=9) and healthy male control subjects (n=13) were recruited into the study after obtaining informed consent. Briefly, diabetic subjects were chosen from patients followed at the Kuopio University Hospital Diabetes Clinic. Control subjects were volunteers from the local university student population. Clinical and biochemical data are displayed in Table 2.

### Table 2. Characteristics of diabetic and control subjects for studies 2 and 3.

<table>
<thead>
<tr>
<th></th>
<th>Diabetes (n=9)</th>
<th>Control (n=13)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>23 ± 1.7</td>
<td>23 ± 2.9</td>
</tr>
<tr>
<td>Body mass index (kg·m⁻²)</td>
<td>23.5 ± 2.5</td>
<td>23.3 ± 1.7</td>
</tr>
<tr>
<td>VO2max (ml·kg⁻¹·min⁻¹)</td>
<td>46 ± 6.9</td>
<td>45 ± 6.0</td>
</tr>
<tr>
<td>Exercise frequency (times·week⁻¹)</td>
<td>2.6 ± 1.6</td>
<td>1.2 ± 1.6</td>
</tr>
<tr>
<td>Duration of diabetes (years)</td>
<td>9 ± 5.8</td>
<td>N/A</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>7.3 ± 1.7</td>
<td>N/A</td>
</tr>
<tr>
<td>Daily insulin dose (IU·kg⁻¹)</td>
<td>0.6 ± 0.3</td>
<td>N/A</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
<td>4.3 ± 0.5</td>
<td>4.9 ± 1.0</td>
</tr>
<tr>
<td>HDL (mmol/l)</td>
<td>1.2 ± 0.3</td>
<td>1.5 ± 0.5</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.5 ± 1.2</td>
<td>1.1 ± 0.4</td>
</tr>
</tbody>
</table>

Data are means ± SD. N/A, not applicable. VO2max, maximal oxygen uptake. HDL, high-density lipoprotein.
Diabetic and control subjects were comparable in age, BMI and physical fitness (as measured by VO₂max). Reasons for exclusion included any cardiovascular or pulmonary disease, vitamin supplementation, chronic medication other than insulin and regular participation in organized athletic events or highly intense physical activity. None of the diabetic subjects had clinically evident atherosclerotic disease, nephropathy (overnight urinary albumin excretion was <20 μg•min⁻¹ and serum creatinine was normal), or neuropathy, and only two of them had mild background retinopathy, not requiring laser treatment. Diabetic subjects were in fair glycemic control (the mean HbA1c of the last two measurements over the preceding 6 - 8 months was 7.3% ± 1.7; normal range 4.0 - 6.0%).

Studies 4 and 5. The KIHID Risk Factor Study is a prospective population-based cohort of 2682 men (349). The study population was a random age-stratified sample of men living in Eastern Finland who were 42, 48, 54 or 60 years old at baseline. The KIHID four-year follow-up study included 1,038 subjects who had undergone carotid ultrasound examination in the original study. Both the baseline and the four-year follow-up studies have been described in detail previously (248, 349).

Study 4. Analyses were limited to the 1005 men participating in the four-year follow-up study for whom complete data for assessment of the metabolic syndrome were available. Men with diabetes at baseline (n=47) were excluded from prospective analyses and analyses relating the metabolic syndrome to physical activity and cardiorespiratory fitness. Diabetes at baseline and at the four-year follow-up was defined as fasting blood glucose ≥ 6.1 mmol/l or a clinical diagnosis of diabetes with either dietary, oral or insulin treatment (69, 350) and IFG was defined as fasting blood glucose 5.6-6.0 mmol/l (69).

Study 5. Analyses were limited to the 1005 men participating in the four-year follow-up study for whom complete data for assessment of the metabolic syndrome were available. Men who had the metabolic syndrome or diabetes at baseline were excluded, leaving 612 and 771 men for analyses of the development of the metabolic syndrome as defined by the WHO and the NCEP, respectively.

4.2 Anthropometric measurements

For anthropometric measurements the patients were without shoes and lightly clothed (shorts or warm-up pants). Height was measured to the nearest cm. Body weight was measured to the nearest kg. The BMI (kg body weight•m⁻² height) was calculated.

For percent body fat (Study 1), the sum of biceps, triceps, subscapular and suprailliac skin-fold was measured to the nearest mm with calipers by two trained sports medicine students. Skin-fold measurements for a given subject were taken by the same person both before and after the training period. The skin-fold measurements made by a given person were equally distributed between trained and control groups. Percent body fat was calculated according to age and gender norms (351).
For Studies 4 and 5, waist circumference was taken as the average of two measurements taken after inspiration and after expiration (mean difference between the two measurements ≥ 1.5 cm) at the midpoint between the lowest rib and the iliac crest. Waist-hip ratio was defined as the ratio of waist girth to the circumference of the hips measured at the trochanter major.

4.3 Blood pressure

Blood pressure was taken as the average of three measurements taken at two-min intervals with the subject seated for Studies 1-3. In Studies 4 and 5, blood pressure was measured with a random-zero mercury sphygmomanometer (Hawksley, United Kingdom). The measurement protocol included, after supine rest of five minutes, three measurements in supine, one in standing and two in sitting position at five-minute intervals. The mean of all six measurements was used as the systolic and diastolic blood pressure.

4.4 Definitions of the metabolic syndrome

The metabolic syndrome for men according to the WHO definition (Table 3) was modified for epidemiological studies as proposed by the EGIR (238) and defined as: hyperinsulinemia (fasting insulin levels in the top 25% of the non-diabetic population), IFG or diabetes and the presence of at least two of the following: abdominal obesity, dyslipidemia (triglycerides ≥1.70 mmol/l or HDL <0.9 mmol/l), or hypertension (blood pressure ≥140/90 mm Hg or blood pressure medication) (69). Insulin resistance was also estimated as the bottom fourth of insulin sensitivity as measured by a recently validated index (QUICKI) based on fasting insulin and glucose concentrations ((log insulin) + log (glucose))^{-1} (262). Hypertension was defined according to the EGIR recommendations at a lower level than the original WHO definition for consistence with current WHO-ISH and Sixth Joint National Committee recommendations (238, 240, 241). Microalbuminuria was not included in the definition (238).

Abdominal obesity was defined according to two definitions: 1) according to the original WHO definition - waist-hip ratio ≥0.90 or BMI ≥ 30 kg/m² (69) and 2) modified according to the EGIR recommendation - waist circumference ≥ 94 cm (238).

The metabolic syndrome as defined by the NCEP (Table 3) was three or more of the following: fasting plasma glucose levels ≥6.1 mmol/l (blood glucose levels ≥5.6 mmol/l), serum triglycerides ≥1.7 mmol/l, serum HDL <1.0 mmol/l, blood pressure ≥130/85 mmHg, waist girth >102 cm (70). Use of waist girth >94 cm was suggested for men genetically susceptible to insulin resistance (70).
### Table 3. The modified WHO and NCEP definitions of the metabolic syndrome in men*

<table>
<thead>
<tr>
<th>Modified WHO definition</th>
<th>NCEP definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>- Hyperinsulinemia (upper quartile of the non-diabetic population) or fasting plasma glucose ≥6.1 mmol/l AND</td>
<td>At least three of the following:</td>
</tr>
<tr>
<td>- At least two of the following:</td>
<td>- Fasting plasma glucose ≥6.1 mmol/l</td>
</tr>
<tr>
<td>- Abdominal obesity</td>
<td>- Abdominal obesity</td>
</tr>
<tr>
<td>- Dyslipidemia (serum triglycerides ≥1.70 mmol/l or HDL cholesterol &lt;0.90 mmol/l)</td>
<td>- Serum triglycerides ≥1.70 mmol/l</td>
</tr>
<tr>
<td>- Hypertension (blood pressure ≥140/90 mmHg or medication)</td>
<td>- Serum HDL cholesterol &lt;1.0 mmol/l</td>
</tr>
<tr>
<td><strong>Abdominal obesity</strong></td>
<td>- Blood pressure ≥130/85 mmHg or medication</td>
</tr>
<tr>
<td>- Definition 1 - MSy-WHO WHR</td>
<td><strong>Abdominal obesity</strong></td>
</tr>
<tr>
<td>WHR &gt;0.90 or body mass index ≥30 kg/m²</td>
<td>- Definition 1 - MSy- NCEP 102</td>
</tr>
<tr>
<td>- Definition 2 - MSy-WHO Waist</td>
<td>Waist girth &gt;102 cm</td>
</tr>
<tr>
<td>Waist girth ≥ 94 cm</td>
<td>- Definition 2 - MSy- NCEP 94</td>
</tr>
<tr>
<td></td>
<td>Waist girth &gt;94 cm</td>
</tr>
</tbody>
</table>

* WHO, World Health Organization; NCEP, National Cholesterol Education Program. HDL, high-density lipoprotein; MSy, metabolic syndrome; WHO WHR, WHO definition with waist-hip ratio > 0.90 or body mass index ≥ 30 kg/m²; WHO Waist, WHO definition with waist ≥ 94 cm; NCEP 102, NCEP definition with waist girth > 102 cm; NCEP 94, NCEP definition with waist > 94 cm.

---

**4.5 Evaluation of physical activity**

Physical activity was quantified at baseline in Studies 1 and 5 using the KIHD 12-Month Leisure Time Physical Activity Questionnaire (352), which was modified from the Minnesota Leisure Time Physical Activity Questionnaire (353) for use in the Kuopio Ischemic Heart Disease Risk Factor Study. The KIHD questionnaire is a detailed quantitative questionnaire of the most common conditioning and lifestyle leisure-time physical activities of middle-aged Finnish men. The questionnaire enables the assessment of the duration, frequency and mean intensity of leisure-time physical activity as recalled over the previous 12 months. Low-intensity physical activity was defined as <4.5 metabolic equivalents (METs; 1 MET is defined as the energy expenditure at rest, corresponding to an oxygen uptake of 3.5 ml O₂/kg). Moderate-to-vigorous physical activity was defined as ≥4.5 METs (23). Vigorous physical activity was defined as ≥7.5 METs and includes activities commonly considered to be vigorous (skiing, jogging, ball sports, forestry) at the subjective intensities at which they are widely practiced.

We also attempted to assess leisure time physical activity during Study 1 with diaries, but less than a quarter of the patients recorded physical activity sufficiently during the study.
4.6 Exercise testing

Maximal exercise testing. All subjects in Study 1 underwent a maximal exercise test to determine VO₂max (ml·kg⁻¹·min⁻¹) and maximal exercise capacity (Wₘₐₓ, W·kg⁻¹), using an electrically braked bicycle ergometer (Tunturi EL 400, Turku, Finland), breath-by-breath gas monitoring (Medikro 919, Kuopio, Finland) and continuous electrocardiogram. Testing began at 50 W and was increased by 25 W every two min. Maximal effort was defined subjectively by the subjects’ maximal voluntary effort (heart rate was greater than 85% of predicted maximum in all cases). The tests were carried out before and after 12-16 weeks of training. For some post-training measurements, the oxygen gas analyzer malfunctioned, obviating the VO₂max results for 20 patients (nine in the training group and 11 in the control group). Wₘₐₓ was obtained for all type 1 DM men completing the study, however.

In Studies 2 and 3, all subjects underwent a maximal exercise test to determine VO₂max, using an electrically braked bicycle ergometer (Tunturi EL 400, Turku, Finland), breath-by-breath gas monitoring (Medikro 919, Kuopio, Finland) and continuous electrocardiogram monitoring. Testing began at 60 W and was increased by 30 W every 2 min. Maximal effort was defined subjectively by the subjects’ maximal voluntary effort or objectively (oxygen consumption increase of less than 150 ml/min despite increasing workload).

In Study 5, a graded symptom-limited maximal exercise stress test was carried out on an electrically-braked cycle ergometer (Medical Fitness Equipment 400L, Mearn, The Netherlands). Workload was increased linearly by 20 W/min. Oxygen consumption was measured directly from respiratory gas exchange analysis, as has been previously described (16, 354). VO₂max was defined as the highest value for oxygen uptake or as the plateau in oxygen uptake.

Submaximal exercise test. One to two weeks after the maximal exercise test subjects in Studies 2 and 3 exercised for 40 min at 60% of their VO₂max after a five-min warm up at 60 W. Before exercising all subjects were requested to refrain from intense exercise and alcohol for at least three days before exercise testing, and from smoking for at least 24 h for the one smoker in each group. On the day of exercise, the subjects ate a light, carbohydrate-rich breakfast. Diabetic subjects decreased their usual rapid acting insulin dose as appropriate. Exercise tests were carried out 2-4 h after breakfast.

4.7 Dietary and other assessments

Dietary records. The diabetic men in Study 1 kept a seven-day food record at baseline and after six and 12 weeks. Because of poor compliance with keeping dietary records especially after baseline, however, dietary records after six and 12 weeks were pooled. Food records were over seven consecutive days, during which the diet was considered to be representative by the subjects. Written and oral instructions were given for providing detailed information about the quantity and quality of all items consumed during the seven-day period. Nutrient intake (355) was calculated using the Micro-Nutrica software package for dietary analysis (Social Insurance Institution, Helsinki, Finland).
Other assessments (Studies 4 and 5). Assessments of medical history and medications and family history of diseases was assessed with a self-administered questionnaire. We defined men with CVD as those who reported a history of angina pectoris (according to the London School of Hygiene Cardiovascular Questionnaire (356)) or myocardial infarction, use of medication for CHD, coronary bypass surgery or angioplasty or an abnormal result on an exercise test that was suggestive of myocardial ischemia (ST-segment depression ≥1.0 mm, exercise induced angina or both), cardiac insufficiency, claudication, stroke, cardiomyopathy, arrhythmias or other CVD. A family history of diabetes or CHD was considered to be present when at least one first-degree family member was reported to have diabetes or CHD, respectively.

Smoking was assessed with a self-administered questionnaire assessing current and numbers of cigarettes, cigars and pipefuls of tobacco used per day (357). Alcohol consumption was assessed with a self-reported quantity-frequency questionnaire. The average weekly intake of alcohol was calculated in pure ethanol (g/wk) (67). Various measurements of socioeconomic status based on education, occupation, income, housing tenure and material wealth were incorporated into a summary index of adult socioeconomic status (358).

4.8 Biochemical methods

Blood sample collection and preparation (Study 1). Subjects were asked to refrain from smoking on the day of blood tests and exercise testing and from consuming alcohol for 3 days before blood draws and exercise testing. On a separate day from VO2max testing blood samples were taken both before and after the training period 1.5-4 h after eating a light carbohydrate rich breakfast or lunch in the morning or mid-day. EDTA-blood samples for later determination of HbA1c levels were stored at 4°C and measured within four days of being drawn. Samples for plasma glucose determination were drawn in NaF/K-oxalate tubes and centrifuged for plasma. The plasma was stored at -74°C until determination within two weeks of being drawn. Blood samples for serum lipoprotein, apolipoprotein and triglyceride analyses were centrifuged for serum and stored at -74°C until determination after the completion of the study.

Studies 2 and 3. Routine screening laboratory tests, lipoprotein profiles and HbA1c analyses were determined on blood samples drawn in a fasting state in the morning on a separate day from the exercise tests. Samples for blood glutathione and plasma TBARS assays were taken from an antecubital vein five min before and within two min after exercising at 60% VO2max for 40 minutes. Fingerstick blood glucose determinations were done 10 minutes before and within five minutes after exercise at 60% VO2max in the diabetic group only.

Studies 4 and 5. Subjects were asked to fast for 12 h before blood sampling. They were also asked to refrain from smoking for 12 h and from consuming alcohol for three days before blood draws.
Blood HbA1c and plasma glucose levels (Studies 1-3). Blood HbA1c was measured using liquid cation exchange chromatography (normal range 4.0 - 6.0%). Plasma glucose levels were measured using a glucose oxidase method.

Blood glucose and serum insulin determinations (Studies 4 and 5). Blood glucose was measured at baseline and four-year follow-up using a glucose dehydrogenase method after precipitation of proteins by trichloroacetic acid. The serum samples for insulin determination were stored at -80°C. Insulin was determined with a Novo Biolabs radioimmunoassay kit (Novo Nordisk, Bagsvaerd, Denmark) and at follow-up (Study 5) with a different radioimmunoassay kit (Pharmacia Diagnostics, Uppsala, Sweden).

Serum cholesterol and triglyceride levels (Studies 1-5) were measured enzymatically (CHOD-PAP and GPO-PAP methods). The same method was also used for HDL after removal of LDL and VLDL by dextran sulphate/MgCl₂ (359). For Study 1, LDL was calculated according to Friedewald’s formula (360), which has also been validated in type 1 DM (361).

Serum apolipoprotein levels (Study 1). Analyses of apo A-I and apo B were based on the measurement of immunoprecipitation enhanced by polyethylene glycol (PEG) at 340 nm (362) using a Kone Specific Chemical Analyzer (Kone Ltd., Espoo, Finland).

Blood glutathione analyses (Studies 2 and 3). For total glutathione (TGH) determinations, EDTA-blood was precipitated with perchloric acid and the deproteinized supernatant was used. For blood glutathione disulfide (GSSG), the clear supernatant obtained from EDTA-blood treated with 5-sulfosalicylic acid was neutralized and reacted with 2-vinylpyridine. Treated samples were frozen at -20°C until spectrophotometric determination by the method of Tietze with the reaction mixture as suggested by Adams (181, 363, 364).

Plasma TBARS (Studies 2 and 3). For the determination of TBARS, EDTA-blood was added immediately after being drawn to CHELEX (Bio-Rad Laboratories, CA, USA) treated potassium phosphate buffer containing Na₂EDTA. The mixture was briefly centrifuged to obtain plasma. The plasma was immediately treated with ethanolic butylated hydroxytoluene. Treated plasma was added to 25% trichloroacetic acid to precipitate plasma proteins. The mixture was then placed in a boiling water bath for 30 min to release protein-bound MDA. The tubes were centrifuged to obtain a clear supernatant. The supernatant was frozen at -70°C until TBARS could be determined spectrophotometrically (176, 365, 366).

Determination of erythrocyte Se-GPX, GRD and GST activity (Study 3) was carried out using washed red cell hemolysates. GPX activity was assayed spectrophotometrically with H₂O₂ as a substrate instead of cumene peroxide to detect selenium-dependent GPX activity (181, 367). GRD activity was determined spectrophotometrically with a 10 mmol/l potassium-N-2-hydroxyethylpiperazine-N-2-ethanesulfonic acid buffer (181, 368). GST activity was assayed spectrophotometrically with 1,2-dichloro-4-nitrobenzene as the substrate (181, 369). Enzyme activity was expressed as micromoles per minute per gram of hemoglobin.
Determination of erythrocyte Cu,Zn-SOD activity (Study 3) was based on the reduction of nitroblue tetrazolium in a xanthine-xanthine oxidase dependent superoxide generating system (370). The activity of SOD that could cause a 50% inhibition of superoxide produced by the reduction of nitroblue tetrazolium was defined as 1 unit (U).

Red cell catalase activity (Study 3) was determined by monitoring the decrease in absorbance at 240 nm in the presence of 10 mmol/l H₂O₂ (371). One U of catalase activity was defined as the decomposition of 1 mmol• l⁻¹ H₂O₂ • min⁻¹ at 25° C.

4.9 Statistical methods

SPSS/PC+ and PC Windows v. 6-10 software (SPSS, Chicago, IL) was used for all statistical analyses. Statistical significance was considered to be P<0.05.

Study 1. Variables are expressed as mean ± SD, except for percentages, which are expressed as median (95% confidence intervals [CI]). Differences in the measured variables before and after training were tested between groups using Student’s unpaired t-test and within groups using ANOVA repeated measures after determining normality in distribution of the variables in question. The Mann-Whitney U-test was used to compare relative (%) changes in variables in trained and untrained groups over the training period. Correlation, partial correlation and stepwise multiple regression analysis were used to assess the associations between selected variables at baseline.

Studies 2 and 3. Results for the groups are expressed as means ± SD. Differences between the group means were analysed for significance using the unpaired Student's t-test. Differences within the same group before and after exercise were tested with repeated measures ANOVA. Pearson's correlation and univariate regression analysis was used to assess the associations between selected variables.

Study 4. Differences in baseline clinical and biochemical characteristics among men who had diabetes at baseline, who developed diabetes during follow-up and who remained non-diabetic were tested for statistical significance with one-way ANOVA, and where indicated, the chi-squared test. The association of the metabolic syndrome with the risk of developing diabetes was estimated using logistic regression adjusting for age. Sensitivity and specificity of the definitions of the metabolic syndrome for prevalent and incident diabetes were calculated and then compared using the McNemar test. Receiver-operated characteristics (ROC) analysis was done using continuous variables to derive cut-offs waist circumference corresponding to BMI ≥25 and 30 kg/m². Data are presented as means ± SD, medians (interquartile ranges), or simple percentages. Triglyceride and insulin concentrations were corrected for skewing by log transformation, but are presented as medians (interquartile ranges) using untransformed values.

Study 5. Results for the groups are expressed as means ± SD. Differences between the group means were analyzed for significance using the unpaired Student’s t-test or the Mann-Whitney test as appropriate. Differences within the same group before and after exercise were tested with repeated measures ANOVA. Pearson's correlation and partial correlation analysis
was used to assess the associations between selected variables. Differences in baseline clinical and biochemical characteristics between men who developed the metabolic syndrome during follow-up and those who did not were tested for statistical significance with Student's t-test, the Mann-Whitney U test or the chi-squared test. The association of LTPA and cardiorespiratory fitness with the development of the metabolic syndrome during follow-up was estimated using logistic regression adjusting for potential confounding or mediating factors. Indices of LTPA and cardiorespiratory fitness were categorized into thirds for the logistic regression analyses (except for moderate-to-vigorous leisure-time physical activity, which was categorized based on min/wk according to the CDC/ACSM recommendations), although results were similar using continuous variables (not shown). The covariates for the logistic regression models were forced into the model, although results were similar when using a stepwise model. Durations in min/wk of LTPA are presented as medians (interquartile ranges); other data are presented as means ± SD or simple percentages. Triglyceride and insulin concentrations and durations of LTPA were corrected for skewing using log transformation, but are presented using untransformed values.
5 RESULTS

5.1 Study 1

There were no significant differences with respect to glycemic control, cardiovascular fitness or lipid profile between the trained and control diabetic men before training (Table 4). The level of leisure time aerobic (brisk walking, jogging, skiing, cycling, skating and swimming) and conditioning (ball sports, weight lifting, and downhill skiing in addition to aerobic exercise) physical activity of the study participants for the 12 months prior to the study (Table 2 of the Original Publications, Study 1) was for the most part in accordance with American Diabetes Association recommendations (372).

Total energy intake (training group 2324 ± 418 kcal•d⁻¹, control group 2287 ± 473 kcal•d⁻¹, P=0.36), percent of total energy intake (E%) from fat (training group 33.5 ± 5.7 E%, control group 35.3 ± 8.0 E%, P=0.49) and dietary polyunsaturated/saturated fatty acid ratio (training group 0.48 ± 0.17, control group 0.47 ± 0.17, P=0.86) from the 32 men filling out dietary records were similar between training (n=18) and control (n=14) groups.

Baseline associations of physical fitness, physical activity and other clinical features with the lipid profile. VO₂max (r=-0.41, P=0.002) correlated inversely with age. Measures of cardiovascular fitness were not related to any other lipid profile index.

After adjusting for age, daily energy expenditure on leisure time aerobic activity was associated with VO₂max (r=-0.33, P=0.042) and Wmax (r=0.57, P<0.001). After adjusting for percent body fat, leisure time aerobic activity daily energy expenditure correlated inversely with triglyceride levels (r=-0.33, P=0.049) and positively with the apo A-I/apo B ratio (r=0.34, P=0.048). Associations of daily energy expenditure on aerobic activity did not reach statistical significance. The correlation between daily energy expenditure on aerobic and conditioning activity and between daily energy expenditure on physical activity and duration of physical activity was high (r >0.90). Consequently, substitution of conditioning physical activity for aerobic activity had little effect on the results. Substitution of duration of physical activity for physical activity energy expenditure also yielded similar results.

BMI and percent body fat correlated with LDL-cholesterol (r=0.29, P=0.033 and r=0.45, P=0.001, respectively), total cholesterol (r=0.29, P=0.033 and r=0.46, P=0.001, respectively) and apo B (r=0.33, P=0.015 and r=0.43, P=0.002, respectively). No other associations with lipid or glycemic indices reached significance. HbA₁c correlated with LDL (r=0.27, P=0.050), total cholesterol (r=0.30, P=0.028) and apo B (r=0.26, P=0.058), but not with BMI, percent body fat or indices of cardiovascular fitness.

Determinants of baseline lipid, lipoprotein and apolipoprotein measures. Using stepwise multiple linear regression with percent body fat, HbA₁c and aerobic exercise daily energy expenditure as explanatory variables, percent body fat (partial β 0.46) was determinant of total cholesterol at baseline (adjusted r²=0.25, P=0.009). HbA₁c approached significance (partial β 0.25, P=0.056). Also using percent body fat, HbA₁c and aerobic exercise daily energy expenditure as explanatory variables, percent body fat (partial β 0.44 and 0.42)
explained 19% and 16% of the variation in LDL and apo B at baseline (P=0.001 and 0.003). HbA1c approached significance. BMI explained less of the variance when BMI was substituted for percent body fat. Daily energy expenditure on aerobic exercise (partial β -0.33) and percent body fat (partial β 0.36) explained 19% of the variation in triglyceride levels. Daily energy expenditure on aerobic exercise (partial β 0.34) was also a determinant of the apo A-I/apo B ratio (adjusted r²=0.09, P=0.046). When substituted for aerobic exercise daily energy expenditure, neither maximal exercise capacity nor VO2max significantly explained any of the variation in lipid levels.

Table 4. Baseline and post-training anthropometric and biochemical data for the 42 training and control type 1 diabetic men finishing study 1.

<table>
<thead>
<tr>
<th></th>
<th>Before training</th>
<th></th>
<th>After training</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Training</td>
<td>Control</td>
<td>Training</td>
<td>Control</td>
</tr>
<tr>
<td>VO2max* (ml•kg⁻¹•min⁻¹)</td>
<td>43.4 ± 8.0</td>
<td>42.0 ± 7.2</td>
<td>46.1 ± 6.6⁷</td>
<td>43.4 ± 7.2</td>
</tr>
<tr>
<td>Wmax (W/kg)</td>
<td>3.36 ± 0.65</td>
<td>3.31 ± 0.54</td>
<td>3.55 ± 0.67⁷</td>
<td>3.36 ± 0.52</td>
</tr>
<tr>
<td>Daily insulin dosage (units/kg)</td>
<td>0.68 ± 0.19</td>
<td>0.71 ± 0.18</td>
<td>0.68 ± 0.20</td>
<td>0.70 ± 0.20</td>
</tr>
<tr>
<td>Hemoglobin A1c (%)</td>
<td>8.3 ± 1.1</td>
<td>8.3 ± 1.3</td>
<td>8.0 ± 1.0</td>
<td>8.5 ± 1.6</td>
</tr>
<tr>
<td>Plasma glucose (mmol/l)</td>
<td>10.5 ± 6.0</td>
<td>10.1 ± 4.3</td>
<td>12.1 ± 6.0</td>
<td>11.9 ± 5.8</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24.4 ± 1.9</td>
<td>24.4 ± 2.2</td>
<td>24.3 ± 1.9</td>
<td>24.5 ± 2.3</td>
</tr>
<tr>
<td>Percent body fat</td>
<td>20.5 ± 4.1</td>
<td>18.5 ± 5.0</td>
<td>20.4 ± 4.7</td>
<td>18.2 ± 5.8</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>4.89 ± 0.97</td>
<td>4.71 ± 1.08</td>
<td>4.66 ± 0.94⁴</td>
<td>4.79 ± 1.18</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/l)</td>
<td>3.15 ± 0.81</td>
<td>2.99 ± 0.74</td>
<td>2.88 ± 0.75⁴</td>
<td>2.92 ± 0.80</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/l)</td>
<td>1.21 ± 0.28</td>
<td>1.21 ± 0.40</td>
<td>1.32 ± 0.28⁷</td>
<td>1.31 ± 0.45⁷</td>
</tr>
<tr>
<td>HDL/LDL³</td>
<td>0.41 ± 0.13</td>
<td>0.42 ± 0.12</td>
<td>0.49 ± 0.16⁴</td>
<td>0.46 ± 0.15⁵</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>1.18 ± 0.50</td>
<td>1.12 ± 0.53</td>
<td>1.02 ± 0.50</td>
<td>1.25 ± 0.53</td>
</tr>
<tr>
<td>Apolipoprotein B (g/l)</td>
<td>0.82 ± 0.20</td>
<td>0.78 ± 0.18</td>
<td>0.75 ± 0.19⁴</td>
<td>0.82 ± 0.20</td>
</tr>
<tr>
<td>Apolipoprotein A-I (g/l)</td>
<td>1.42 ± 0.27</td>
<td>1.44 ± 0.34</td>
<td>1.50 ± 0.25⁷</td>
<td>1.54 ± 0.38⁷</td>
</tr>
<tr>
<td>HDL/Apolipoprotein A-I</td>
<td>0.84 ± 0.08</td>
<td>0.84 ± 0.10</td>
<td>0.88 ± 0.07⁷</td>
<td>0.84 ± 0.10</td>
</tr>
<tr>
<td>Apo A/I/apo B⁴</td>
<td>1.83 ± 0.51</td>
<td>1.90 ± 0.47</td>
<td>2.09 ± 0.52⁷</td>
<td>1.96 ± 0.51</td>
</tr>
</tbody>
</table>

Data are means ± SD
*VO2max, maximal oxygen uptake. ¹P<0.05, ²P<0.005, ³P<0.001, before vs. after training. ¹Wmax, maximal exercise capacity. ¹HDL/LDL, HDL:cholesterol/LDL:cholesterol. ⁴Apo A-I/apo B, apolipoprotein A-I/apolipoprotein B.

Effect of endurance training. After 12-16 weeks of endurance training, VO2max and Wmax increased significantly only in the training group (Table 4). In the training group, total and LDL-cholesterol and apo B decreased, and HDL, apo A-I and the HDL/LDL and apo A/I/apo B ratios increased. In contrast, only HDL, apo A-I and the HDL/LDL ratio increased in the control group. Results for all analyses are essentially identical when substituting HDL/total cholesterol for HDL/LDL. There were no significant changes in BMI, percent body fat, HbA1c.
or daily insulin dosage in either group. For the 23 men completing the study who filled out
dietary records after six or 12 weeks, no changes in dietary polyunsaturated
saturated fatty acid ratio occurred in either the trained (n=10) or untrained
(n=13) group (P>0.3 for all variables).

When comparing the relative changes brought about by the 12-to-16 week endurance
training program between the training and control groups, favorable changes in HDL/LDL,
apo B, apo A-I/apo B and triglyceride levels were significantly greater in the training group
(Table 4). Of note, triglyceride levels increased 19% in the control group, although this
change was not significant when comparing pre- and post-training levels.

**Baseline HDL/LDL and the effect of endurance training on lipid levels.** To examine
the possible role of baseline lipid status in the response of the lipid profile to regular physical
exercise, we divided the training and control groups into two subgroups based on the training
group median HDL/LDL value (Table 5 in **Study 1** of the Original Publications). As can
clearly be seen, the relative changes in the HDL/LDL and apo A-I/apo B ratios produced by
training are more prominent in type 1 DM men with low HDL/LDL levels at baseline.
Smaller or no differences were found when dividing into subgroups based on median HDL or
LDL baseline levels (data not shown).

**5.2 Studies 2 and 3**

**Maximal oxygen consumption**
The mean VO2max in both groups was similar (Table 2 on page 36).

**Fingerstick blood glucose levels in the diabetic group before and after exercise.** Pre-
and post-exercise blood glucose levels were 10 ± 5 mmol/l and 8 ± 5 mmol/l, respectively.

![Graph of plasma TBARS and GSSG levels](image)

**Figure 3.** Mean levels of (a) plasma thiobarbituric acid reacting substances (TBARS) and (b) glutathione
dissulfide (GSSG) before and after exercise for the diabetic and control groups. Error bars represent SD.

1 Diabetic vs. control group, P=0.005, Mann-Whitney. 2 Diabetic group, before vs. after exercise, P=0.001.
3 Control group, before vs. after exercise: P=0.012. 4 Before vs. after exercise, P<0.005.
Plasma TBARS levels before and after exercise. At rest, the diabetic group had a greater than 2 fold higher mean plasma TBARS (P=0.002; Figure 3a). Mean plasma TBARS increased significantly approximately 50% in both groups with exercise (diabetic group, P=0.001; control group, P=0.012). Mean blood TGSH did not change with exercise (Table 2).

Blood total glutathione levels before and after exercise. Blood TGSH levels were increased in the diabetic group both before and after exercise (Table 2 of Study 2 in the Original Publications). Mean blood TGSH remained unchanged in response to exercise in both groups. Mean GSSG, however, increased by at least 50% in both groups (Figure 3b; diabetic group, P=0.004; control group, P=0.002). The GSSG/TGSH ratio showed a similar response to exercise in both groups (diabetic group; P=0.007, control group; P=0.002).

Erythrocyte glutathione dependent enzymes at rest. Red cell GRD activity at rest was 15% higher in the diabetic group (P=0.049, Figure 1a of Study 3 in the Original Publications). Neither Se-GPX nor GST activity differed between the two groups (Figures 1b and 1c of Study 3 in the Original Publications).

Erythrocyte Cu,Zn-SOD and catalase activities at rest. Erythrocyte Cu,Zn-SOD and catalase activities at rest were significantly lower in the diabetic group (P=0.007 and P=0.023, Figure 2 of Study 3 in the Original Publications).

Erythrocyte glutathione dependent enzyme activities after exercise. Postexercise red cell Se-GPX activity rose modestly with exercise in the control group (P=0.003, Figure 1b of Study 3 in the Original Publications), but not in the diabetes group. Post-exercise Se-GPX activity was higher in the control group compared to the diabetic group (P=0.046). Postexercise GRD activity was also higher in diabetic than in non-diabetic men (P=0.032). Exercise did not significantly affect GRD or GST activity in either group.

Erythrocyte Cu,Zn-SOD and catalase activities after exercise. There were no significant exercise induced changes in either Cu,Zn-SOD or catalase activity in either group (Figure 2 of Study 3 in the Original Publications).

Correlations and univariate regression analysis. A strong inverse correlation between resting plasma TBARS and VO2max was found in the diabetic group (r=-0.82, P=0.006; Figure 4). Adjustment for triglyceride levels at rest (r=-0.82, P=0.024) or HbA1c (r=-0.76, P=0.027) using partial correlation analysis had only minimal effects on the correlation. Post-exercise plasma TBARS did not correlate with VO2max (Figure 2b of Study 2 in the Original Publications). A strong positive correlation between the relative plasma TBARS increase (the ratio of post-exercise TBARS to pre-exercise TBARS) in response to exercise and oxygen consumption during exercise (60% VO2max) was found in the diabetic subjects (r=0.81, P=0.008; (Figure 2c of Study 2 in the Original Publications). Adjustment for triglyceride levels or HbA1c had little effect on the correlation. These correlations were not present in the control group.
At rest red cell GST activity correlated inversely with blood HbA1c activity ($r=-0.79$, $P=0.012$). There were no other significant correlations between the antioxidant enzymes and serum lipid levels, prevailing glucose levels, HbA1c, or VO2max in the diabetic group. Plasma TBARS levels also did not correlate with any of the erythrocyte antioxidant enzyme activities.

5.3 Study 4

Baseline. Men with diabetes at baseline and men developing diabetes during the four-year follow-up were heavier and more dyslipidemic, hypertensive and hyperinsulinemic at baseline (Table 1 in Study 4 of the Original Publications). The overwhelming majority of the 51 men developing diabetes (88%) had a BMI $\geq 25$ kg/m$^2$ (overweight or obese as defined by the National Institutes of Health and the WHO (68, 284), although most men remaining non-diabetic were also overweight. Most men who subsequently developed diabetes had a BMI $<30$ kg/m$^2$, although more men developing diabetes were obese.

Similarly, almost all men developing diabetes had a waist-hip ratio $>0.9$ although the majority of men who did not develop diabetes also had a waist-hip ratio $>0.9$ (Table 1 in Study 4 of the Original Publications). Only 59% of men developing diabetes had a waist circumference $\geq 94$ cm at baseline. Less than a third of the men who developed diabetes had a waist girth $>102$ cm.

Because the 94 and 102 cm waist circumference cut-offs were derived at least in part from a cross-sectional population study from the Netherlands in which those cut-offs corresponded to a BMI of 25 and 30 kg/m$^2$ (373), respectively, we repeated ROC analyses to derive cut-offs for this population. In this cohort, the BMI cut-offs of $\geq 25$ and 30 kg/m$^2$ corresponded to a waist girth $\geq 87$ cm (sensitivity = 0.84 and specificity = 0.84) and 96 cm (sensitivity = 0.86, specificity = 0.88), respectively. The cut-off of 87 cm was as sensitive as BMI $\geq 25$ kg/m$^2$ (0.90 vs. 0.88) in identifying men who developed diabetes during follow-up.
Association of the metabolic syndrome with development of diabetes. Men having the WHO definition of the metabolic syndrome with adiposity defined as waist-hip ratio >0.90 or BMI ≥30 kg/m² had a nearly nine-fold greater likelihood of developing diabetes than men without the metabolic syndrome (Figure 5). Furthermore, sensitivity (0.83 and 0.67) and specificity (0.78-0.80) for detecting prevalent and incident diabetes was quite high (Table 4). Use of the insulin sensitivity index (QUICKI) to estimate insulin resistance resulted in a slightly higher sensitivity (0.69), specificity (0.82) and odds ratio (OR) (10.4) of the WHO definition for incident diabetes (not shown).

![Figure 5. Age-adjusted odds ratios and 95% confidence intervals for the development of diabetes after a four-year follow up in middle-aged Finnish men followed since the late 1980s as predicted by four definitions of the metabolic syndrome: a modified World Health Organization (WHO) definition with adiposity defined as waist-hip ratio >0.9 or body mass index ≥30 kg/m² (WHO WHIR), a modified WHO definition with abdominal obesity defined as waist girth ≥94 cm (WHO Waist), the National Cholesterol Education Program (NCEP) definition with abdominal obesity defined as waist circumference >102 cm (NCEP 102 cm) and the NCEP definition with abdominal obesity defined as waist girth >94 cm (NCEP 94 cm).](image)

Men fulfilling the WHO definition of the metabolic syndrome with adiposity defined as waist girth ≥94 cm were 7.0 times more likely to develop diabetes during follow-up (Figure 5). The metabolic syndrome with waist girth ≥94 cm had a clearly lower sensitivity (0.68 and 0.57) for prevalent and incident diabetes, and was only slightly more specific (0.81-0.83) (Table 4). We repeated analyses using the waist girth cut-off corresponding to BMI ≥25 kg/m² in this population, 87 cm. The sensitivity, specificity and OR for the prediction of diabetes was virtually identical to the WHO definition based on waist-hip ratio >0.90 or BMI ≥30 kg/m² (not shown). Even defining adiposity as BMI ≥25 kg/m² or waist ≥102 cm, a definition proposed by the National Institutes of Health for screening in the presence of other risk factors (284), gave nearly identical results (not shown).

The NECP definition of the metabolic syndrome detected only 61 and 41% of prevalent and incident diabetes, although specificity was quite high (0.89-0.90) (Table 5). The likelihood of men with the metabolic syndrome as defined by the NCEP to develop diabetes was high (Figure 5).

Because the NCEP also pointed out that some genetically susceptible men with only mild increases in abdominal obesity (waist circumference 94–102 cm) can develop multiple metabolic risk factors and should similarly benefit from intervention, we repeated analyses with waist circumference >94 cm. The prevalence increased from 11 to 18%, with an OR of 5.0 for developing diabetes during follow-up (Figure 5). Sensitivity for prevalent (0.72) and incident (0.49) diabetes improved (Table 5). Again, because a waist circumference ≥87 cm
corresponds to a BMI ≥25 kg/m² in this population, we repeated analyses using waist girth ≥87 cm. The prevalence increased to 23%, and the sensitivity, specificity and OR for the prediction of diabetes with waist as 87 cm was 0.59, 0.79 and 5.1, respectively (not shown).

**Table 5.** Prevalence of the definitions of the metabolic syndrome and their sensitivity and specificity for prevalent (a) and incident (b) cases of diabetes mellitus in middle-aged Finnish men.

### A. Prevalent diabetes

<table>
<thead>
<tr>
<th>Metabolic syndrome definition</th>
<th>Prevalence of metabolic syndrome (n/N,%</th>
<th>Prevalence of diabetes (n/N,%</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO, WHR ≥ 0.91</td>
<td>250/1005 (24.9%)</td>
<td>47/1005 (4.7%)</td>
<td>0.83</td>
<td>0.78</td>
</tr>
<tr>
<td>WHO, waist ≥ 94 cm</td>
<td>212/1005 (21.1%)</td>
<td>47/1005 (4.7%)</td>
<td>0.68</td>
<td>0.81</td>
</tr>
<tr>
<td>NCEP, waist &gt;102 cm</td>
<td>138/1005 (13.7%)</td>
<td>47/1005 (4.7%)</td>
<td>0.61</td>
<td>0.89</td>
</tr>
<tr>
<td>NCEP, waist &gt;94 cm</td>
<td>206/1005 (20.5%)</td>
<td>47/1005 (4.7%)</td>
<td>0.72</td>
<td>0.82</td>
</tr>
</tbody>
</table>

### B. Incident diabetes

<table>
<thead>
<tr>
<th>Metabolic syndrome definition</th>
<th>Incidence of diabetes during follow-up (n/N,%</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>WHO, WHR ≥ 0.91</td>
<td>211/958 (22.0%)</td>
<td>51/958 (5.3%)</td>
<td>0.67</td>
</tr>
<tr>
<td>WHO, waist ≥ 94 cm</td>
<td>180/958 (18.8%)</td>
<td>51/958 (5.3%)</td>
<td>0.57</td>
</tr>
<tr>
<td>NCEP, waist &gt;102 cm</td>
<td>109/958 (11.4%)</td>
<td>51/958 (5.3%)</td>
<td>0.41</td>
</tr>
<tr>
<td>NCEP, waist &gt;94 cm</td>
<td>167/958 (18.0%)</td>
<td>51/958 (5.3%)</td>
<td>0.49</td>
</tr>
</tbody>
</table>

* WHO, World Health Organization. ¹WHR, waist-to-hip ratio. ²NCEP, National Cholesterol Education Program.
Differences in sensitivity of the WHO definition with WHR >0.90 vs. the WHO definition with waist ≥94 cm, at baseline P=0.016, at follow-up, P=0.063; vs NCEP definition with waist >102 cm, at baseline P=0.006, at follow-up, P<0.001; vs. NCEP definition with waist >94 cm, at baseline P=0.18, at follow-up, P=0.022.
Differences in specificity of the WHO definition with WHR >0.90 vs. all other definitions at baseline and follow-up, P<0.001. Statistical significance was calculated with the McNemar test. In corresponding analyses with impaired fasting glycemia excluded from the definitions, sensitivity for especially the NCEP definitions was decreased (WHO definition with waist-to-hip ratio >0.90 or body-mass index ≥30 kg/m², sensitivity 0.55; WHO definition with waist >94 cm, sensitivity 0.49; NCEP definition with waist >102 cm, sensitivity 0.31; NCEP definition with waist >94 cm, sensitivity 0.37), with almost no effect of specificity [not shown].

**Clustering of insulin resistance and components of the metabolic syndrome.** Over 95% of men with the metabolic syndrome as defined by the WHO had hyperinsulinemia. Conversely, over 80% of the men with insulin resistance had the metabolic syndrome with adiposity as defined by the WHO, emphasizing the clustering of insulin resistance and other components of the metabolic syndrome. At baseline, 11% of men had the metabolic syndrome by both the NCEP definition using the lower 94 cm cut-off for waist circumference and the WHO definition based on waist-hip ratio >0.9, of whom 23 (21%) developed diabetes.
5.4 Study 5

Baseline measures. The 107 men who developed the metabolic syndrome by the end of
the 4-year follow-up were heavier and more hypertensive, dyslipidemic and hyperinsulinemic
than the 505 men who did not already at baseline (Table 6). They also had slightly higher
blood glucose levels and a higher prevalence of CVD.

| Table 6. Baseline characteristics of the 107 men who developed the metabolic syndrome during follow-up and the 505 men who did not. |
|---|---|---|---|
| At 4-year follow-up | Metabolic syndrome - | Metabolic syndrome + |
| N | 505 | 107 |
| Age, y | 50.9 ± 6.6 | 52.7 ± 6.1 | 0.005* |
| Cardiovascular disease, % | 29 | 44 | 0.001 |
| Smokers, % | 33 | 31 | 0.70 |
| Blood pressure medication, % | 11 | 31 | <0.001 |
| Systolic blood pressure, mmHg | 128.8 ± 14.0 | 135.3 ± 15.6 | <0.001 |
| Diastolic blood pressure, mmHg | 85.6 ± 9.7 | 90.6 ± 8.9 | <0.001 |
| Body mass index, kg/m² | 25.5 ± 3.2 | 28.0 ± 4.4 | <0.001 |
| Waist-to-hip ratio | 0.92 ± 0.06 | 0.96 ± 0.05 | <0.001 |
| Serum HDL cholesterol, mmol/l | 1.36 ± 0.30 | 1.23 ± 0.26 | <0.001 |
| Serum triglycerides, mmol/l | 1.14 ± 0.56 | 1.48 ± 0.96 | <0.001 |
| Fasting blood glucose, mmol/l | 4.4 ± 0.4 | 4.6 ± 0.4 | 0.002 |
| Fasting serum insulin, pmol/L | 56.3 ± 18.1 | 73.6 ± 22.9 | <0.001 |
| VO₂max†, ml•kg⁻¹•min⁻¹ | 33.6 ± 7.9 | 28.5 ± 7.2 | <0.001 |
| Total ‡LTPA, min/wk | 393 (231 – 618) | 320 (164 – 501) | 0.006 |
| LTPA <4.5 METs§, min/wk | 195 (84 – 370) | 143 (58 – 303) | 0.032 |
| LTPA ≥ 4.5 METs, min/wk | 147 (63 – 255) | 121 (43 – 211) | 0.030 |
| LTPA ≥ 7.5 METs, min/wk | 31 (7 – 91) | 12 (0 – 42) | <0.001 |

Values are means ± SD except for LTPA variables, which are presented as medians and interquartile ranges. *Mann-Whitney U-test. †VO₂max, maximal oxygen uptake. ‡LTPA, leisure-time physical activity. §METs, metabolic equivalents.
About 25% of the men engaged in ≤60 min/week of at least moderate LTPA (≥4.5 METs) over the previous year. Nearly 40% roughly met the ACSM and Surgeon General recommendations for physical activity (at least 30 min of moderate-intensity exercise on most days of the week, calculated here as ≥3 h/wk). Men who did not develop the metabolic syndrome had a higher VO₂max and engaged in more LTPA, especially vigorous LTPA.

**Baseline age-adjusted partial correlations.** Low-intensity LTPA was not correlated with cardiorespiratory fitness, whereas moderate and especially vigorous physical activity were (age-adjusted correlation of VO₂max with LTPA <4.5 METs, r=0.01, P=0.81; with LTPA ≥4.5 METs, r=0.24, P<0.001; with LTPA ≥7.5 METs, r=0.40, P<0.001).

**Association of leisure-time physical activity with development of the metabolic syndrome.** In logistic regression analyses adjusting only for age (model 1), men with duration of total LTPA in the upper third were less likely to develop the metabolic syndrome than men whose duration of total LTPA was in the lower third (Table 7). Similarly, men engaging in ≥3 hours a week of LTPA ≥4.5 METs were 48% less likely to develop the metabolic syndrome than sedentary (≤60 min moderate intensity exercise /wk) men. At least 60 min of vigorous LTPA appeared to reduce the likelihood to develop the metabolic syndrome by nearly two thirds. Although duration of low-intensity LTPA in the upper third appeared to decrease the likelihood of developing the metabolic syndrome by 40%, the association was not significant.

ORs for total LTPA, LTPA ≥4.5 METs, and LTPA ≥7.5 METs for development of the metabolic syndrome were not attenuated in logistic regression models adjusting for age and BMI (model 2) and for age, BMI and other potentially confounding variables (model 3, Table 7). The ORs also remained virtually unchanged after adjustment for potentially mediating variables included in the definition of or related to the metabolic syndrome (model 4, Table 7). The 95% CIs widened, however, and the associations for total LTPA and LTPA ≥4.5 METs were no longer significant. In contrast, men participating in ≥60 min vigorous exercise/wk were still nearly two thirds less likely to develop the metabolic syndrome than sedentary men (P=0.009 for the trend).

Inclusion of low-intensity LTPA or VO₂max as a continuous variable in the regression models had little effect on the ORs for moderate and vigorous LTPA (not shown). Exclusion of smokers or men with CVD at baseline also had no qualitative effect on the associations, although some of the associations were no longer significant because of reduced statistical power.

**Association of physical activity with development of the metabolic syndrome in high-risk men.** Moderate and vigorous LTPA appeared to be especially effective in decreasing the likelihood of developing the metabolic syndrome in a particularly high-risk group of 286 men (hyperinsulinemia at baseline or two or more of the following: adiposity, dyslipidemia or hypertension). The OR (95%CI) for LTPA ≥4.5 METs, >3 h/wk vs. ≤60min/wk, was 0.45 (0.22-0.94), and for LTPA ≥7.5 METs, upper vs. lower third, 0.25 (0.11-0.55) (model 4).
Table 7. Odds ratios for development of the metabolic syndrome in middle-aged Finnish men according to categories of baseline leisure-time physical activity (LTPA) and cardiorespiratory fitness.

<table>
<thead>
<tr>
<th>LTPA (min/wk)</th>
<th>tertiles</th>
<th>OR, model 1*</th>
<th>OR, model 2†</th>
<th>OR, model 3‡</th>
<th>OR, model 4§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total LTPA</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;270 min/wk</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>270–486 min/wk</td>
<td>2</td>
<td>0.72 (0.43-1.19)</td>
<td>0.66 (0.38-1.15)</td>
<td>0.73 (0.44-1.22)</td>
<td>0.83 (0.45-1.52)</td>
</tr>
<tr>
<td>≥487 min/wk</td>
<td>3</td>
<td>0.52 (0.30-0.89)</td>
<td>0.55 (0.31-0.98)</td>
<td>0.53 (0.31-0.92)</td>
<td>0.54 (0.28-1.04)</td>
</tr>
<tr>
<td>Trend (P)</td>
<td></td>
<td>0.055</td>
<td>0.10</td>
<td>0.073</td>
<td>0.18</td>
</tr>
<tr>
<td>Low-intensity LTPA (&lt;4.5 METs, min/wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;111 min/wk</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>111–270 min/wk</td>
<td>2</td>
<td>0.86 (0.52-1.42)</td>
<td>0.93 (0.54-1.60)</td>
<td>0.90 (0.54-1.49)</td>
<td>0.97 (0.52-1.79)</td>
</tr>
<tr>
<td>≥271 min/wk</td>
<td>3</td>
<td>0.60 (0.35-1.03)</td>
<td>0.62 (0.35-1.10)</td>
<td>0.61 (0.35-1.05)</td>
<td>0.66 (0.34-1.28)</td>
</tr>
<tr>
<td>Trend (P)</td>
<td></td>
<td>0.17</td>
<td>0.23</td>
<td>0.20</td>
<td>0.41</td>
</tr>
<tr>
<td>Moderate and vigorous LTPA (≥4.5 METs, min/wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤60 min/wk</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>61–180 min/wk</td>
<td>2</td>
<td>0.82 (0.49-1.37)</td>
<td>0.74 (0.42-1.30)</td>
<td>0.82 (0.48-1.38)</td>
<td>0.86 (0.46-1.60)</td>
</tr>
<tr>
<td>≥180 min/wk</td>
<td>3</td>
<td>0.52 (0.30-0.89)</td>
<td>0.52 (0.29-0.92)</td>
<td>0.52 (0.30-0.90)</td>
<td>0.55 (0.29-1.04)</td>
</tr>
<tr>
<td>Trend (P)</td>
<td></td>
<td>0.047</td>
<td>0.078</td>
<td>0.058</td>
<td>0.16</td>
</tr>
<tr>
<td>Vigorous LTPA (≥7.5 METs, min/wk)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;10 min/wk</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>10–59min/wk</td>
<td>2</td>
<td>0.64 (0.39-1.05)</td>
<td>0.58 (0.34-0.98)</td>
<td>0.65 (0.39-1.08)</td>
<td>0.60 (0.33-1.09)</td>
</tr>
<tr>
<td>≥60min/wk</td>
<td>3</td>
<td>0.37 (0.21-0.65)</td>
<td>0.32 (0.18-0.59)</td>
<td>0.37 (0.21-0.66)</td>
<td>0.36 (0.19-0.70)</td>
</tr>
<tr>
<td>Trend (P)</td>
<td></td>
<td>0.002</td>
<td>0.001</td>
<td>0.003</td>
<td>0.009</td>
</tr>
<tr>
<td>VO_{2max} ‡ (ml·kg⁻¹·min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤28.9</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>29.0–35.6</td>
<td>2</td>
<td>0.52 (0.31-0.86)</td>
<td>0.59 (0.34-1.01)</td>
<td>0.53 (0.31-0.89)</td>
<td>0.84 (0.45-1.56)</td>
</tr>
<tr>
<td>≥35.7</td>
<td>3</td>
<td>0.24 (0.13-0.47)</td>
<td>0.36 (0.18-0.73)</td>
<td>0.25 (0.13-0.49)</td>
<td>0.70 (0.31-1.58)</td>
</tr>
<tr>
<td>Trend (P)</td>
<td></td>
<td>&lt;0.001</td>
<td>0.014</td>
<td>&lt;0.001</td>
<td>0.69</td>
</tr>
</tbody>
</table>

* adjusted for age category. † adjusted for age category and body mass index. ‡ adjusted for age category, body mass index, adult socioeconomic status, presence of cardiovascular disease, smoking (not at all, 1-19 cigarettes per day or 20 or more cigarettes per day) and alcohol consumption (abstainers, low intake and high intake according to g/wk consumption). § adjusted for age category; body mass index; waist-hip ratio; use of antihypertensive medications; systolic and diastolic blood pressure; and concentrations of high-density lipoprotein, triacylglycerol, insulin and glucose; family history of diabetes mellitus. ‡ METs, metabolic equivalents; † VO_{2max}, maximal oxygen consumption.
Associations of cardiorespiratory fitness with development of the metabolic syndrome. Men with high cardiorespiratory fitness were nearly two thirds less likely to develop the metabolic syndrome in models adjusting only for age (Table 7). Importantly, little or no attenuation in the ORs was seen when adjusting for age and BMI or further for other potentially confounding variables (model 3). Extensive adjustment for variables closely associated with the metabolic syndrome (model 4) attenuated the association, however, suggesting that much of the association of cardiorespiratory fitness with development of the metabolic syndrome is mediated through these variables.

We stratified the men into low and high VO2max groups by age group median VO2max to examine whether the association between vigorous LTPA and development of the metabolic syndrome is modified by cardiorespiratory fitness (Figure 6). Cases numbers are the actual case numbers by cardiorespiratory fitness level and adjusted over categories of physical activity duration for numbers of men per category; age category; BMI; waist-hip ratio; family history of diabetes; use of antihypertensive medications; systolic and diastolic blood pressure; and concentrations of high-density lipoprotein, triglycerides, insulin and glucose. It is clear that men who are less fit and sedentary represent a high-risk group for development of the metabolic syndrome, with a 7-fold increased likelihood of developing the metabolic syndrome compared with fit, active men.

![Figure 6](image_url) The adjusted number of cases of the metabolic syndrome (MSy) at the four-year follow-up according to duration (min/wk) of vigorous leisure-time physical activity in fit and unfit men. In unfit men, \( P \) for the most active vs. least active category was 0.022, \( P \) for the trend 0.025. The association in fit men was not significant, presumably because of the low incidence.

Moderate or vigorous leisure-time physical activity and cardiovascular fitness and the metabolic syndrome as defined by the NCEP. Of the 771 men who did not have the metabolic syndrome as defined by the NCEP or diabetes at baseline, 215 men had the metabolic syndrome at the 4-year follow-up. After adjustment for major potentially
confounding factors (model 3), LTPA reduced the likelihood for development of the metabolic syndrome (the OR for LTPA ≥4.5 METs, ≥3 h/wk vs. ≤60min/wk, 0.63 (95%CI 0.41-0.97), and for LTPA ≥7.5 METs, upper vs. lower third, 0.45 (95%CI 0.29-0.70). In analyses adjusting for potentially mediating variables (model 4), the OR for LTPA ≥4.5 METs, ≥3 h/wk vs. ≤60min/wk was 0.60 (95%CI 0.37-0.99), and for LTPA ≥7.5 METs, upper vs. lower third, 0.48 (95%CI 0.29-0.77).

After adjustment for major potentially confounding factors (model 3), the OR for development of the metabolic syndrome for VO_{2max}, upper third vs. lower third, was 0.27 (95%CI 0.15-0.48). In analyses adjusting for potentially mediating variables (model 4), the OR for VO_{2max}, upper vs. lower third, was 0.45 (95%CI 0.23-0.88).
6 DISCUSSION

6.1 Study design and methods

Study 1. The subjects in Study 1 were recruited from the North Savo and North Carelian hospital districts from patients followed in the hospital out-patient clinics and area health centers. They were not representative of male type 1 diabetic patients in general, because patients with evident atherosclerotic disease, microalbuminuria (overnight urinary albumin excretion <20 μg/min and normal serum creatinine), more than mild background retinopathy or mild signs of neuropathy (missing Achilles reflexes) were excluded. Because recruitment was for an exercise intervention study, the study sample was probably biased towards more physically active individuals, as suggested by the fact that, based on the KIHD 12-month leisure-time physical activity questionnaire, most men met the American Diabetes Association recommendations for physical activity. For the same reason they may have been more compliant than most type 1 diabetic patients. Had the patients been less physically active, the effect of aerobic training on fitness and the lipid profile would probably have been more prominent. Results also cannot be extrapolated to women or patients with complications. A common problem in exercise intervention studies, the drop-out rate was high. Similar numbers of trained and control group participants dropped out, however, and there were no significant differences between trained and control finishers in relevant clinical, anthropometric and biochemical variables. The results nonetheless suggest that in at least motivated type 1 diabetic men largely without complications, a structured aerobic exercise program can improve the lipid profile even in already active individuals.

Inclusion of serum apolipoprotein concentrations provided additional support for the lipoprotein results. Only one other study, which was uncontrolled, has measured apolipoproteins in response to aerobic exercise training in type 1 diabetes (31).

In contrast to most previous studies, Study 1 was randomized and controlled, and the sample size was large enough to detect changes in lipids, lipoproteins and apolipoproteins that might be expected to occur from an exercise intervention program. Furthermore, based on trials in non-diabetic individuals, the duration and intensity of the planned aerobic exercise training may have been expected to have an effect on the lipid profile.

Measurement of physical capacity in Watts and VO₂max allowed objective assessment of the improvement in fitness with training. Subjects were also asked to fill out training diaries in order to assess compliance with the training program, but diaries were inconsistently filled out, and no assessment through diaries was possible. Without this information, the degree of compliance with the training program cannot be reliably assessed. Accurate statements about the amount, frequency and intensity of running exercise needed to bring about the changes in the lipid profile seen in this study cannot therefore be made.

Study 1 also has some other important limitations. Blood samples were taken at only two time points, before and after exercise training. Percent body fat was estimated by skin fold measurements rather than by the more accurate but less practical underwater weighing or dual
energy X-ray absorptiometry techniques. Compliance with completing food records was poor, and obviates detection of subtle dietary changes and their possible impact during the exercise program. Insulin regimens were recorded before and after the exercise intervention, but the importance of accurate reporting was not stressed.

Studies 2 and 3. The type 1 diabetic men and control men of Studies 2 and 3 were recruited from the Kuopio University Hospital outpatient diabetes clinic and from the University of Kuopio student population. They therefore can by no means be considered representative. Because oxidative stress may be elevated in type 1 diabetes as a consequence of microvascular damage, patients with significant microvascular complications were excluded. Even though the control subjects were recruited from the student population, no difference between the diabetic and control men in age, frequency of moderate to strenuous exercise, lipid profile or smoking habits were noted.

Measurement of oxidative stress is difficult and for the most part indirect (374, 375). In the TBARS assay, thiobarbituric acid (TBA) is reacted with MDA, a product of lipid peroxidation, to form TBARS, which can be measured spectrophotometrically. Although not very specific (374, 375), in vitro TBARS has been shown to be closely associated with hydroperoxides, and plasma TBARS to be closely correlated ($r=0.73$) with LDL susceptibility to oxidation (376). Vitreal TBARS and HPLC-measured MDA have been found to correlate highly ($r=0.94$) in diabetic patients (377). Nonetheless, measurement of serum TBARS is non-specific. For that reason, it is generally recommended that more than one measure of lipid peroxidation be used (374, 375).

As in most human studies, samples were limited to blood. Red cells and plasma may not necessarily reflect conditions within the artery wall, where the major pathogenic processes of atherosclerosis are presumed to take place.

Studies 4 and 5. The subjects of these studies were middle-aged men participating in the Kuopio Ischaemic Heart Disease Risk Factor Study (KIHDS), a prospective population-based study (349). The study population comprised a random age-stratified sample of 2,682 men living in Eastern Finland who were 42, 48, 54 or 60 years old at baseline between 1984 and 1989. The participation rate in both the baseline and follow-up examinations was high. Detailed assessment of cardiovascular and metabolic risk factors and potentially confounding risk factors was carried out.

Another strength of this study is the use of proposed definitions of the WHO and NCEP for study 5, rather than our own definitions. Arbitrary definitions derived by individual researchers may be subject to greater variability and make comparison of results between studies difficult. There is also a greater possibility that definitions of individual researchers may be improperly influenced by the findings of the study, i.e., a particular definition is chosen because it enhances a particular finding.

Overall obesity was measured by BMI and body fat distribution by waist circumference and waist-hip ratio. These are only crude measures of adiposity and body fat distribution, but acceptable for population studies (68).
The original WHO definition of the metabolic syndrome recommends that insulin resistance be measured by the euglycemic hyperinsulinemic clamp. This is not possible for most epidemiological studies and many clinical studies. Hyperinsulinemia as a surrogate for insulin resistance has been considered to be adequate for epidemiological studies (260). Use of HOMA or QUICKI has been suggested to improve the estimation of insulin resistance (261, 262). For similar reasons, IFG was used instead of IGT, even though the two groups only partially overlap (378).

Accurate assessment of habitual LTPA is problematic in epidemiological studies. Although data entry for the validated KIHD 12-month Leisure-time Physical Activity Questionnaire (379) is time-intensive, the questionnaire provides detailed information on frequency, duration and intensity of physical activity as recalled for the preceding 12 months. The rather high age-adjusted correlation of cardiorespiratory fitness with duration of vigorous LTPA, and conversely, absence of such a correlation with low-intensity physical activity are evidence for the validity of the questionnaire. When readministered 12 months later, LTPA in the KIHD questionnaire was quite repeatable (intraclass correlation coefficient 0.58, reflecting both the variability inherent in the questionnaire and that of LTPA itself) (379). The KIHD 12-month LTPA Questionnaire seems to quantify LTPA well enough to allow for evaluation of current physical activity recommendations with respect to the metabolic syndrome, and compares well with other questionnaires (331, 380). Even so, the associations in this study between LTPA and development of the metabolic syndrome are probably underestimations because of the inherent imprecision of physical activity questionnaires.

A strength of these studies is that \( \text{VO}_{2\text{max}} \) was measured directly using respiratory gas exchange analysis during a maximal symptom-limited cycle ergometer exercise test under standardized conditions, which is an accurate and highly reproducible measure of cardiorespiratory fitness (381).

A major limitation is the absence of women and elderly from the cohort. Furthermore, the study design does not allow generalization to other races. Also, follow up was only four years. A longer follow up would be particularly helpful in Study 4, where the number of incident cases of diabetes was relatively small (n=51).

Inference of causality based on single observational studies is not possible. Varying degrees of epidemiologic, trial and biological evidence for the favorable effect of moderate and vigorous physical activity on insulin sensitivity and other components of the metabolic syndrome are available (20-26). Nonetheless, confirmation of the results should be sought from other prospective cohort studies and preferably randomized controlled trials.
6.2 Main findings

6.2.1 Study 1. Randomized controlled study assessing the effect of regular aerobic exercise on the lipid profile in men with type 1 diabetes mellitus

This study is to our knowledge the first randomized controlled study of adequate size assessing the effect of regular aerobic exercise on the lipid profile in type 1 DM. The 12-16 week endurance exercise program produced favorable changes in lipid, lipoprotein and apolipoprotein levels in type 1 diabetic men who for the most part already met the American Diabetes Association recommendations for physical exercise (348) at baseline. These changes were independent of effects on body mass or composition and glycemic control, and occurred despite a rather modest improvement in maximal oxygen consumption.

The level of aerobic activity as measured by daily energy expenditure was associated inversely with triglyceride levels and directly with the apo A-I/ apo B ratio, even after controlling for adiposity and glycemic control. Percent body fat correlated positively with total and LDL cholesterol, apo B and triglyceride levels. Maximal oxygen consumption was not associated with any of the lipid profile indices. This suggests that although physical activity and physical fitness are interrelated, physical activity may have a greater bearing on lipid levels, independently of body composition in type 1 diabetes. In contrast to our results, Austin et al. (382) found negative correlations between VO_{2max} and total and LDL-cholesterol and between BMI and HDL in 59 adolescent boys and girls with type 1 DM. The reason for the discrepancy in results may be in part due to the younger age group and inclusion of females in their study. A stronger association of leisure-time physical activity with the lipid profile is also in contrast to findings in non-diabetic men participating in the population-based KIHD study (67). Additional information of the relationship among physical activity, cardiorespiratory fitness and the lipid profile in type 1 diabetes is needed.

At the end of the exercise program the relative increase in HDL/LDL and especially apo A-I/apo B was greater in the training group, with no changes occurring in body composition, body mass, glycemic control, dietary energy and fat intake or insulin dosage. LDL and apo B also decreased significantly during the training period only in the training group, although only the relative change in apo B significantly differed from the control group. The 12-16 week training program also significantly increased cardiovascular fitness, as demonstrated by modest gains in VO_{2max} and W_{max} in the training group. In non-diabetic individuals, increased vigorous aerobic exercise and improved fitness have often been associated with similar improvements in the lipoprotein and apolipoprotein levels, frequently accompanied by increases in HDL cholesterol, but not always in the absence of weight loss (23-25, 108, 112, 383). In the few studies in type 1 diabetic patients, study size has frequently been too small or the exercise intervention too modest to draw firm conclusions about the effect of aerobic exercise on the lipid profile (28-31). In an earlier study, an aerobic exercise program decreased LDL by 14% and increased HDL by 10% in 20 type 1 diabetic men and women (31). Corresponding changes in apo B and apo A-I were also found. We found that HDL, apo
A-I and the HDL/LDL ratio increased also in the control group, underscoring the need for a control group in lipid intervention studies. The reasons for the increase in the control group are not clear. HDL and LDL have been shown to vary seasonally (384), however, with the least favorable changes in cold months (our study began in February and ended in June). We also cannot rule out that the control group did not spontaneously increase the amount of physical activity during the spring. The increase in HDL cholesterol and apo A with aerobic exercise reported by Lehmann et al. (31) may therefore be in part due to lack of a control group and seasonal variation in lipids in addition to concomitant weight loss and decreased percent body fat.

Patients with low HDL/LDL ratios at the beginning of the training program had the most favorable response to regular training. Of the various lipid indices, the HDL/LDL or HDL/total cholesterol ratio is the best single predictor of cardiovascular risk (98, 385) and improvement of prognosis in response to lipid lowering therapy (385). More favorable changes in patients with low HDL/LDL levels are therefore clinically important because these are the patients standing to gain the most benefit by such changes.

Training furthermore increased the HDL/apo A-I ratio, with no change occurring in the control group. The HDL/apo A-I ratio is considered to be representative of the HDL2/HDL3 cholesterol ratio, since HDL2 cholesterol has much less protein content than HDL3 (99). Although controversial (98), HDL2 is often held to be more antiatherogenic than HDL3 cholesterol (97, 99). Training in non-diabetic subjects most often has preferentially increased the HDL2 subfraction (105). In contrast, Lehmann et al. (31) found increased HDL3 cholesterol and unchanged HDL2 levels with training in an uncontrolled exercise intervention in type 1 DM patients. No other studies have examined the effect of regular physical exercise on HDL subfractions in type 1 DM.

The relative change in triglyceride levels was also more favorable in the trained men, although part of the difference between the trained and untrained men can be attributed to the increase in triglyceride levels in the untrained men. The favorable effect of training on triglyceride levels may be in part a spurious finding, since the samples were taken postprandially. Still, changes in HDL/LDL correlated inversely with changes in triglyceride levels and changes in exercise performance measured in Watts tended to be determinant of changes in triglyceride levels, suggesting that training was beneficial.

Features of the metabolic syndrome are also common in type 1 diabetic men (74). Strong evidence suggests that many of the changes in the lipid profile and other features of the metabolic syndrome that aerobic exercise programs produce are mediated by the improvements in insulin sensitivity that may occur even in the absence of weight loss (23, 108, 315, 321, 386), increasing skeletal muscle lipoprotein lipase activity and decreasing hepatic triglyceride lipase activity (108, 321). Aerobic training has been shown to increase insulin sensitivity in type 1 diabetic patients (28, 29). No changes in the daily insulin requirement of the type 1 diabetic men were noted, but this may be due to imprecision in the assessment of their daily insulin requirements. Regular physical exercise may also favorably
affect reverse cholesterol transport by decreasing cholesterol ester transferase protein levels (387) and increasing lecithin:acylcholoseral transferase activity (388), preferentially increasing HDL₂ levels.

6.2.2 Studies 2 and 3. Altered antioxidant enzyme defenses and increased resting and exercise induced oxidative stress in young, insulin-dependent diabetic men

TBARS. A greater than two-fold higher level of plasma TBARS, a widely used, indirect measure of lipid peroxidation, was found in healthy young men with type 1 diabetes both at rest and after exercise, suggesting increased oxidative stress. The most striking findings were the strong inverse correlation between resting plasma TBARS and VO₂max and the strong positive correlation between the relative increase in plasma TBARS and VO₂max, present only in the diabetic group, and apparently independent of glycemic control or lipid levels at rest.

The more than two-fold higher level of resting plasma TBARS in the diabetic men suggests increased oxidative stress in type 1 diabetes and agrees with many (46, 51, 135, 148, 150, 153) but not all (137, 140, 152, 156, 157) studies finding increased plasma TBARS or MDA in diabetic patients. The diabetic subjects in this study were in moderate glycemic control and with few complications. Our findings are consistent with other studies reporting elevated plasma TBARS or MDA even in type 1 diabetic patients without complications (153, 154).

Diabetic and control groups showed a similar, approximately 50% increase in plasma TBARS to 40 min sustained exercise at 60% VO₂max, although absolute post-exercise plasma TBARS were remarkably elevated in diabetic vs. control subjects. Increased plasma TBARS in response to exercise is in agreement with many studies examining sustained exercise-induced oxidative stress (115, 176, 178, 389, 390). Although free fatty acid or triglyceride levels were not measured immediately before or after exercise, net plasma triglyceride and free fatty acid levels of fatty acids containing two double bonds (the minimum for significant TBARS formation) or more remained virtually unchanged in response to 30 and 60 min of variable intensity exercise (391). Therefore, it is unlikely that lipid changes during exercise are a significant cause of exercise induced TBARS formation.

The rise in plasma TBARS relative to resting values correlated directly with oxygen consumption during exercise (at 60% VO₂max). VO₂max did not correlate with absolute post-exercise TBARS. Although it may at first seem paradoxical that more fit diabetic men had greater relative increases in plasma TBARS, the more fit subjects consumed up to 1.6 times as much oxygen during exercise than the less fit, subjecting them to greater pro-oxidant forces. That the correlations between VO₂max, resting plasma TBARS and relative rise with exercise were not present in the control group in the present study nor reported in previous studies (176, 389, 390) may be because of the variability in the less fit control subjects or their much lower resting levels of oxidative stress. Fitness may also play a more central role as a determinant of resting oxidative stress in diabetes.
The relationship between oxidative stress and antioxidant defenses is complex. Upregulation of antioxidant defenses can occur in response to increased oxidative stress or result in decreased oxidative stress (178). Exercise training in non-diabetic animals has been shown, however, to reduce indices of lipid peroxidation in heart (392) and skeletal muscle (180). Training in animals has also been shown to have favorable effects on oxidative stress and antioxidant status as measured by TGSH and GSSG (181, 392, 393) and GPX, catalase and superoxide dismutase activities (181, 394). VO_{2\text{max}} has also been found in healthy young men to correlate with superoxide dismutase and catalase activities in the vastus lateralis muscle (395). Although no relationship of VO_{2\text{max}} with glutathione and its enzymes, superoxide dismutase or catalase was noted, regular physical activity and higher VO_{2\text{max}} may nonetheless lower oxidative stress by strengthening antioxidant defenses and permitting more efficient use of oxygen. Another mechanism may be through insulin resistance, because insulin resistance may also be associated with increased oxidative stress in addition to decreased VO_{2\text{max}} (62-64).

Glutathione and glutathione-dependent enzymes. The diabetic group had a higher resting level of blood TGSH than control subjects. The GSSG/TGSH ratio was similar in both groups. Higher blood TGSH levels in diabetes are in contrast to other human studies finding either lower (127, 131) or unchanged levels (126, 129). Elevated erythrocyte GRD activity in the diabetic group may partially explain increased total blood glutathione stores found in the diabetic group by increasing conversion of GSSG to GSH, since excess GSSG is exported out of the cell (396).

In contrast to our study, most other human studies in which decreased blood TGSH levels have been found have been in older, mainly non-insulin-dependent subjects (131) or poorly defined populations (127). In a study investigating platelet glutathione levels in type 1 diabetic patients only slightly older than in our study and in moderate glycemic control, TGSH content was similar and GRD activity elevated in type 1 diabetes (126). In the type 1 diabetic patients without nephropathy, TGSH levels were nearly 20% higher than control subjects, although no statistical comparison was reported (126). Increased GRD activity in type 1 diabetes is consistent with some (397, 398), but not all (134) previous reports.

Induction of the polyol pathway by hyperglycemia has been proposed as a major mechanism leading to the depletion of glutathione reported in some other studies (399, 400). Our study and those of others (126, 397) suggest that at least in some diabetic populations, polyol pathway induced decreases in the NADPH/NADP+ ratio or other abnormalities in thiol metabolism do not occur to such an extent to prevent upregulation of blood TGSH levels and red cell GRD activity. Thus the higher blood glutathione levels found in the diabetic group in the present study may be in part because the clinical characteristics of the diabetic population in our study differed markedly from those in previous studies. Young otherwise healthy men with type 1 diabetes may partially compensate for increased oxidative stress by upregulation of the glutathione system.
No differences at rest between diabetic and control men were found in erythrocyte Se-GPX and GST activities. Decreased (142) and unchanged (397) Se-GPX activity in type 1 diabetes has been previously reported. Interestingly, GST was the only enzyme that was inversely correlated with HbA1c levels. In vitro studies in rat liver have shown decreased GST activity in a hyperglycemic medium (401). Human studies assessing erythrocyte GST activity in type 1 diabetes are limited. Ståhlberg and Hietanen (134), however, also found no difference in GST activity between diabetic and control subjects.

The increase in blood GSSG and GSSG/TGSH in response to exercise in this study also indicates increased oxidative stress resulting from exercise, although there were no significant differences in these indices between diabetic and control groups. Acute changes in glutathione redox status have been considered to be a particularly sensitive indicator of oxidative stress (176, 181, 402-404). This response of GSSG and GSSG/TGSH to acute exercise is also in agreement with previous studies by us (176, 181, 402) and others (403, 404). We have found no other studies published assessing exercise-induced oxidative stress in diabetes.

The erythrocyte selenium-dependent GPX response to exercise seemed to be impaired in the diabetic group. Se-GPX activity rose significantly with exercise only in the control group, and unlike at rest, Se-GPX activity was higher in the control men compared to the diabetic men. The explanation for lack of significant upregulation of Se-GPX in the diabetic group is unclear. Upregulation of GPX in response to acute exercise has been found in skeletal muscle in animal experiments (405), although in some human studies, no changes in erythrocyte GPX activity were reported (406-408). Red cell GRD and GST activity were unaffected by exercise.

**Red cell catalase and Cu,Zn-SOD activity.** Our finding of decreased red cell Cu,Zn-SOD activity in type 1 diabetes at rest agree with some (61, 139, 142), but not all (397) previous studies. Decreased Cu,Zn-SOD activity coupled with increased superoxide production (43, 44) could predispose to increased oxidative stress, especially if not compensated with increased catalase or Se-GPX activity. Superoxide may react with other reactive oxygen species such as nitric oxide to form highly toxic species such as peroxynitrite, in addition to direct toxic effects (35). Alternatively, superoxide can be dismutated to much more reactive hydrogen peroxide, which through the Fenton reaction can then lead to highly toxic hydroxyl radical formation (44). Thus, decreased catalase activity could also contribute to the increased oxidative stress as measured by plasma TBARS found in the diabetic men. Elevated glucose (401) and hydrogen peroxide levels (409) have also been shown to inactivate catalase.

Red cell GRD, GST, catalase and Cu,Zn-SOD activity were unaffected by exercise. The response of erythrocyte catalase activity to acute exercise in our study agrees with some previous studies also showing no change after exercise (406-408). Because catalase is localized to peroxisomes or microperoxisomes (138), it would be expected that GPX, located in the cytosol and mitochondria, would be more sensitive to exercise-induced peroxide and peroxyl radical formation. The response of erythrocyte Cu,Zn-SOD activity to exercise has been very inconsistent in the literature (410).
6.2.3 Study 4. The metabolic syndrome and development of diabetes mellitus: application and validation of recently suggested definitions of the metabolic syndrome in a prospective cohort study

The WHO definition of the metabolic syndrome with waist-hip ratio >0.9 or BMI ≥30 kg/m² was the most sensitive of the definitions, detecting over four fifths of prevalent and two thirds of incident cases of diabetes with good specificity (0.78-0.80). The NCEP definition with adiposity defined as waist girth >102 cm missed most incident cases of diabetes, although it was the most specific. All four definitions identified individuals at high risk for developing diabetes during follow up in this population-based cohort of middle-aged men.

The WHO definition of the metabolic syndrome used in this study was modified largely according to the recommendations of the EGIIR (238). The WHO definition of the metabolic syndrome included insulin resistance as measured by the euglycemic hyperinsulinemic clamp and IGT (69). Clamp studies are not well suited for most epidemiological studies, and for many studies glucose tolerance tests are not possible. Our study suggests that the recommendation by the EGIR to estimate insulin resistance with hyperinsulinemia instead of clamp studies and to use of IFG instead of IGT for the definition of the metabolic syndrome is valid for epidemiological studies. The recently validated insulin sensitivity index QUICKI, closely related to HOMA (262), slightly increased the sensitivity and specificity for prevalent and incident diabetes. Hypertension was defined according to the EGIIR recommendations at a lower level (≥140/90) than the original WHO definition (≥160/90) for consistence with current WHO-ISH and Sixth Joint National Committee recommendations (238, 240, 241). Also as recommended by the EGIIR (238), microalbuminuria was not included in the definition. The EGIIR also recommended that triglycerides ≥2.0 or HDL <1.0 mmol/l be used for the definition of dyslipidemia (238). A triglyceride cut-off of ≥1.70 mmol/l has been recommended by both the WHO and the NCEP. Definitions of the metabolic syndrome using this cut-off are slightly more sensitive for predicting diabetes than those using triglycerides ≥2.0 mmol/l. HDL cut-offs of 0.9 vs. 1.0 mmol/l have little effect on the prevalence of the metabolic syndrome or its sensitivity or specificity for diabetes. We therefore used the original WHO definition of dyslipidemia.

One of the most controversial aspects of the metabolic syndrome is the definition of adiposity. The WHO definition of the metabolic syndrome with adiposity as defined by waist-hip ratio >0.9 or BMI ≥30 kg/m² detected diabetes well, detecting 83 and 67% of prevalent and incident cases of diabetes with a specificity of 0.78-0.80. The WHO definition of the metabolic syndrome with adiposity modified according to the EGIIR as waist circumference ≥94 cm performed less well, present at baseline in only 68 and 57% of prevalent and incident cases of diabetes. The NCEP definition with adiposity defined as waist circumference >102 cm was quite specific but insensitive, detecting only 61 and 41% of prevalent and incident diabetes. The NCEP recommendations suggest that some men may be genetically predisposed
to the metabolic syndrome already at lower levels of abdominal obesity, with waist circumferences between 94-102 cm (70). Using a cut-off of 94 cm for waist girth improved sensitivity of the definition to 0.72 and 0.49 for prevalent and incident diabetes, with decreased but still good specificity (0.82-0.84). This suggests that the genetic susceptibility of waist 94-102 cm for the metabolic syndrome could be generalized to all middle-aged men, at least in the Finnish population.

The 94 and 102 cm cut-offs for waist circumference are influenced by a Netherlands cross-sectional study in which these cut-offs corresponded to a BMI of ≥25 and ≥30 kg/m² (373). A waist girth cut-off of 87 cm corresponded to a BMI ≥25 kg/m² in the non-diabetic KIHD cohort, underscoring the well-described (68) variable and population-specific relation of waist circumference to BMI, even in northern European populations. Substituting a waist girth cut-off of 87 cm improved sensitivity of the NCEP definition to 0.59 for new-onset diabetes, with decreased, but still quite high specificity (0.79). Similarly, defining adiposity as waist girth ≥87 cm or even as BMI ≥25 kg/m² or waist girth ≥102 cm (level for action in the presence of other risk factors as recommended by the National Institutes of Health) for the modified WHO definition of the metabolic syndrome was more sensitive than defining adiposity as waist ≥94 cm and as sensitive as defining adiposity as waist-hip ratio >0.9 or ≥30 kg/m² in detecting prevalent and incident diabetes.

Even mild overweight, especially in the presence of insulin resistance, increases the risk for diabetes (68, 289). Both the WHO and the NECP definitions of the metabolic syndrome are based on insulin resistance, the WHO definition directly, and the NCEP definition indirectly through markers or correlates of insulin resistance. Failure to consider even mild overweight or abdominal obesity in the presence of insulin resistance or markers of insulin resistance as a significant risk factor could be a significant shortcoming from both a clinical and public health perspective, missing most persons at risk for developing an increasingly common disease such as diabetes, which carries a high morbidity and mortality.

Even though mild disturbances in glucose metabolism are a central feature of the metabolic syndrome, including a measure of hyperglycemia in the definitions of the metabolic syndrome is problematic when using diabetes as an endpoint. Even when excluding IFG from the definitions, the sensitivity of the WHO definitions was still fairly high (0.49-0.55), whereas the sensitivity of the NECP definitions was only 0.31-0.37. The relatively greater effect of removing hyperglycemia from the NECP definitions is mainly due to the absence of a measure of insulin resistance (e.g. hyperinsulinemia). Excluding hyperglycemia from the definitions did not affect specificity.

The WHO definition of the metabolic syndrome based on waist-hip ratio >0.9 or BMI ≥30 kg/m² was common, present in slightly more than one fifth of all non-diabetic men at baseline. Over 95% of men with the metabolic syndrome had hyperinsulinemia. Conversely, over 80% of the men with insulin resistance had the metabolic syndrome with adiposity as defined by the WHO, emphasizing the clustering of insulin resistance and other components of the metabolic syndrome. The metabolic syndrome with the EGIR definition of adiposity
(waist ≥94 cm) was less prevalent, about 19% of men at baseline. The NCEP definition with adiposity defined as >102 cm was much less common, present in about 11% of men at baseline. Using a cut-off of 94 cm for waist girth increased prevalence to 18%.

At baseline, 11% of men had the metabolic syndrome by both the NCEP definition using the lower 94 cm cut-off for waist circumference and the WHO definition based on waist-hip ratio >0.9, of whom 23 (21%) developed diabetes. This concurrence occurred even though the WHO and NCEP used rather different approaches to defining the metabolic syndrome (the former based strongly on insulin resistance and the latter only on numbers of features related to insulin resistance), again emphasizing the clustering of components of the metabolic syndrome. Despite the concurrence of the WHO and NCEP definitions, the absence of a measure of insulin resistance (e.g. hyperinsulinemia) in the definition may also partly explain the lower sensitivity of the NECP definitions in detecting prevalent and incident diabetes.

An obvious shortcoming of using type 2 diabetes as an endpoint for evaluating the sensitivity of the metabolic syndrome is that few data are available of the proportion of type 2 diabetes cases that are expected to have the metabolic syndrome prior to development of diabetes. Even so, type 2 diabetes is closely related to the metabolic syndrome, and can in large part be considered an end-stage manifestation of the metabolic syndrome. Therefore, definitions of the metabolic syndrome can be compared by their sensitivity and specificity for detecting new cases of incident diabetes in a prospective cohort study design. Roughly 5-10% of middle-aged diabetic patients have latent autoimmune diabetes of the adult (79), in which insulin secretion is the primary defect, and the overall proportion of diabetic patients thought to have insulin resistance before onset of diabetes has been estimated to be 75-85% (411, 412). If this were taken into account, the sensitivity of the WHO and NCEP definitions in detecting prevalent and incident diabetes in which the metabolic syndrome may be expected to precede diagnosis would be even higher.

### 6.2.4 Study 5. Low levels of leisure-time physical activity and cardiorespiratory fitness predict development of the metabolic syndrome

Study 5 is to our knowledge the first study reporting the association of physical inactivity with the development of the metabolic syndrome. Moderate and vigorous LTPA decreased the risk for development of the metabolic syndrome as defined by an adaptation of the WHO definition by up to nearly two-thirds in this population-based cohort of middle-aged men, even after extensive adjustment for potential mediating and confounding factors, and especially in high-risk men. Moreover, findings were qualitatively similar even when using two rather different definitions of the metabolic syndrome, one based heavily on insulin resistance or hyperinsulinemia (WHO) (69) and the other only on features related to insulin resistance (NCEP) (70). Cardiorespiratory fitness was also inversely associated with development of the metabolic syndrome independently of obesity and other important confounding factors.
The CDC-ACSM guidelines for physical activity recommend that all adults should engage in at least 30 minutes of moderate-intensity physical activity per day to prevent chronic diseases, including type 2 diabetes and CVD (23). Moreover, the report emphasized 'lifestyle' activity. Our findings suggest that men complying with the CDC-ACSM recommendations are nearly half as likely to develop the metabolic syndrome. Of importance, this decreased likelihood was independent of BMI and other potential confounders such as CVD, smoking and current socioeconomic status. When additional adjustment for potentially mediating factors such as baseline insulin, glucose, lipid and blood pressure levels, the 95% CIs widened such that the association was no longer significant in the entire cohort. In a high-risk subgroup of men with either baseline hyperinsulinemia or two or more features of the metabolic syndrome (hypertension, dyslipidemia or overall or abdominal obesity), however, the protective effect of moderate or vigorous physical activity was significant even when adjusting for potentially mediating factors. More dramatic reductions in risk were accrued by men engaging in ≥60 min vigorous activity/wk compared to sedentary men. The decrease in likelihood of the metabolic syndrome in men engaging in regular moderate and vigorous exercise was also independent of low-intensity physical activity and cardiorespiratory fitness. Previously, the likelihood of the clustering of metabolic factors was reported to increase with the intensity of physical activity in a cross-sectional study of 711 working men who presented for preventive assessment (325).

In our study, low-intensity leisure-time physical activity was not associated with development of the metabolic syndrome. Consistently, low-intensity physical activity has been less strongly associated with the risk of most chronic diseases than moderate or vigorous physical activity in most previous studies (23, 65, 67).

An important finding of the present study was that men with VO_{2max} in the upper third were 65-75% less likely to develop the metabolic syndrome than those with VO_{2max} in the lower third, even after adjustment for important confounding factors such as age, BMI, CVD, smoking and current socioeconomic status. The apparent benefit of cardiorespiratory fitness in preventing the metabolic syndrome was substantially attenuated after adjustment for other potential mediating factors such as blood pressure, triglyceride and HDL cholesterol levels and insulin and glucose levels. Directly measured or estimated VO_{2max} has been consistently associated with many components of the metabolic syndrome, including insulin resistance, serum HDL and triglyceride levels and blood pressure (67, 258, 324, 325, 413). Our findings suggest that high cardiorespiratory fitness is strongly protective against the metabolic syndrome, but that the benefit may be largely mediated by components of or closely related to the metabolic syndrome. Interestingly, findings were qualitatively similar even when using the NCEP definition of the metabolic syndrome, which is based only on features related to insulin resistance (70). Despite marked attenuation of the strong inverse association of fitness with the development of the metabolic syndrome by the NCEP definition when adjusting for potentially mediating factors, the association remained significant. Our findings agree with two recent cross-sectional studies showing an association of low cardiorespiratory fitness and
clustering of metabolic factors (324, 325). Although moderate and vigorous physical activity are important determinants of VO$_{2\text{max}}$, a strong genetic component is also present (191). This raises the question of whether physical activity has similar effects in fit and unfit individuals.

Men who were both less fit and sedentary represented a high-risk group, with a 7-fold increased likelihood of developing the metabolic syndrome compared to fit men engaging in at least 60 min of vigorous activity a week. Even modest amounts of vigorous physical activity in less fit men appeared to dramatically decrease the likelihood of developing the metabolic syndrome, even though active but unfit men were still about 2.5-fold more likely to develop the metabolic syndrome than active fit men. From a clinical perspective, measurement of VO$_{2\text{max}}$ in sedentary men may provide an efficient means to target even individuals with relatively few metabolic risk factors who may benefit from more intensive interventions including lifestyle or structured physical exercise in the prevention of the metabolic syndrome and its consequences.
7 SUMMARY AND CONCLUSIONS

1. The 12-16 week endurance exercise program improved lipid, lipoprotein and apolipoprotein levels in type 1 diabetic men who for the most part already met the American Diabetes Association recommendations for physical exercise at baseline. The most favorable training induced changes in the HDL/LDL and apo A-I/apo B ratios were in patients with low baseline HDL/LDL levels, likely the group with the most benefit to be gained by such changes. Changes in the lipid profile occurred without changes in body fat or composition, glycemic control, or daily insulin dosage. Encouragement of regular vigorous leisure-time physical activity in otherwise healthy motivated type 1 diabetic patients with dyslipidemia may improve the lipid profile even in physically active patients.

2. Higher plasma TBARS, an indirect measure of lipid peroxidation, was found in healthy young men with type 1 diabetes both at rest and after exercise, suggesting increased oxidative stress. A strong inverse correlation between resting plasma TBARS and VO2max was found in the diabetic men, which could imply a protective effect of physical fitness against lipid peroxidation. Improved measures of lipid peroxidation and an exercise intervention study in sedentary diabetic patients would be necessary to test this hypothesis.

3. Otherwise healthy young diabetic men had lower erythrocyte Cu,Zn-SOD and catalase activity, but higher blood total glutathione concentrations and increased red cell GRD activity. Coupled with increased plasma TBARS and blood total glutathione levels in the diabetic men, these changes may reflect increased susceptibility to oxidative stress and compensatory adaptations of glutathione homeostasis in response to increased oxidative stress. Exercise-induced red cell Se-GPX upregulation appeared to be impaired in the diabetic men, which could predispose to exercise-induced oxidative stress.

4. The WHO and NCEP definitions of the metabolic syndrome appear valid, identifying individuals with a 5-9-fold increased likelihood of developing diabetes during the four-year follow-up in this population-based cohort of middle-aged Finnish men. The modified WHO definition based on waist-hip ratio >0.9 was the most sensitive in detecting prevalent and incident diabetes and had good specificity. The NCEP definition of the metabolic syndrome with adiposity defined as waist girth >102 cm was the most specific, but did not detect most cases of incident diabetes. Defining adiposity as waist circumference >94 cm improves the sensitivity of the NCEP definition. Use of hyperinsulinemia or the QUICKI insulin sensitivity index as a surrogate for insulin resistance is sufficient for epidemiological studies. Further application of these definitions in other populations is necessary.

5. Compliance with the current CDC-ACSM recommendations for physical activity decreased the likelihood for development of the metabolic syndrome during a four-year follow-up in middle-aged men, especially in high-risk groups. Vigorous leisure-time physical activity provided additional benefits. Cardiorespiratory fitness was also strongly inversely associated with development of the metabolic syndrome, although possibly not independently of mediating factors. These findings support the CDC-ACSM recommendations for regular
physical activity in the prevention of the metabolic syndrome. Measurement of VO$_{2\text{max}}$ in sedentary men with risk factors may provide an efficient means for targeting individuals who would benefit from interventions to prevent the metabolic syndrome and its consequences. Intervention at an early phase in even relatively low-risk men may dramatically reduce the risk for development or progression of metabolic disturbances that eventually culminate in chronic and progressive diseases such as diabetes and atherosclerosis.

These studies support promotion of moderate and vigorous leisure-time physical activity to improve dyslipidemia and cardiorespiratory fitness and possibly decrease lipid peroxidation in otherwise healthy type 1 diabetic men, and in middle-aged non-diabetic men, to decrease the risk for development of the metabolic syndrome and thereby prevent chronic and progressive diseases such as diabetes and atherosclerosis.
8 FUTURE DIRECTIONS

Compelling evidence linking e.g. AGEs, oxidative stress, cytokines and adhesion molecules to various pathological conditions in \textit{in vitro} and animal models of diabetes exists, but more knowledge on the mechanisms of oxidative stress in relation to hyperglycemia and insulin resistance is needed. Furthermore, the causal role of oxidative stress in the development of diabetic micro- and macrovascular complications remains to be shown. The meaning of exercise-induced oxidative stress from a health perspective, and whether aerobic training decreases resting or exercise-induced oxidative stress are still unclear.

The lack of specificity of various measures of oxidative stress is a major problem facing all investigators in free radical research. Furthermore, many studies have relied solely or mainly on TBARS as an index of lipid peroxidation. Additional indices of lipid peroxidation should be employed. Measures of protein or DNA oxidative damage have been virtually totally ignored and should also be used. To assess whether improved physical fitness decreases resting oxidative stress in type 1 diabetes, an aerobic training trial in sedentary diabetic patients would be necessary.

Further studies of the metabolic syndrome as defined by the WHO and the NCEP and its association with type 2 diabetes and CVD are needed. The relationship between overall obesity as measured by BMI and measures of abdominal obesity such as waist girth and waist-hip ratio, and in turn their relationships with components of the metabolic syndrome, vary among populations. Furthermore, the prevalence of abdominal and overall obesity varies widely between populations. It may well be that these differences among populations also affect the associations of the different definitions of the metabolic syndrome with diabetes and CVD.

Strong though varying degrees of epidemiologic, trial and biological evidence for the favorable effect of moderate and vigorous exercise on insulin sensitivity and other components of the metabolic syndrome are available. Furthermore, recent evidence from the Finnish Diabetes Prevention Study and U.S. Diabetes Prevention Program suggest that even relatively modest lifestyle interventions can have a major impact in decreasing the risk for diabetes in glucose-intolerant individuals. Although our study suggests that regular moderate and vigorous physical activity may prevent the metabolic syndrome itself, no trials with lifestyle interventions using the metabolic syndrome as an endpoint have yet been carried out. The long-term efficacy of such interventions clinically and at the population level in the treatment and prevention of the metabolic syndrome and its consequences warrant further research.
REFERENCES

49. Tsai EC, Hirsch IB, Brunzell JD, Chait A. Reduced plasma peroxyl radical trapping capacity and increased susceptibility of LDL to oxidation in poorly controlled IDDM. Diabetes 1994;43:1016-1014.
78. Shaw JE, Zimmet PZ, McCarty D, de Courten M. Type 2 diabetes worldwide according to the new classification and criteria. Diabetes Care 2000;23 Suppl 2:B5-10.


185. Atalay M. Antioxidant Responses to Physical Exercise-Induced Oxidative Stress. In Department of Physiology, Publications of the University of Kuojo D. Medicina 158, 1998, p. 76 + appendix
186. Khanna S: Thiol Antioxidants: Protection Against Oxidative Stress and Redox Regulation of Cellular Responses. In Department of Physiology, Publications of the University of Kuojo C. Natural Sciences and Environmental Sciences, 1998, p. 80 + appendix


296. Boden WE. High-density lipoprotein cholesterol as an independent risk factor in cardiovascular disease: assessing the data from Framingham to the Veterans Affairs High-Density Lipoprotein Intervention Trial. Am J Cardiol 2000;86:19L-22L.


ORIGINAL PUBLICATIONS 1 – 5