Oxidative stress and health status in patients with insulin-dependent diabetes mellitus

J.H. Assink

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Oxidative stress and health status in patients with insulin-dependent diabetes mellitus

Oxidatieve stress en gezondheidsbeleving van patiënten met insuline-afhankelijke diabetes mellitus

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Voor hen die mij zeer dierbaar zijn

I

General introduction and outline of the thesis

INTRODUCTION

Before the discovery of insulin by Banting and Best, insulin-dependent (type 1 or juvenile) diabetes mellitus was an atrocious disease, characterised by a unquenchable thirst and leading to an inevitable death within a short period of time. After this period, as insulin treatment became available in 1923, life expectancy of patients with insulin-dependent diabetes mellitus (IDDM) increased dramatically, but this longer survival was found to be accompanied by the development of microvascular complications.

These complications, characterised by angiopathy of the microvascular system, clinically affect, primarily, the eyes, the kidneys and the peripheral nervous system[1]. Diabetic retinopathy, of which the macular and proliferative forms can result in blindness, develops in more than 90% of the IDDM patients[2]. Diabetic nephropathy, an important cause of end-stage renal disease in the Netherlands, develops in 20-40% [2-5]. Diabetic neuropathy, which is associated with an increased risk of foot ulcers and amputation, is found in approximately 60% of the patients[6].

Microvascular complications occur in patients with IDDM as well as in other types of diabetes (e.g secondary diabetes and non-insulin-dependent diabetes mellitus), which all share chronic hyperglycaemia as the primary feature. Therefore, it has long been suspected that chronically elevated glucose levels play a causal role in the pathogenesis of these complications. Many studies have been published suggesting that strict metabolic control has a preventive effect on the development of diabetic nephropathy[2,3,7-9], retinopathy[8,10] and neuropathy[11,12]. Often, however, these studies were regarded as being too short-term or too small to provide a definite proof of the 'hyperglycaemia hypothesis'. Furthermore, in some studies even a deterioration of diabetic retinopathy during the first years of strict metabolic control was described[7,9,13].

However, in 1993 the hyperglycaemia hypothesis was confirmed by the results of the Diabetes Control and Complications Trial (DCCT)[14-16], a large multicenter trial which showed that strict metabolic control, achieved by an

intensive insulin regime, effectively delays the onset and slows the progression of microvascular complications. Given this endorsement of the 'hyperglycaemia hypothesis', to achieve (near) normoglycaemia has become the main objective of care in IDDM. However, it is still questionable whether such strict metabolic control can be realized in routine diabetes care[17].

Despite intensive treatment and strict metabolic control, a number of patients will still develop microvascular complications[16,18]. Furthermore, overt nephropathy and advanced retinopathy do not seem to be influenced by average blood glucose concentrations at all[13,19]. These findings suggest that the development of diabetic complications is also dependent on other factors[20–22]. In addition, the pathophysiological mechanism by which poor metabolic control predisposes to the microvascular complications is not completely understood. Many mechanisms and factors have been or are still under investigation: hereditary factors[23–26], dietary factors[27–30], blood pressure[31–34], haemodynamic factors[35,36], endothelial factors[37,38], the polyol pathway[39,40], growth factors[17], and Advanced Glycosylation End products[40–42]. Still, these studies did not came up with a definite answer up to now.

OXIDATIVE STRESS

Another recently proposed mechanism, which might be involved in the development and progression of microvascular complications, is based on the tissue damage resulting from the formation of free radicals. Free radicals are highly reactive particles, since these molecules contain an unpaired electron in their outer orbit. The formation of these radicals is a biological process, which takes place, for example, when oxygen is reduced to water in the respiratory chain in the mitochondria of living cells or during enzymatic synthesis of prostaglandins and leukotrienes[43,44]. Under normal physiological conditions the cell is protected against an overproduction of radicals by several anti-oxidant systems. Based on their action these anti-oxidant systems could be classified as either preventive or chain breaking systems. In the latter, the anti-oxidative property of the substances involved (e.g. uric acid, tocopherols, beta-carotene) is based on their ability to form a more stable reaction product after the reaction with a free radical, thereby interrupting the oxidative process. Preventive anti-oxidants (e.g. ceruloplasmin, ferritin, transferrin) are preventing the formation

of free radicals by binding substances that act as catalysts in the formation of free radicals. These anti-oxidant systems are located intra- as well as extracellularly and involve enzymatic as well as non-enzymatic reactions. In case of a disturbed balance between the anti-oxidant defence systems and the formation of free radicals (oxidative stress), free radicals have been suggested to play a role in the development of insulin-dependent diabetes mellitus[45] as well in other diseases [46].

In diabetes, the formation of free radicals can be induced by increased glucose concentration through non-enzymatic glycation of protein substrates[47], auto-oxidative glycation[48], activation of protein kinase C[49] and increased polyol pathway activity[50]. In addition, diabetes is known to induce chances in the content and activity of cellular anti-oxidant enzymes[51-55].
Free radicals, which are not trapped by anti-oxidants during the periods of oxidative stress, are suggested to disturb endothelial dependent vasorelaxation, stimulate growth factors, induce the expression of adhesion molecules, promote the blood coagulation, and contribute to the formation of Advanced Glycosylation Endproducts[56,57]. All these mechanisms have been implicated in the development of microvascular complications. Furthermore, by modifying molecules, free radicals may form cytotoxic substances, which may directly induce endothelial damage[58]. In addition, intervention studies mostly performed in diabetic rats have shown a protective effect of various anti-oxidants on the development of microvascular complications[59-61].
Given these findings, increased oxidative stress could be a mechanism involved in the development of microvascular complications in patients with IDDM. Since oxidative stress is not exclusively related with hyperglycaemia, but is also influenced by other factors like dietary factors (variation of intake of anti-oxidants) or hereditary factors (regulation of enzymatic anti-oxidants), it might also account for the development of microvascular complications under strict metabolic control.
Because in vivo measurement of free radicals is difficult given their reactivity, short half-life, and very low concentrations[62], various methods have been developed to investigate indirectly whether such oxidative processes occur in reality. Substances indicating oxidative damage to DNA (oxidized DNA bases), proteins (protein carbonyls) and lipids can be measured. Another indirect indicator of oxidative stress is given by measurement of anti-oxidant status[63]. To study the relation between oxidative stress and microvascular complications lipid peroxidation and its consequences could be useful. Two of these methods are the measurement of malondialdehyde, a degradation product of lipid

peroxides and the susceptibility of low-density lipoprotein (LDL) to in vitro oxidation. The latter has recently been proposed as an appropriate measure of the response to the in vivo oxidative stress, which is thought to occur within the matrix of the vessel wall close to the endothelium[64, 65].
In the first part of this thesis the results of a study will be described focusing on the presence of oxidative stress in IDDM patients and its possible role in the development in microvascular complications. Oxidative stress has been measured by anti-oxidant status, the susceptibility of LDL to in vitro oxidation and serum malondialdehyde concentrations. In the second chapter the study population is introduced by describing the relationship between metabolic control, serum lipids and the presence of microvascular complications. Chapter 3 presents the results of a comparison of serum anti-oxidant status between IDDM patients and healthy controls. The presence of oxidative stress assessed by the susceptibility of LDL to in vitro oxidation and malondialdehyde concentrations is described in chapter 4, whereas chapter 5 presents the association of these two parameters with metabolic control and microvascular complications.

HEALTH STATUS

Since 1948, when the World Health Organization defined health as being not only the absence of disease and infirmity, but also the presence of physical, mental and social well being[66], quality of life issues have become steadily more important in health care practice and research[67].
Quality of life is notoriously difficult to capture in a comprehensive definition. It could best be regarded as an umbrella concept that encompasses health status as well as satisfaction in a broader range of domains such as environment, economic resources, relationships, work, and leisure time. The concept health status differs from 'quality of life' in that the latter is broader and also includes socio-economic aspects and perceptions of a person's immediate environment[68]. Because health status represents what a patient perceives as one of the outcomes of medical care, it is regarded as an important adjuvant to traditional biological and clinical outcome measures.

Insulin-dependent diabetes mellitus is a chronic disease which affects many aspects of everyday life. To maintain satisfactory blood glucose level in order to avoid hyperglycaemic complaints and hypoglycaemic events, food intake,

exercise and insulin dose must be balanced from hour to hour. In addition, there is a great risk of developing one or more microvascular complications. This ever-present responsibility for daily balance and worries about future health and well-being may pose an extra burden to a IDDM patients' daily life. As described before, the importance of strict metabolic control for the prevention as well as the progression of long-term diabetic complications has been shown for patients with insulin dependent diabetes mellitus[16]. As reported by the DCCT, the main side effects of strict metabolic control are considerable weight gain and a threefold increased risk of serious hypoglycaemia[16, 69]. To many patients, these side effects may be an unacceptable price for strict metabolic control, because of both physical and emotional consequences. Furthermore, stricter metabolic control may require more intensive therapy, such as multiple daily doses of insulin (by manual injection or by insulin pump) and frequent measurements of blood glucose values. This regime may have its own negative effects on a patient's health status.

In the second part of this thesis the results of a study are described, which was performed in connection with the main investigation. In this study the association between metabolic control, insulin replacement therapy and their consequences on health status is investigated. In chapter 6 the effects of metabolic control and diabetic complaints on health status are described. The consequences of insulin therapy and self-control are assessed in chapter 7. Finally, the consequences of the results of this thesis are discussed in chapter 8 and compiled in chapter 9.

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2

Metabolic control, lipid profile, and microvascular complications in a Dutch population of IDDM patients

SUMMARY

In IDDM, there is a definite relationship between the level of metabolic control (HbA1c levels) and the development and progression of diabetic microvascular complications. Still, it is known from clinical practice that HbA1c is not always the best predictor for the development of complications. Other variables related to metabolic control may better reflect the risk for the development of diabetes complications in these patients. We studied the association between different measures of metabolic control, lipids and diabetes complications in a group of 281 patients with insulin-dependent diabetes mellitus (153 men and 128 women) with a mean age of 38.2 years (SD 12.4) and diabetes duration of 17.2 years (10.7).

Metabolic control, as assessed by HbA1c and glycosylated apolipoprotein B, was only weakly correlated with serum lipid concentrations. LDL-phenotype was not associated with metabolic control. Fifty-seven patients (20.2%) were categorized as having nephropathy, 146 patients (52.0%) as having diabetic neuropathy and 93 patients (33.1%) as having diabetic retinopathy. Metabolic control, most serum lipids and LDL-phenotype were not significantly related to the presence of any microvascular complication, diabetic nephropathy, retinopathy or neuropathy. In patients with macro-albuminuria or using ACE-inhibitors triglycerides were increased compared to micro- and normo-albuminuric patients (1.67 mmol/l (0.36) vs. 1.14 mmol/l (0.09) and 1.12 mmol/l (0.05), $p \leq 0.05$ after adjustment for age and gender). Lipoprotein [a] was significantly lower in patients with any microvascular complication (regression coefficient -51.6 mg/ml (95% confidence interval [-99.8; -3.4])). These associations were essentially the same after further adjustment for duration of diabetes, HbA1c and fasting conditions. In conclusion, metabolic control was not associated with the presence of micro-vasculair complications, whereas triglycerides were increased in patients with diabetic nephropathy. These results may support to the hypothesis that serum lipid disturbances might be an additional risk factor in microvascular complications in general, and diabetic nephropathy in particular.

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INTRODUCTION

Insulin-dependent diabetes mellitus (IDDM) afflicts an increasing number of subjects worldwide. In The Netherlands, it is estimated that approximately 45.000 subjects suffer from this disease, often acquired at an early age[1] and associated with an increased morbidity and mortality, mainly due to the presence of diabetic complications[2]. Although non-insulin-dependent diabetes is far more common, the risk of especially microvascular complications (retinopathy, nephropathy, neuropathy) in IDDM is such that its treatment merits much attention.

In IDDM, there is a definite relationship between the level of metabolic control (HbA1c levels) and the development and progression of diabetic microvascular complications[3]. The mechanisms by which the diabetic state per se or lack of glycemic control predispose to the development of diabetic complications is incompletely understood. From clinical practice it is known that even a normal (or a near to normal) HbA1c does not totally preclude the development of diabetic complications. This apparent discrepancy might be explained by HbA1c being not the best indicator of glycemic control for all patients. Other glycosylated proteins like apolipoprotein B or advanced glycosylation end products (AGE)) may be more appropriate measures. Also, other variables related to metabolic control, notably lipids may better reflect the risk for the development of diabetes complications in these patients. We studied the association between different measures of metabolic control, lipids and diabetic complications in a large population of IDDM patients.

PATIENTS AND METHODS

Patients

The present study was conducted at the outpatient clinic of the 'De Weezenlanden' hospital in Zwolle, a middle-sized town in the eastern part of the Netherlands. From January 1995 to January 1996, 293 consecutive IDDM patients were invited to participate in the study. IDDM was defined as starting insulin replacement therapy within six months after the first sign of diabetes mellitus and before the age of 30 years, or the absence of C-peptide secretion. Twelve patients refused to participate in the study, so the presented results are

based on the remaining 281 patients. The study protocol was approved by the hospital scientific and ethical committees and all patients gave their informed consent.

Study protocol

Patients were examined according to a standardized protocol by one trained physician. Smoking habits, alcohol use, as well as used medication, including and frequency of injections were recorded. Body mass index was calculated as weight (kg) divided by the square of height (m^2). Body fat distribution was assessed by the ratio of waist and hip circumferences. Blood pressure measurements were performed after five and seven minutes of rest in the supine position using a precalibrated standard mercury sphygmomanometer. Diastolic blood pressure was recorded at the disappearance of the Korotkoff sounds (phase V). The mean of the two measurements was used in the analysis. Hypertension was defined as systolic blood pressure of 140 mmHg or over and/or diastolic blood pressure of 90 mmHg or over[4]. Patients who were treated with antihypertensive medication were also considered hypertensive.

Blood sampling was performed after 30 minutes rest in supine position. In 122 subjects (79 males, 43 females) blood sampling was performed under fasting conditions, before the morning dose of insulin was administered. As most participating patients lived at some distance from the hospital, the blood samples of the other 159 patients were collected after their usual morning insulin dose and normal breakfast.

The clinical chemistry assays for total serum cholesterol, HDL-cholesterol and triglycerides were performed on a Hitachi 717 chemistry analyzer based on commercially available techniques (Boehringer Mannheim, Mannheim, Germany). Total serum cholesterol was determined by means of the CHOD-PAP method. HDL-cholesterol was measured after precipitation with sodium phosphotungstate-Mg^{2+}. Triglycerides were determined by means of the GPO-PAP method. LDL-cholesterol was calculated according the formula of Friedewald[5]. Apolipoprotein A-I, and apolipoprotein B and lipoprotein [a] were determined by commercially available immunochemistry techniques on the Beckman Array Analyzer (Beckman, Fullerton, California).

Metabolic control was assessed by measuring glycosylated haemoglobin A1c (HbA1c) and glycosylated apolipoprotein B. HbA1c was measured by affinity chromatography (Pierce columns, Glyco test II) (upper limit of normal 6.0%)[6]. Glycosylated apolipoprotein B, as percentage of total apolipoprotein B was determined, according to Panteghini[7].

The procedure for assessing LDL-phenotype was based on the method described by Mc Namara et al.[8], with the following modifications. Plasma from each subject was rapidly thawed, diluted ten times with a physiological salt solution and mixed with a solution containing 40% sucrose and 0.1% bromophenol blue. One microliter of this sample was loaded onto the applicator of the Pharmacia Phastsystem containing 4-15% nondenaturing polyacrylamide gradient gels and the appropriate buffer strips (Pharmacia) and subjected to electrophoresis for 18 hours at 225 V and 15 °C. After electrophoresis the gels were silver stained using the Biorad Silver Stain kit. The electrophoretic patterns were examined independently by two examiners to assign LDL-subclass phenotypes. There was never a disagreement between the two examiners assigning LDL-subclasses. LDL-subclass patterns were defined as phenotype A (major subclass >25.5 nm), phenotype B (major subclass <25.5 nm) or phenotype A/B (intermediate phenotype) according to Austin et al.[9,10].

In twenty-four hour urine samples, collected by the patients the day before their outpatient visit, albumin was measured, using an immunonephelometric technique on the Array immune analyzer (Beckman, Fullerton, California). Urinary albumin excretion rate (UAER) was calculated and divided into three categories: normo-albuminuria: UAER <30 mg/24h; micro-albuminuria: 30 mg< UAER <300 mg/24h; macro-albuminuria: UAER >300 mg/24h. Seven patients with normo-albuminuria and fourteen patients with micro-albuminuria used ACE-inhibitors. The hospital files showed that in all these 21 patients the indication for this treatment was albuminuria repeatedly over 100 mg/24h. Therefore, these patients were categorised as macro-albuminuric. Serum as well as urine creatinine concentrations were measured kinetically on the Hitachi 717, as described by Jaffé[11]. Creatinine clearance rate was calculated according to the Cockroft formula[12].

In order to assess the degree of diabetic retinopathy the examination included direct and indirect ophthalmoscopy through dilated pupils by an experienced ophthalmologist. Retinopathy was graded according to national standards in three categories: no retinopathy, background retinopathy and pre-proliferative or proliferative retinopathy[13].

Neuropathy was assessed by vibration threshold measurement using a Somedic vibrameter type IV and by sensibility-testing using monofilaments according to Semmes-Weinstein (monofilaments by Gillis W Long Hansons disease centre Carreville USA). The vibration threshold was determined according to a standardized procedure at six test sites: the dorsomedial aspect of the first metatarsal bone, the flat surface just above the medial malleolus and the dorsum of the metacarpal bone of the index finger for both left and right site of the body. Vibration measurement was defined as abnormal when one of the measurements was two standard deviations above the reference value for age and localization.

Sensibility-testing was performed at five regions on both legs and classified as abnormal when in one of the regions pressure of monofilament 5.07 was not felt[14]. Peripheral neuropathy was defined as at least one abnormal vibration measurement or sensibility test-result. Severity of peripheral neuropathy was graded as the number of abnormal tests.

Data analysis

The chi-square test was used to compare frequencies. Analysis of variance was performed to test for differences between groups. Partial correlation coefficients were calculated to describe the relations between serum lipids and age, BMI, WHR and parameters of metabolic control. Multiple linear regression analyses were used to analyse the other associations between continuous measures. Two-sided P-values ≤ 0.05 were considered to be statistically significant. All analyses were performed using SPSS 6.1.3 for Windows.

RESULTS

Clinical characteristics

The clinical characteristics of the study population are summarised in Table 1. Systolic blood pressure increased significantly with age and duration of diabetes (regression coefficients 0.64 mmHg per year (SEM 0.08) and 0.31 mmHg per year (0.10), respectively after adjustment for gender, and age and gender). Both systolic and diastolic blood pressure were associated with body mass index (regression coefficient 1.30 mmHg per kg/m^2 (0.31) and 0.51 mmHg per kg/m^2 (0.15)) as well as with waist hip ratio (regression coefficient 29.4 mmHg per cm/cm (14.4) and 17.6 mmHg per cm/cm (7.1), respectively all four $p \leq 0.05$ after adjustment for age and gender).

Current smokers had significantly higher WHR (0.96 (0.01) vs 0.94 (0.01)), but lower BMI (24.1 kg/m^2 (0.31) vs. 25.0 kg/m^2 (0.24)). Systolic and diastolic blood pressure were lower in smokers (135.0 mmHg (1.60) vs. 141.0 mmHg (1.42) and 81.0 mmHg (0.76) vs. 83.8 mmHg (0.63)). These differences were essentially the same after further adjustment for BMI or WHR. The number of cigarettes smoked daily was inversely associated with BMI (regression coefficient -0.64 kg/m^2 per cigarette (0.31) $p \leq 0.05$ after adjustment for age and gender). Smokers had a shorter duration of diabetes (14.9 years (0.9) vs. 18.3 years (0.8)). Alcohol use was not related with BMI, WHR or blood pressure.

Renal function, as assessed by creatinine clearance was better in men compared to women (Table 1) and significantly inversely associated with age and duration of diabetes (regression coefficients -1.30 ml/min per year (0.10); and -0.24 ml/min per year (0.14)). Albumin/ creatinine ratio was significantly associated with age, systolic blood pressure as well as creatinine clearance (regression coefficients 0.36 per year (0.12); 0.50 per mmHg (0.08) and -0.16 per ml/min (0.07) respectively. Renal function was not related to diastolic blood pressure, waist/ hip ratio, the consumption of alcohol, or smoking.

Metabolic control and lipids

Metabolic control, as assessed by HbA1c and glycosylated apolipoprotein B did not significantly differ between men and women, although there appeared to be a tendency in females for worse metabolic control. No difference could be found regarding the various schemes of insulin administration (2-3 or 4 times

Table 1. Clinical characteristics of the study population

	Men	Women	Total
Number of patients	153	128	281
Age (years)	39.5 (12.5)	36.6 (12.1)	38.2 (12.4)
Duration of diabetes (years)	17.0 (11.5)	17.4 (9.7)	17.2 (10.7)
Body mass index (kg/m2)	24.5 (3.1)	25.0 (3.3)	24.7 (3.2)
Waist hip ratio	0.98 (0.06)	0.90 (0.08)***	0.94 (0.08)
Systolic blood pressure (mmHg)	139.1 (18.4)	139.0 (18.6)	139.1 (18.4)
Diastolic blood pressure (mmHg)	83.2 (8.2)	82.6 (8.5)	82.9 (8.3)
Hypertension	64 (41.8%)	46 (35.9%)	110 (39.1%)
Current smokers	54 (35.3%)	37 (28.9%)	91 (32.4%)
Number of cigarettes/ day	11.4 (8.1)	15.2 (9.9)*	12.9 (9.0)
Alcohol users	111 (72.5%)	61 (47.7%)***	172 (61.2%)
Number of units/ week	10.0 (7.8)	6.8 (6.6)**	8.9 (7.5)
Frequency of insulin injections			
- 2 or 3 times a day	25 (16.3%)	9 (7.0%)***	34 (12.1%)
- 4 times a day	102 (66.7%)	73 (57.0%)	175 (62.3%)
- CSII/ CIPII	26 (17.0%)	46 (36.0%)***	72 (25.6%)
Creatinine clearance (ml/min)	115.1 (25.8)	97.8 (25.6)***	107.0 (27.1)
Albumin /creatinine ratio	6.08 (21.88)	5.92 (27.75)	6.01 (24.68)
HbA1c(%)	8.07 (1.99)	8.49 (1.76)	8.27 (1.90)
Glycated apolipoprotein B (%)	3.12 (0.91)	3.00 (0.92)	3.07 (0.91)
Total cholesterol (mmol/l)	4.92 (1.07)	5.05 (0.93)	4.98 (1.01)
HDL-cholesterol (mmol/l)	1.26 (0.30)	1.53 (0.39)***	1.39 (0.37)
LDL-cholesterol (mmol/l)	3.10 (0.89)	3.02 (0.83)	3.06 (0.86)
Triglycerides (mmol/l)	1.28 (1.19)	1.07 (0.66)	1.18 (0.99)
Apolipoprotein A (mg/l)	1.30 (0.21)	1.48 (0.25)***	1.38 (0.25)
Apolipoprotein B (mg/l)	0.90 (0.25)	0.88 (0.23)	0.89 (0.24)
Lipoprotein [a] (mg/l)	139.7 (197.9)	108.9 (153.9)	125.6 (179.4)
LDL-phenotype			
- type A	121 (79.1%)	93 (72.7%)	214 (76.2%)
- type A/B (intermediate)	25 (16.3%)	31 (24.2%)	56 (19.9%)
- type B	7 (4.6%)	4 (3.1%)	11 (3.9%)

CSII= continuous subcutaneous insulin infusion.
CIPII= continuous intra-peritoneal insulin infusion.
Values are number of patients or means with percentage or standard deviations between parentheses. Difference between men and women : * = $p \leq 0.05$, ** = $p \leq 0.001$, *** = $p \leq 0.001$

daily, and CSII). Blood pressure, BMI, WHR, duration of diabetes and renal function were also not related with of metabolic control. In contrast to HbA1c, glycosylated apolipoprotein B was significantly associated with age (regression coefficient 0.009 % per year (0.004)). Current smokers had significantly lower levels of glycosylated apolipoprotein B (2.88 % (0.08) vs. 3.16% (0.07)). Glycosylated apolipoprotein B was associated with HbA1c (regression coefficient 0.15 % per percent (0.03), $p \leq 0.05$ after adjustment for age and gender)

Fasting did not influence most lipid levels, except for HDL-cholesterol and apolipoprotein A, which were significantly lower under fasting conditions (1.30 mmol/l (0.03) vs. 1.45 mmol/l (0.03) and 1.33 mmol/l (0.02) vs. 1.43 mmol/l (0.02), respectively). Patients using alcohol had higher levels of HDL-cholesterol and lower levels of triglycerides (1.42 mmol/l (0.03) vs. 1.33 (0.03) and 1.09 mmol/l (0.05) vs. 1.33 mmol/l (0.13) both $p \leq 0.05$ after adjustment for age, gender and fasting condition). Total serum cholesterol and apolipoprotein B were significantly associated with the number of alcohol consumptions (regression coefficient 0.03 mmol/l per consumption per week (0.01) and 0.005 mmol/l per consumption per week (0.002). Serum lipid concentrations did not differ between current smokers and non smoking patients. Urinary albumin excretion, as assessed by albumin/ creatinine ratio, was associated with total serum cholesterol, apolipoprotein B and triglyceride concentrations (regression coefficients 0.005 mmol/l (0.002); 0.001 mg/ml (0) and 0.007 mmol/l (0.002), respectively $p \leq 0.05$ after adjustment for age, gender and fasting conditions). Most lipid parameters were associated with age, BMI, WHR and the parameters of metabolic control (Table 2). No significant association was found between lipid concentrations and creatinine clearance or duration of diabetes. These results were essentially the same when the analyses were restricted to patients of whom fasting blood was obtained.

Sixty-seven patients (23.8%) were classified as having an abnormal LDL-phenotype (type B or intermediate type (A/B)). LDL-phenotype was not associated with age, gender, fasting condition, BMI, WHR, duration of diabetes, smoking, alcohol consumption, renal function, metabolic control, or the use of lipid lowering drugs. Total serum cholesterol and apolipoprotein B concentrations were higher in patients with an abnormal LDL-phenotype (5.1 mmol/l (0.1) vs. 4.6 mmol/l (0.1) and 0.93 mg/ml (0.02) vs. 0.77 mg/.ml (0.03)).

Table 2. Relationship between lipid concentrations, clinical characteristics and metabolic control

	Age	BMI	WHR	HbA1c	GapoB	Cholt	HDL	LDL	Tg	ApoA	ApoB
Total cholesterol (Cholt)	0.32*	0.21*	0.19*	0.17*	0.28*						
HDL-cholesterol (HDL)	0.21*	-0.18*	-0.12*	-0.05	0.07	0.13*					
LDL-cholesterol (LDL)	0.27*	0.17*	0.18*	0.16*	0.33*	0.91*	-0.11				
Triglycerides (Tg)	0.04	0.31*	0.20*	0.15*	-0.10	0.37*	-0.31*	0.25*			
Apolipoprotein A (ApoA)	0.15*	-0.04	-0.08	0.06	0.07	0.33*	0.75*	0.09	-0.02		
Apolipoprotein B (ApoB)	0.18*	0.19*	0.23*	0.19*	0.24*	0.82*	-0.21*	0.87*	0.28*	0.03	
Lipoprotein [a]	-0.03	-0.06	-0.07	0.03	0.04	0.09	0.01	0.14*	-0.08	-0.04	0.12*

BMI= body mass index,; WHR= waist hip ratio; GapoB= glycosylated apolipoprotein B.

Values are partial correlation coefficients adjusted for age, gender and fasting condition. * = $p \leq 0.05$

Diabetic complications

The prevalence of the diabetic complications is shown in Table 3. Table 4 summarizes the association between the presence of any microvascular complication, the parameters of metabolic control and lipid concentrations. Mean level of HbA1c or glycated apolipoprotein B did not differ between patients with or without any microvascular complication, nephropathy, retinopathy, neuropathy only. In patients with abnormal vibration threshold HbA1c was significantly increased compared to those with no abnormal test (8.64% (0.18) vs. 8.00% (0.14)). Metabolic control did not differ between patients with or without abnormal sensibility testing .

Most serum lipid concentrations were not related with the presence of any microvascular complication, retinopathy, nephropathy or neuropathy only. In patients with macro-albuminuria or using ACE-inhibitors triglycerides were increased compared to micro- and normo-albuminuric patients (1.67 mmol/l (0.36) vs. 1.14 mmol/l (0.09) and 1.12 mmol/l (0.05), $p \leq 0.05$ after adjustment

Table 3. Prevalence of diabetic complications

	Men	Women	Total
any microvascular complications			
- no complications	46 (30.1%)	47 (36.7%)	93 (33.1%)
- 1 complication	55 (35.9%)	52 (40.6%)	107 (38.1%)
- 2 complications	33 (21.6%)	22 (17.2%)	55 (19.6%)
- 3 complications	19 (12.4%)	7 (5.5%)	26 (9.2%)
retinopathy			
- no retinopathy	103 (67.3%)	85 (66.4%)	188 (66.9%)
- back ground	32 (20.9%)	34 (26.6%)	66 (23.5%)
- pre-/ proliferative	18 (11.8%)	9 (7.0%)	27 (9.6%)
nephropathy			
- normo-albuminuria	116 (75.8%)	108 (84.4%)	224 (79.7%)
- micro-albuminuria	16 (10.5%)	9 (7.0%)	25 (8.9%)
- macro-albuminuria +ACE- inhibitor	21 (13.7%)	11 (8.6%)	32 (11.4%)
peripheral neuropathy			
- 0 abnormal test	62 (40.5%)	73 (57.0%)	135 (48.1%)
- 1 abnormal test	48 (31.4%)	35 (27.4%)	83 (29.5%)
- 2 abnormal tests	43 (28.1%)	20 (15.6%)	63 (22.4%)

Values are number of patients with percentage between parentheses.

for age, gender and fasting conditions). Lp[a] was significantly lower in patients with any microvascular complication (Table 4). These associations were essentially the same after further adjustment for duration of diabetes, HbA1c, LDL-phenotype and fasting conditions. LDL-phenotype was not associated with the presence of retinopathy, neuropathy, nephropathy or any microvascular complication.

Table 4. Association between degree of metabolic control, lipid concentrations and absence or presence of complications

	Model 1		Model 2	
Any microvascular complications				
- HbA1c	0.33	[-0.14; 0.79]		- - - - - -
- Glycosylated apolipoprotein B	-0.03	[-0.28; 0.21]		- - - - - -
- Total serum cholesterol	-0.11	[-0.36; 0.13]	-0.05	[-0.31; 0.21]
- HDL-cholesterol	-0.03	[-0.12; 0.06]	-0.02	[-0.11; 0.08]
- LDL-cholesterol	-0.12	[-0.34; 0.10]	-0.10	[-0.33; 0.13]
- Triglycerides	0.08	[-0.09; 0.26]	0.14	[-0.04: 0.32]
- Apolipoprotein A	-0.02	[-0.08: 0.04]	0.01	[-0.06; 0.07]
- Apolipoprotein B	-0.02	[-0.09; 0.04]	-0.02	[-0.08; 0.05]
- Lipoprotein [a]	-51.6	[-99.8; -3.4]	-61.5	[-111.7;-11.2]

Values are regression coefficients with 95% confidence intervals between parentheses.
Model 1 parameters of metabolic control and lipid concentrations adjusted for age and gender .
Model 2 parameters of metabolic control and lipid concentrations adjusted for age gender, duration of disease and metabolic control.

DISCUSSION

The results of this study in 281 IDDM patients show that the presence of any microvascular complication was not associated to metabolic control, LDL-phenotype nor to most of the serum lipids. Triglyceride concentrations were increased in patients with nephropathy.

The laboratory assays used in our study are according to international standards and applied to an unselected group of diabetic patients and control subjects. Although most blood samples were obtained in the non-fasting state, adjusting

for the fasting condition or limiting the analysis to subjects with a fasting blood sample revealed the same associations.
The prevalence of microvascular complications in this Dutch IDDM population is in general comparable to other population studies[15,16], as are most of the risk factors for these complications. Our patients were on average older and had a longer duration of diabetes compared to the patients, that participated in the studies previously mentioned. Therefore differences in methodological procedures are the most obvious explanation for the slightly lower prevalances of nephropathy and retinopathy in these studie compared to our results.

Most of our results regarding serum lipid concentrations are in agreement with results of other studies as summarised by Brunzell and Lopes [17,18]. Although the lipid profile of the patients participating in our study was measured on a yearly basis, sixteen (5.6%) patients had increased serum lipids concentrations (no lipid lowering medication and total cholesterol concentration above 6.5 mmol/l, or triglycerides above 2.2 mmol/l) under fasting conditions. In twenty three (7%) patients the serum lipids were increased under nonfasting conditions and had to be measured again. In addition, ten patients (3.5%) were classified as having LDL-phenotype B, although their lipid concentrations were with the normal ranges. Given its role in the development of macrovascular complications regular assessment of lipid profile is important in the care of IDDM patients.

The absence of an association between metabolic control and diabetes complications in our transversal study, is in agreement with baseline data of the DCCT[3], which reported glycosylated haemoglobin levels of 8.8% for the primairy intervention group (no complications) and 9.0% for the secondairy intervention group. A possible explanation for the absence of this association could be that a single measurement of HbA1c is an imprecise estimate of an individual usual glycaemic control, given the reported variability of glycated haemoglobin concentrations [19]. An other explanation could be the small range of HbA1c in this study. Strict metabolic control (HbA1c within normal range) was achieved in 33 patients (12%). Good metabolic control, defined as an HbA1c lower than 7.2% (20% above the upper limit of normal) was reached in 84 (29%) of the patients. These results are comparable with a recently described group of IDDM patients in The Netherlands[20].
A third reason, and in our opinion the most obvious explanation for the absence of this association, could be that patients who are aware of their diabetic complications are more likely to improve their control as a consequence of this

knowledge. The finding that in patients with an abnormal vibration threshold HbA1c levels were increased support of this theory.

The main interest for lipid profile disturbances in patients with IDDM is based on the associated risk of atherosclerosis. As shown by the results from our study some serum lipids are associated, independently from metabolic control, with microvascular complications. These results are in agreement with the findings reported by previous studies[16,21,22]. It should be emphasized however, that the presented data cannot provide evidence regarding a causal relationship between the serum lipids and diabetic complications since it was a cross-sectional study. Therefore, prospective studies, investigating the role of lipids and lipids lowering in the development of microvascular complications would be of value.

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3

Serum anti-oxidants in insulin-dependent diabetes mellitus and diabetic complications

SUMMARY

Oxidative stress is postulated to be increased in patients with diabetes mellitus. Serum anti-oxidants play a role in protecting vascular endothelium against oxidative injury, and provide a possible defence against diabetic angiopathy. We studied serum anti-oxidant concentrations in 281 patients with insulin-dependent diabetes mellitus and 98 control subjects, as well as the association between anti-oxidants and microvascular complications. Retinol, transferrin and uric acid concentrations were significantly decreased in diabetes patients compared to controls (1.83 µmol/l (0.04) vs 2.31 µmol/l (0.06); 2.24 g/l (0.02) vs. 2.50 g/l (0.04); 0.25 mmol/l (0) vs. 0.31 mmol/l (0.01), $p \leq 0.05$ respectively). No differences were found for alpha- and gamma-tocopherol, beta-carotene, ferritin and ceruloplasmin. In diabetes patients uric acid, gamma-tocopherol and transferrin were significantly associated with glycosylated apolipoprotein B, a parameter of short term metabolic control (regression coefficients -0.01 mmol/l per percent (0.004), 0.25 mmol/l per percent (0.12) and -0.06 g/l per percent (0.02)). HbA1c was only significantly related with alpha-tocopherol and gamma-tocopherol (regression coefficients 0.85 µmol/l per percent (0.29) and 0.15 µmol/l per percent (0.04), respectively). Most anti-oxidants were significantly associated with lipid concentrations. Patients with nephropathy had increased levels of retinol, ferritin and uric acid compared to other diabetic patients (1.75 µmol/l (0.04) vs 2.16 µmol/l (0.14); 83.4 µg/l (4.4) vs. 128.0 µg/l (19.1); 0.24 mmol/l (0.04) vs. 0.28 mmol/l (0.01)). No significant associations were found for the other anti-oxidants. Retinopathy and neuropathy were not associated with any of the anti-oxidants measured. In conclusion, some relationships between serum anti-oxidants, metabolic control and serum lipids were found. However, the contrasting findings of decreased anti-oxidants levels in diabetes patients compared to control subjects and increased levels in diabetes patients with microvascular complications, suggest that the investigated anti-oxidants are not involved in the etiology of microvascular complications.

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INTRODUCTION

It has been postulated that diabetes mellitus is characterised by increased oxidative stress[1,2]. Results from studies in animals and humans have suggested that oxidative cell injury caused by free radicals contributes to the development of both macroangiopathy[1,3,4] and microangiopathy[1,5,8]. Changes in the intracellular level and activity of anti-oxidant enzymes, like superoxide dismutase or glutathione peroxidase, are known to occur in diabetes mellitus.[9-12]. Extracellular fluids contain several anti-oxidants that delay or inhibit the oxidative process by their presence at a lower concentration than the oxidizable substrate[13]. For this reason serum anti-oxidants can play a role in protecting vascular endothelium against oxidative injury, and provide a possible defence against diabetic angiopathy.

Previous studies[14-22] on serum anti-oxidants in diabetic patients were limited to a single category of individual anti-oxidants. Other researchers reported only on subgroups of diabetes patients, such as children[23], or made no distinction between IDDM and NIDDM patients. We conducted a study to compare serum concentrations of various anti-oxidants between patients with insulin-dependent diabetes mellitus and subjects without diabetes mellitus. In addition, the relationship between these serum anti-oxidants and the presence of microvascular complications was assessed, within the group of diabetic patients.

PATIENTS AND METHODS

Study population

The present study was conducted at the outpatient clinic of the 'De Weezenlanden' hospital in Zwolle, a middle-sized town in the eastern part of the Netherlands. From January 1995 to January 1996, 293 consecutive IDDM patients were invited to participate in the study. IDDM was defined as the start of insulin therapy within six months after the first sign of diabetes mellitus and before the age of 30 years, or the absence of C-peptide secretion. In the same period 106 consecutive healthy men and women (control group), who were admitted for minor surgery (e.g. cosmetic surgery, sterilisation or arthroscopy), were invited to participate in the study. Health in the latter group was defined

as no current diseases except the indication for surgery (ASA classification category 1) and not using medication, except oral contraceptives. Twelve diabetes patients and 8 control subjects refused to participate in the study, so the presented results are based on the remaining 281 diabetic patients and 98 control subjects. The study protocol was approved by the hospital medical ethical committee and all patients gave their informed consent.

Measurements

Patients were examined according to a standardised protocol by one trained physician. Smoking habits, alcohol use, as well as used medication, including insulin dose and frequency of injections, were recorded. Body mass index was calculated as weight (kg) divided by square of height (m^2). Body fat distribution was assessed by the ratio of waist and hip circumferences. Blood pressure measurements were performed after five and seven minutes of rest in the supine position using a precalibrated standard mercury sphygmomanometer. Diastolic blood pressure was recorded at the disappearance of the Korotkoff sounds (phase V). The mean of the two measurements was used in the analysis. Hypertension was defined as systolic blood pressure of 140 mmHg or over and/or diastolic blood pressure of 90 mmHg or over[24]. Patients who were treated with antihypertensive medication were also considered hypertensive.

Blood sampling was performed after 30 minutes rest in supine position. In 122 diabetes patients (79 males, 43 females) blood sampling was performed under fasting conditions, before the morning dose of insulin was administered. As most participating patients lived at some distance from the hospital, the blood samples of the other 159 diabetes patients were collected after their usual morning insulin dose and normal breakfast. Blood sampling in the control subjects was never under fasting conditions.

Glycosylated haemoglobin A1c (HbA1c) was measured by affinity chromatography (Pierce columns, Glyco test II) (upper limit of normal 6.0%)[25]. The routine clinical chemistry assays for total serum cholesterol, HDL-cholesterol and triglycerides were performed on a Hitachi 717 chemistry analyzer based on commercially available techniques (Boehringer Mannheim, Mannheim, Germany). Total serum cholesterol was determined by means of the CHOD-PAP method. HDL-cholesterol was measured after precipitation with

sodium phosphotungstate-Mg^{2+}. Triglycerides were determined using the GPO-PAP method. LDL-cholesterol was calculated according the formula of Friedewald[26]. Glycosylated apolipoprotein B, as percentage of total apolipoprotein B was determined according to Panteghini[27].

Plasma vitamin A (retinol), beta-carotene and vitamin E (alpha-tocopherol and gamma-tocopherol) were determined simultaneously by means of HPLC in one analytical run, essentially according to Zaman et al.[28], with the following modifications: instead of a programmable UV-VIS detector, a 996 Photo Diode Array Detector (Waters Associates, Milford, USA) was used, and in the extraction procedure methanol and hexane instead of ethanol and hexane. To prevent oxidation of the tocopherols during the extraction procedure, butylated hydroxytoluene (BHT), pyrogallol, vitamin C and EDTA were added. Transferrin, ceruloplasmin and uric acid were determined according to routine procedure immunonephelometrically on an Array immune analyzer (Beckman, Fullerton, California). Ferritin was routinely analysed with the ferritin Enzymun test on the ES 607 analyzer (Boehringer Mannheim, Mannheim, Germany)

In twenty-four hour urine samples, collected by the diabetes patients the day before their outpatient visit, albumin was measured, using an immunonephelometric technique on the Array immune analyzer (Beckman, Fullerton, California). Urinary albumin excretion rate (UAER) was calculated and divided into three categories: normo-albuminuria: UAER <30 mg/24h; micro-albuminuria: 30 mg/24h < UAER <300 mg/24h and macro-albuminuria: UAER >300 mg/24h. Seven patients with normo-albuminuria and fourteen patients with micro-albuminuria used ACE-inhibitors. The hospital files showed that in all these 21 patients the indication for this treatment was albuminuria repeatedly over 100 mg/24h. Therefore, these patients were categorised as macro-albuminuric. Serum as well as urine creatinine concentrations were measured kinetically on the Hitachi 717, as described by Jaffe[29]. Creatinine clearance rate was calculated according to the Cockroft formula[30].

In order to assess the degree of diabetic retinopathy the examination included direct and indirect ophthalmoscopy through dilated pupils by an experienced ophthalmologist. Retinopathy was graded according to national standards in three categories: no retinopathy, background retinopathy and pre-proliferative or proliferative retinopathy[31].

Neuropathy was assessed by vibration threshold measurement using a Somedic vibrameter type IV and by sensibility-testing using monofilaments according to Semmes-Weinstein (monofilaments by Gillis W Long, Hansons Disease Centre, Carreville, USA). The vibration threshold was determined according to a standardised procedure at six test sites: the dorsomedial aspect of the first metatarsal bone, the flat surface just above the medial malleolus and the dorsum of the metacarpal bone of the index finger for both the left and right side of the body. Vibration measurement was defined as abnormal when one of the measurements was two standard deviations above the reference value for age and localization. Sensibility-testing was performed at five regions on both legs and classified as abnormal when in one of the regions pressure of monofilament 5.07 was not felt[33]. Peripheral neuropathy was defined as at least one abnormal vibration measurement or sensibility test result. Severity of peripheral neuropathy was graded as the number of abnormal tests.

Data analysis

The chi-square test was used to compare frequencies. Analysis of variance (ANOVA) was performed to test for differences between groups. In addition, multiple regression analyses were performed to adjust for possible confounders. Partial correlation coefficients were calculated to describe the relations between anti-oxidants and age, body mass and serum lipids adjusted for potential confounding factors, notably age and gender. For serum triglyceride and LDL-cholesterol concentrations additional calculations were performed limiting the analyses to subjects of whom fasting blood was obtained. After adjustment for age and gender two-sided P-values ≤ 0.05 were considered to be statistically significant. All analysis were performed using SPSS 6.1.3 for Windows.

RESULTS

IDDM patients versus control subjects

The clinical characteristics of diabetes patients and controls are summarised in Table 1. Both systolic and diastolic blood pressure were higher in diabetic patients. Although there was a tendency in the control subjects for a worse lipid spectrum compared to diabetes subjects, only the difference for serum HDL-

Table 1. Clinical characteristics of the study population

	IDDM patients		Control group	
Number of patients	281		98	
Gender (Men)	153	(54.4%)	49	(50.0%)
Age (years)	38.2	(0.7)	38.2	(1.0)
Body mass index (kg/ m^2)	24.7	(0.2)	25.5	(0.4)
Duration of diabetes (years)	17.2	(0.6)	n.a.	
Systolic blood pressure (mmHg)	139.1	(1.1)	125.6	(1.0)***
Diastolic blood pressure (mmHg)	82.9	(0.5)	78.1	(0.7)***
Current smokers	91	(32.4%)	40	(40.8%)
Number of cigarettes/ day	12.9	(1.0)	13.7	(1.5)
Never alcohol users	109	(38.8%)	33	(33.7%)
HbA1c (%)	8.27	(0.11)	4.62	(0.06)***
Total cholesterol (mmol/l)	4.98	(0.06)	5.18	(0.10)
HDL-cholesterol (mmol/l)	1.39	(0.02)	1.29	(0.03)*
LDL-cholesterol (mmol/l)	3.06	(0.05)	3.22	(0.09)
Triglycerides (mmol/l)	1.18	(0.06)	1.37	(0.07)

Values are number of patients or means with standard errors between parentheses. N.a.= not applicable. Difference between diabetes patients and control subjects adjusted for age and gender: *= $p \leq 0.05$, ***= $p \leq 0.001$

cholesterol was found to be statistically significant. This difference could not be attributed to the fact, that the blood sampling of control patients was not performed under fasting conditions or to a difference for one of the other clinical characteristics. The anti-oxidant concentrations in the diabetic patients and the control subjects are shown in Table 2. Uric acid, retinol and transferrin were significantly lower in diabetes patients. After further adjustment for fasting condition, and creatinine clearance, body mass index, waist hip ratio and serum lipids or restricting the analyses to patients without hypertension, these differences remained essentially the same.

Table 2. Antioxidant-concentrations in IDDM patients and healthy controls

	IDDM patients	Control group
Uric Acid (mmol/l)	0.25 (0)	0.31 (0.01)***
Alpha-tocopherol (µmol/l)	31.34 (0.56)	32.22 (0.87)
Gamma-tocopherol (µmol/l)	2.99 (0.11)	2.85 (0.14)
Beta-carotene (nmol/l)	324.8 (16.0)	271.6 (18.1)
Retinol (µmol/l)	1.83 (0.04)	2.31 (0.06)***
Ceruloplasmin (g/l)	0.38 (0.01)	0.37 (0.01)
Ferritin (µg/l)	92.7 (45.3)	106.9 (11.0)
Transferrin (g/l)	2.24 (0.02)	2.50 (0.04)***

Values are means with standard errors between parentheses.
Difference between IDDM patients and control group adjusted for age and gender: ***= $p \leq 0.001$

Anti-oxidants in IDDM patients.

Beta-carotene, ceruloplasmin and transferrin concentrations were significantly higher in women than in men with diabetes (379.4 nmol/l (SE 30.1) vs. 278.8 nmol/l (14.0); 0.46 mmol/l (0.01) vs. 0.31 mmol/l (0.01); and 2.40 g/l (0.46) vs. 2.11 g/l (0.02), respectively). Uric acid (0.22 mmol/l (0.01) vs. 0.27 mmol/l (0.01)) and ferritin levels (52.4 µg/l (4.4) vs. 126.5 µg/l (8.2)) were lower. Most anti-oxidants were strongly associated with age and body mass index (Table 3). No relationship was present between any of the anti-oxidant levels and blood pressure, daily insulin doses and the number of cigarettes smoked. Hypertension, smoking and fasting condition were not associated with anti-oxidant levels. Diabetes patients drinking alcohol, had increased levels of uric acid and ceruloplasmin (0.26 mmol/l (0.05) vs. 0.23 mmol/l (0.06); 0.36 g/l (0.01) vs. 0.42 g/l (0.02), respectively $p \leq 0.05$ adjusted for age and gender). Only retinol was associated with the number of alcohol consumptions (regression coefficient 0.01 µmol/l per alcohol consumption per week (0.05); $p \leq 0.05$ after adjustment for age and gender). Duration of diabetes showed a significant inverse relation with transferrin (regression coefficient) -0.006 g/l per year (0.002)).

Metabolic control, as estimated by HbA1c, was not related to the levels of most anti-oxidants. However, alpha-tocopherol and gamma-tocopherol showed a positive association: coefficients of linear regression 0.85 µmol/l per percent (0.29) and 0.15 µmol/l per percent (0.04), respectively $p \leq 0.05$ after adjustment for age and gender). Glycosylated apolipoprotein B was significantly associated with gamma-tocopherol, uric acid and transferrin (regression coefficients 0.25 µmol/l per percent (0.12); -0.01 mmol/l per percent (0.004)and -0.06 g/l per percent (0.02), respectively. Most anti-oxidants were significantly associated with lipid concentrations (Table 3). These relations remained essentially the same after further adjustment for body mass index, waist hip ratio or fasting condition or limiting the analyses to subjects of whom fasting blood was obstained. None of the anti-oxidants was related to lipoprotein [a] concentrations.

Diabetic complications.

Ninety-three patients (33.1%) had diabetic retinopathy (27 (29.3%) with pre-proliferative or proliferative retinopathy), 57 patients were categorised as having nephropathy (32 (56.1%) using an ACE-inhibitor or having macro-albuminuria) and 146 patients as having diabetic neuropathy (63 (43.2%) of these patients had

Table 3. Relationship between age, body mass index, lipid and antioxidant-concentrations in patients with insulin-dependent diabetes mellitus

	Age	Body-mass index	Cholesterol	HDL-cholesterol	LDL-cholesterol	Triglycerides
Uric Acid	0.08	0.23*	0.15*	-0.23*	0.15*	0.31*
Alpha-tocopherol	0.28*	0.14*	0.48*	0.002*	0.43*	0.55*
Gamma-tocopherol	-0.06	0.27*	0.23*	-0.12*	0.23*	0.31*
Beta-carotene	0.23*	-0.10	0.11	0.19*	0.06	-0.04
Retinol	0.06	0.10	0.32*	-0.05	0.22*	0.31*
Ceruloplasmin	-0.16*	0.18*	0.17*	0.07	0.05	0.24*
Ferritin	0.19*	0.23*	0.09	-0.11*	0.08	0.42*
Transferrin	-0.28*	0.09	0.18*	0.08	0.11	0.05

Values are partial correlation coefficients after adjustment for age, gender and fasting condition (age only for gender): * = $p \leq 0.05$

Table 4. Antioxidant concentrations in IDDM patients by the number of microvascular complications

		Diabetic complications			
	No complications	1 complication	2 complications	3 complications	Any complications
Uric Acid (mmol/l)	0.25 (0.01)#	0.23 (0.01)*	0.26 (0.01)‡	0.30 (0.01)#*‡	0.25 (0.01)
Alpha-tocopherol (µmol/l)	31.06 (0.81)	31.19 (0.81)	32.33 (1.74)	31.92 (1.83)	31.63 (0.73)
Gamma-tocopherol (µmol/l)	2.86 (0.18)	3.09 (0.17)	3.23 (0.29)	2.52 (0.27)	3.06 (0.14)
Beta-carotene (nmol/l)	306.8 (19.4)	306.8 (40.8)	332.3 (45.8)	371.3 (62.5)	354.0 (28.0)
Retinol (µmol/l)	1.78 (0.05)#	1.77 (0.05)*	1.75 (0.09)‡	2.45 (0.27)#*‡	1.86 (0.06)
Ceruloplasmin (g/l)	0.38 (0.02)	0.39 (0.01)	0.38 (0.02)	0.37 (0.02)	0.38 (0.01)
Ferritin (µg/l)	81.9 (7.7)#	88.9 (6.23)*	110.8 (17.7)	134.1 (22.4)#*	98.6 (7.0)
Transferrin (g/l)	2.31 (0.04)	2.22 (0.04)	2.21 (0.06)	2.11 (0.06)	2.20 (0.03)

Values are means with standard errors of the mean between parentheses.
[#] difference between patients without diabetic complications and patients with three complications adjusted for age and gender: p≤ 0.05
[*] difference between patients with one and three diabetic complication adjusted for age and gender: p≤ 0.05
[‡] difference between patients with two and three diabetic complication adjusted for age and gender: p≤ 0.05

two abnormal tests). More than twenty-five percent of the patients had two or more microvascular complications. Patients with all three microvascular complications had significant higher concentration of ferritin, retinol and uric acid compared to those with two or less complications (Table 4). Retinopathy and neuropathy were not associated with any differences in anti-oxidant concentrations. Patients with only diabetic nephropathy had increased concentrations of retinol, ferritin and uric acid (1.75 µmol/l (0.04) vs 2.16 µmol/l (0.14); 83.4 µg/l (4.4) vs. 128.0 µg/l (19.1); 0.24 mmol/l (0.04) vs., 0.28 mmol/l (0.01), $p \leq 0.05$ adjusted for age and gender). For these three anti-oxidants a trend was found: showing higher concentrations with increasing UAER. These differences did not change markedly after further adjustment for metabolic control and duration of disease. The results were the same when instead of a classification corrected for ACE-inhibition, patients were classified only by UAER. Retinol was also associated with creatinine clearance (regression coefficient -0.02 µmol/l per ml/min (0.002) $p \leq 0.05$ after adjustment for age and gender). The albumin/ creatinine ratio was significantly related with retinol, ferritin and uric acid (regression coefficients 0.01 µmol/l (0.002); 0.69 µg/l (0.19) 0.001 mmol/l (0.001), respectively. After further adjustment for body mass index, waist hip ratio or creatinine clearance these relations were essentially the same.

DISCUSSION

The results of this study show that uric acid, retinol and transferrin concentrations are decreased in patients with insulin-dependent diabetes mellitus. Short term metabolic control, as measured by glycosylated apolipoprotein B[33], was associated with uric acid, gamma-tocopherol and transferrin levels. HbA1c was only significantly related with alpha-tocopherol and gamma-tocopherol. Microvascular complications, notably nephropathy, were associated with an increase in uric acid, retinol and ferritin levels. Most anti-oxidants were associated with lipid concentrations.

Blood samples were only obtained under fasting conditions in a subgroup of the diabetes population and in none of the control subjects. However, adjustment for fasting condition, or limiting the analyses to subjects with a fasting blood

sample revealed the same associations. In the present study laboratory assays used were according to international standards and performed on blood samples of an unselected group of diabetes patients and healthy subjects, without knowledge of their clinical characteristics. Therefore, in our opinion selection bias has not influenced the results of this study.

The lower uric acid levels found in diabetes patients compared to controls, as its relation with gender, age, metabolic control and lipids are in agreement with other studies[34-37]. Serum uric acid levels have been shown to be largely determined by genetic factors, purine production and renal function[15]. Results of, previous studies on metalion chelating and oxidizing anti-oxidants (ceruloplasmin ferritin and transferrin) have shown increased[17, 23], unchanged[38-40] and decreased[41] levels in IDDM patients. The conflicting results of these studies might be explained by the small sample sizes (n<30), and the differences in analytical procedures. The relationships found for alpha- and gamma-tocopherol, retinol and beta-carotene are in agreement with other studies[22,42-44]. Results that contrast with those found in our study, are mostly from studies, using older analytical procedures (e.g. measuring only total tocopherol levels)[45-47].

With respect to microvascular complications and the serum anti-oxidants examined in our study, we only found two reports of similar work. One study reported uric acid as a risk factor for proliferative retinopathy[48], in the other study increased alpha-tocopherol levels were associated with increased urinary albumin excretion rate but not with diabetic retinopathy[14] In the latter study retinol levels were also found to be increased in patients with macro-albuminuria, as in our study.

In insulin-dependent diabetes mellitus there is a clear relationship between metabolic control and the development and progression of microvascular complications[49]. Although the pathophysiology of these complications is probably multifactorial, results of in vitro[50], animal[51], and human studies [5] suggest that oxidative stress through an increased formation of free radicals might be involved in the development of complications. Increased generation of reactive oxygen metabolites such as superoxide anion and hydrogen peroxide has been shown to occur in diabetes mellitus in association with hyperglycaemia[1]. Increased glucose concentration can induce formation of free radicals through non-enzymatic glycation of protein substrates[52], auto-oxidative glycation[53], activation of protein kinase C[54] and increased polyol pathway[55]. These reactive

oxygen species, if not trapped by anti-oxidants, are suggested to disturb endothelial dependent vasorelaxation, stimulate growth factors, induce the expression of adhesion molecules, activate the blood coagulation, and contribute to the formation of advanced glycosylated end-products. All these mechanisms have been implicated in the development of microvascular complications[5,6]. Furthermore by modifying molecules, free radicals may form cytotoxic substances, which can directly induce endothelial damage. In addition diabetes is known to induce chances in the content and activity of cellular anti-oxidant enzymes[9-12].

As they are active at different places, depending on the chemical properties of the anti-oxidants, changes in concentrations of anti-oxidants should be seen as local alterations of anti-oxidant defences. Absolute levels of individual anti-oxidants might not be the only factor that determine anti-oxidant defences. Activity of these anti-oxidants could be enhanced or depressed. Furthermore, most anti-oxidants are regulated by more control mechanisms, in which the formation of radical active species is in general not the most important. For example, as stated earlier uric acid levels are mainly determined by purine production, genetic and renal factors[13]. In studies by Tsai[16] and Asayama[15] chain breaking as well as preventive anti-oxidant functions in general were shown to be decreased in subjects with poorly controlled IDDM, despite normal or even increased levels of individual anti-oxidants. This supports the view of Jones[23] that no conclusions can be drawn from levels of individual serum anti-oxidants as indicator of oxidative stress.

Our study shows a decrease of some anti-oxidative substances in IDDM patients. Despite this, the data lend no support to the view, that low levels of anti-oxidative substances are associated with the presence of diabetic microvascular complications. In diabetic nephropathy, levels were even increased and similar to those in non-diabetic subjects. If the anti-oxidants measured in our study would play an important pathogenetic role, one would expect the opposite. It is difficult to accept, that low levels of anti-oxidants initially lead to microvascular damage, and subsequently by some unknown mechanism, anti-oxidants would rise to counteract further damage. In spite of the limitations inherent to a cross-sectional study our results suggest that the anti-oxidants investigated are not relevant in the occurence of microvascular complications.

The hypothesis that oxidative stress, induced by hyperglycaemia, causes endothelial damage and is the final common pathway in the development of both macroangiopathy and microangiopathy, is not supported by our cross-sectional study. Prospective studies, to the role of both anti-oxidants and pro-oxidants (notably free radicals) in endothelial damage, may confirm these findings.

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Lipid peroxidation in insulin-dependent diabetes mellitus: comparison with healthy controls

SUMMARY

Oxidative stress is postulated to be increased in patients with diabetes mellitus. Because in vivo measurement of free radicals is difficult to establish, various methods have been developed to investigate indirectly whether such oxidative processes occur in vivo. Two of these methods are the measurement of plasma malondialdehyde concentration, as a reflection of lipid peroxidation and the susceptibility of LDL to in vitro oxidation.

We studied these two parameters in 281 patients with insulin-dependent diabetes mellitus and 98 subjects without diabetes mellitus.

Age, gender, plasma levels of total serum cholesterol, LDL-cholesterol, triglyceride and LDL-phenotype did not significantly differ between the two groups. HDL-cholesterol concentrations were significantly higher in diabetic patients compared to controls (1.39 mmol/l (0.02) vs. 1.29 mmol/l (0.03) mean (SEM). With regard to composition of LDL-cholesterol, the amount of LDL triglycerides was significantly increased in diabetic patients, while the amount of cholesterol esters was decreased (7.8 % (0.1) vs. 6.9 % (0.2) and 28.5 % (0.2) vs. 29.5 (0.4) both $p \leq 0.05$). In line with this, the size of LDL-subclass 1 particles was found to be significantly increased in IDDM patients compared to the control subjects (26.59 nm (0.03) vs. 26.42 nm (0.05)). There was no difference in the in vitro susceptibility of LDL to oxidation, maximum rate of oxidation and MDA concentrations between IDDM patients and control subjects.

Our results do not support the presence of increased oxidative stress in patients with insulin-dependent diabetes mellitus, as assessed by the susceptibility of LDL to in vitro oxidation or plasma malondialdehyde concentrations.

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INTRODUCTION

Oxidative stress is postulated to be increased in patients with diabetes mellitus [1,2]. Results from several studies, both in animals and in humans, have suggested that oxidative cell injury (in particular to endothelial cells) by free radicals contributes to the development of both macroangiopathy[1,3,4] and microangiopathy[1,5-8]. Because in vivo measurement of free radicals is difficult given their reactivity, short half-life, and very low concentrations[9], various methods have been developed to investigate indirectly whether such oxidative processes occur in vivo.

The susceptibility of LDL to in vitro oxidation has recently been proposed as a measure of the response of LDL to the in vivo oxidative stress, which is thought to occur within the matrix of the vessel wall close to the endothelium[10]. Whereas the results of the susceptibility of LDL to oxidation are inconclusive[10-14], concentrations of malondialdehyde, a marker of general lipid peroxidation and oxidative stress, have been found to be increased principally in patients with non-insulin-dependent diabetes mellitus[6,15-19].

We compared the susceptibility of LDL for oxidation and malondialdehyde concentrations between patients with insulin-dependent diabetes mellitus (IDDM) and subjects without diabetes mellitus.

PATIENTS AND METHODS

Study population

The present study was conducted at the outpatient clinic of the 'De Weezenlanden' hospital in Zwolle, a middle-sized town in the eastern part of The Netherlands. From January 1995 to January 1996, 293 consecutive IDDM patients were invited to participate in the study. IDDM was defined as the start of insulin therapy within 6 month after the first sign of diabetes mellitus and before the age of 30 years, or the absence of C-peptide secretion. In the same period 106 consecutive healthy men and women (controls), who were admitted for minor surgery (e.g. cosmetic surgery, sterilization or artheroscopy), were invited to participate in the study. Health in the latter group was defined as no current diseases, except the indication for surgery (ASA classification category 1), and not using medication, except oral contraceptives. Twelve diabetic patients

and 8 controls subjects refused to participate in the study, so the presented results are based on the remaining 281 diabetic patients and 98 controls subjects. The study protocol was approved by the hospital medical ethical committee and all patients gave informed consent.

Measurements

Patients were examined according to a standardized protocol. Smoking habits, alcohol use, as well as used medication, were recorded. Body mass index was calculated as weight (kg) divided by square of height (m^2). Blood pressure measurements were performed after five and seven minutes of rest in the supine position using a precalibrated standard mercury sphygmomanometer. The mean of the two measurements was used in the analysis. Hypertension was defined as systolic blood pressure of 140 mmHg or over and/or diastolic blood pressure of 90 mmHg or over[20]. Patients using antihypertensive medication were also considered to be hypertensive.

Blood sampling was performed after 30 minutes rest in the supine position. In 122 diabetes patients (79 males, 43 females) blood sampling was performed under fasting conditions, before the morning dose of insulin was administered. As most participating patients lived at some distance from the hospital, the blood samples of the other 159 diabetes patients were collected after their usual morning insulin dose and normal breakfast. Blood sampling in control subjects was never under fasting conditions. The blood samples to be used for LDL-oxidation and determination of malondialdehyde, were collected in EDTA Vacutainer tubes, placed on ice immediately and cooled to 4 °C. Plasma, prepared for the determination of malondialdehyde, was protected against oxidation by addition of 2 mg/ml reduced glutathione and 1.2 mg/ml butylated hydroxytoluene (final concentrations) as well as by flushing the empty space of the tubes with nitrogen. Except for the routine clinical chemistry, all blood samples stored at -80 °C. The entire blood processing procedure was completed within one hour.

The procedure for preparation and oxidation of LDL was adapted from the method as described by Esterbauer et al.[21] with modifications as described previously[22,23]. Briefly, from each subject 2 ml of frozen plasma was rapidly thawed and used for isolation of LDL by ultracentrifugation at 4 °C in the presence of 10 µM EDTA. To minimize the time between isolation and

oxidation and to prevent the loss of lipophilic anti-oxidants[24], LDL was not dialysed[22,23,25]. By omitting dialysis a more stable LDL preparation is obtained, which can be stored in the dark at 4 °C under nitrogen for several days without affecting resistance time (lagtime) and maximum rate of oxidation (propagation rate)[26]. This improves the precision of the method, since each LDL preparation could be oxidized consecutively in triplicate.

The kinetics of LDL-oxidation were followed by continuously monitoring the change of absorbance at 234 nm. Absorbance curves of LDL-preparations obtained from six diabetes patients and two control subjects were determined in parallel. Each LDL-preparation was oxidized in three consecutive oxidation runs on the same day; the values shown for resistance time, maximum rate of oxidation and maximal diene production are means of these three values.

The intra-assay coefficients of variation for resistance time and maximum rate of oxidation were 2.6% and 3.1%, respectively, upon oxidation of the same LDL- samples in three consecutive runs on one day. The inter-assay coefficients were 4.9% and 7.4% respectively, and were obtained by determining the oxidation of LDL-samples from the same subjects prepared on different days. In every oxidation run one reference LDL-sample, prepared from a reference plasma stored at -80 °C, was used as a control. Oxidation runs with a deviation higher than 10% from the values of former reference measurements were omitted.

By using this standardized method, resistance time and maximum rate of oxidation do not differ between LDL prepared from plasma frozen in liquid nitrogen and that from freshly collected plasma from the same subject. In addition, no differences in these parameters were found upon storage of plasma at -80 °C up to 18 month[22,23,26].

The procedure for measuring LDL-particle size was adapted from the method described by Mc Namara et al.[27], with the following modifications. Plasma from each subject was rapidly thawed, diluted ten times with a physiological salt solution and mixed with a solution containing 40% sucrose and 0.1% bromophenol blue. One microliter of this sample was loaded onto the applicator of the Pharmacia Phastsystem containing 4-15% non-denaturing polyacrylamide gradient gels and the appropriate buffer strips (Pharmacia) and was subjected to electrophoresis for 18 hours at 225 V and 15 °C. After electrophoresis the gels were silver stained using the Biorad Silver Stain kit. High molecular weight standards (thyroglobulin (size 17.0 nm) and ferritin (size 12.2 nm) (Pharmacia)) were used together with a reference serum containing

alpha 2- macroglobulin (20.0 nm) and LDL-particles with a size of 26.0 nm. After staining, the gels were scanned with an LKB Ultrascan 2202 laser densitometer (Pharmacia). In general, two major LDL-subclasses were observed and in some cases a third subclass could be identified. The migration distances of the two major LDL-subclasses were compared with those of the standards (ferritin, thyroglobulin, alpha 2- macroglobulin) and with the upper band of the reference LDL. The migration distances of the two major LDL-bands were used to calculate the LDL-particle sizes. The inter-assay variation for the upper and lower bands of the reference LDL was 0.1 nm and 0.2 nm, respectively. The electrophoretic patterns were examined independently by two examiners to assign LDL-subclass phenotypes. LDL-subclass patterns were defined as phenotype A (major subclass >25.5 nm), phenotype B (major subclass <25.5 nm) or phenotype A/B (intermediate phenotype) according to Austin et al.[28, 29]. Plasma malondialdehyde concentrations were determined in plasma lipid extracts, using 1,3-diethyl-2-thiobarbituric acid, exactly as described by Hoving et al.[30].

Routine clinical chemistry

The clinical chemistry assays for total serum cholesterol, HDL-cholesterol and triglycerides were performed on a Hitachi 717 chemistry analyzer based on commercially available techniques (Boehringer Mannheim, Mannheim, Germany). Total serum cholesterol was determined by means of the CHOD-PAP method. HDL-cholesterol was measured after precipitation with sodium phosphotungstate-Mg^{2+}. Triglycerides were determined using the GPO-PAP method. LDL-cholesterol concentrations were calculated according the formula of Friedewald[31]. Phospholipid concentrations in LDL were determined using a commercially available colour reagent (Wako Chemicals, Neuss, Germany). One hundred microliter of LDL-sample (0.25 mg protein/ml) and 750 µl colour reagent were mixed for 10 minutes at 37 °C and the concentration was measured at a wavelength of 500 nm. The protein content of LDL-preparations was measured according to Lowry et. al[32].

HbA1c was measured by affinity chromatography (Pierce columns, Glyco test II) (upper limit of normal 6.0%)[33]. Serum creatinine concentration were measured kinetically on the Hitachi 717, as described by Jaffé[34]. Creatinine clearance was calculated according to the Cockcroft formula[35].

Data analysis

The chi-square test was used to compare frequencies. Analysis of variance

(ANOVA) was performed to test for differences between groups. In addition multiple linear regression analyses were performed to adjust for possible confounders. Two-sided P-values ≤ 0.05 were considered to be statistically significant. All analyses were performed using SPSS 6.1.3 for Windows.

RESULTS

The clinical characteristics of diabetic patients and control subjects are summarized in Table 1. Although there was a tendency in control subjects for a less beneficial lipid profile compared to diabetic subjects, only the difference of serum HDL-cholesterol was found to be statistically significant. This difference could not be attributed to the fact, that the blood sampling of control patients was not performed under fasting conditions or to a difference for one of the other clinical characteristics. LDL-phenotype did not differ between diabetic patients and control subjects.
With regard to composition of LDL-cholesterol, the triglyceride fraction of LDL was significantly increased in diabetic patients compared to control patients, while the cholesterol ester fraction was found to be decreased.
Malondialdehyde concentrations and the susceptibility of LDL for oxidation, did not differ significantly between diabetic patients and control subjects. These results were essentially the same when diabetic patients, of whom fasting blood was obtained, were compared with control subjects or when patients with hypertension were removed from the analysis. In addition, blood sampling conditions (fasting versus non-fasting), the use of lipid lowering drugs and anti-oxidant suppletion or the presence of hypertension in IDDM patient were not associated with any difference in malondialdehyde concentrations or the susceptibility of LDL to oxidation.

DISCUSSION

The results of this study showed no difference in the vitro susceptibility of LDL between IDDM patients and control subjects. There was also no difference in maximum rate of oxidation, oxidation maximum and MDA concentrations. Although all blood samples of the control subjects were obtained in the non-

Table 1. Clinical characteristics of the study population

	IDDM patients	Control group
Number of patients	281	98
Age (years)	38.2 (0.7)	38.2 (1.0)
Men (%)	153 (54.4%)	49 (50.0%)
Body mass index(kg/m^2)	24.7 (0.2)	25.5 (0.4)
Duration of diabetes (years)	17.2 (0.6)	n.a.
Systolic blood pressure (mmHg)	139.1 (1.1)	125.6 (1.0)***
Diastolic blood pressure (mmHg)	82.9 (0.5)	78.1 (0.7)***
Current smokers	91 (32.4%)	40 (40.8%)
Number of cigarettes/ day	12.9 (1.0)	13.7 (1.5)
Never alcohol users	109 (38.8%)	33 (33.7%)
Creatinine clearance (ml/min)	107.0 (1.6)	111.3 (2.6)
HbA1c (%)	8.27 (0.11)	4.62 (0.06)***
Total cholesterol (mmol/l)	4.98 (0.06)	5.18 (0.10)
HDL-cholesterol (mmol/l)	1.39 (0.02)	1.29 (0.03)*
LDL-cholesterol (mmol/l)	3.06 (0.05)	3.22 (0.09)
Triglycerides (mmol/l)	1.18 (0.06)	1.37 (0.07)
LDL-phenotype		
- type A	214 (76.2%)	81 (82.7%)
- type B	56 (19.9%)	15 (15.3%)
- type A/B	11 (3.9%)	2 (2.0%)
LDL-particle size		
- LDL subclass 1 (nm)	26.59 (0.03)	26.42 (0.05)**
- LDL subclass 2 (nm)	24.35 0.03)	24.33 (0.02)

Values are number of patients or means with percentage or standard errors between parentheses. n.a.= not applicable.
Difference between IDDM patients and control subjects, after adjustment for age and gender: * = $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

fasting state and in diabetic patients under fasting conditions as well as in the non-fasting state, limiting the analysis to subjects with a non-fasting blood sample revealed the same associations. In addition, blood sampling condition in IDDM patients was not associated with any difference in malondialdehyde concentrations or susceptibility of LDL to oxidation.

In our study the assays used, are according to international standards and applied to an unselected group of diabetic patients and control subjects. Although all control patients, were classified as ASA classification category 1, indicating no current diseases except the indication for minor surgery and not using any medication, we were surprised to find a less favourable lipid profile in control patients. This difference was not associated with fasting conditions or the use of lipid lowering medication in five of the IDDM patients. A possible explication for this result might be the difference in diet between IDDM patients and the normal population.

Table 2. Parameters of lipid peroxidation and LDL composition

	IDDM patients		Control group	
Malondialdehyde (µmol/l)	1.52	(0.02)	1.52	(0.02)
LDL oxidation resistance				
- resistance time (min)	97.8	(0.6)	96.1	(0.8)
- maximum rate of oxidation (nmol/mg/min)	10.6	(0.1)	10.5	(0.1)
- max diene production (nmol/mg)	451.1	(2.3)	446.5	(3.9)
LDL composition (percentage)				
- cholesterol ester	28.5	(0.2)	29.5	(0.4)*
- free cholesterol	9.7	(0.1)	9.7	(0.2)
- phospholipid	25.0	(0.2)	25.0	(0.3)
- protein	29.1	(0.2)	29.0	(0.3)
- triglyceride	7.8	(0.1)	6.9	(0.2)***

Values are number of patients or means with percentage or standard errors between parentheses.
Difference between IDDM patients and control subjects, after adjustment for age and gender: *= $p \leq 0.05$, ***= $p \leq 0.001$

The results of our study are in contrast with previously reported results regarding the susceptibility of LDL to oxidation[11-14,36-40], as well as for malondialdehyde concentrations[6,18,19], but in agreement with the results of the more recent studies for both parameters[10,41,42]. The differences between these studies might result from the small sample size of most of these studies (n< 60), or from the fact that in most studies examining LDL-oxidation, NIDDM patients or a mixture of IDDM and NIDDM patients were compared with healthy controls[12-14,37-39]. The decrease in oxidation resistance in these studies may therefore be based on the well-known diminished resistance to oxidation of small LDL-particles, which are commonly found in NIDDM patients[13]. In our study no difference in LDL-phenotype was found between IDDM patients and control subjects, while the size of LDL-subclass 1 was smaller in controls.
An other explanation might be methodological differences of the oxidation procedure[12-14,39]. Babiy[13] and his coworkers used gamma ray radiation in stead of copper to oxidize LDL, while in a study performed by Gugliucci[14] both LDL and VLDL were oxidized in vitro. Given the absence of a correlation between the susceptibility of LDL and HbA1c level[10,11], a difference in metabolic control seems to be of minor importance.

The susceptibility of LDL to in vitro oxidation is influenced by its anti-oxidant content[22,23,44,45], and its lipid and fatty acid composition[25,44,46,47]. Although we did not measure anti-oxidant concentrations in LDL, we found a small but significant difference in LDL-lipidcomposition, between the control subjects and IDDM patients, which was also reflected in LDL-size. These differences, however, did not result in a significant difference in resistance time or maximum rate of oxidation.

Given our findings, one could reject the hypothesis that oxidative stress is increased in patients with insulin-dependent diabetes mellitus, but as reviewed by Giugliano[1], there are many other findings strongly supporting this hypothesis. In diabetes, the formation of free radicals may be enhanced by increased glucose concentration through non-enzymatic glycation of protein substrates[48], auto-oxidation of glucose[49], activation of protein kinase C[50] and increased activity of the polyol pathway[51]. In addition, diabetes is known to induce changes in the content and activity of cellular anti-oxidant enzymes[52-56]. Therefore it is more likely that the assays used in this studie do not reflect oxidative stress in IDDM patients, properly. In intervention studies with high doses of vitamine E (25 mg/day) or with fish oil (5 g/day) in non-diabetic

subjects the susceptibility of LDL to oxidation, assesed by the same methods as in our study was significantly decreased[22,25]. However, for observational studies like ours, this method may not be sensitive enough.

In conclusion, the hypothesis that oxidative stress is increased in patients with IDDM and might be the common pathway in the development of microvascular complications, is not supported by the results of our cross-sectional study, using malondialdehyde and the susceptibility of LDL to in vitro oxidation as parameters of oxidative stress.

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Lipid peroxidation and microvascular complications in insulin-dependent diabetes mellitus

SUMMARY

In insulin-dependent diabetes mellitus there is a clear relationship between metabolic control (HbA1c levels) and the development and progression of microvascular complications. Although the pathophysiology of these complications is probably multifactorial, oxidative stress through increased tissue damage by formation of free radicals, might be involved in the development of diabetes complications. We studied the role of oxidative stress in the development of these complications by assessing the susceptibility of LDL for oxidation and serum malondialdehyde concentrations, in a group of 281 patients with insulin-dependent diabetes mellitus.

Fifty-seven patients (20.2%) were categorized as having nephropathy, 146 patients (52.0%) as having diabetic neuropathy and 93 patients (33.1%) as having diabetic retinopathy. More than twenty-five percent of the diabetes patients had two or more microvascular complications. No significant difference in malondialdehyde or oxidation resistance was found between patients with or without microvascular complications, nor by the number of microvascular complications. In addition, nephropathy, retinopathy and neuropathy were not associated with any difference in malondialdehyde level or the susceptibility of LDL oxidation.

Metabolic control, as assessed by HbA1c was not related to malondialdehyde concentrations or to the oxidation resistance of LDL. In contrast glycosylated apolipoprotein B was significantly associated with lagtime (regression coefficient 1.85 min per percent (0.59)), but not with malondialdehyde concentrations.

The results of this cross-sectional study do not support a role of oxidative stress in the presence of microvascular complications in patients with insulin-dependent diabetes mellitus, at least not when assessed by the susceptibility of LDL to in vitro oxidation or malondialdehyde concentration. In addition, we could not confirm a direct association between these parameters of lipid peroxidation and HbA1c.

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INTRODUCTION

In insulin-dependent diabetes mellitus (IDDM), there is a definite relationship between the level of metabolic control (HbA1c levels) and the development and progression of diabetic microvascular complications[1]. The mechanisms by which this lack of glycaemic control predisposes to the development of diabetic complications are incompletely understood. Despite intensive treatment and strict metabolic control, a number of patients still develops microvascular complications[1,2].

A recently proposed mechanism, which might be responsible for microvascular complications, is based on tissue damage induced by free radicals. This damage could occur in case of a disturbed balance between the formation of free radicals and the anti-oxidants defence systems acting against it (oxidative stress). Since oxidative stress is not exclusively related with hyperglycaemia[3], but is also influenced by other factors like dietary factors (the intake of anti-oxidants) or hereditary factors (regulation of enzymatic anti-oxidants) it may account for the development of microvascular complications under strict metabolic control.

The susceptibility of LDL to in vitro oxidation has recently been proposed as a parameter of the response of LDL to in vivo oxidative stress, which is thought to occur within the matrix of the vessel wall, close to the endothelium[4]. Whereas the results of the association between diabetes mellitus and in vitro susceptibility of LDL to oxidation are inconclusive[4-8], concentrations of malondialdehyde, a marker of general lipid peroxidation, have been found to be increased in diabetic patients[3]. We studied the relationships between the susceptibility of LDL for oxidation, malondialdehyde concentrations, and metabolic control as well as the prevalence of microvascular complications in IDDM patients.

PATIENTS AND METHODS

Study population

The present study was conducted at the outpatient clinic of the 'De Weezenlanden' hospital in Zwolle, a middle-sized town in the eastern part of The Netherlands. From January 1995 to January 1996, 293 consecutive IDDM patients were invited to participate in the study. IDDM was defined as the start

of insulin therapy before the age of 30 years within 6 months after diagnosing diabetes mellitus, or the absence of C-peptide secretion. Twelve patients refused to participate in the study, so the presented results are based on the remaining 281 patients. The study protocol was approved by the hospital medical ethical committee and all patients gave their informed consent.

Measurements

Patients were examined according to a standardized protocol by one trained physician. Smoking habits, and used medication, including frequency of injections were recorded. Blood pressure measurements were performed after five and seven minutes of rest in the supine position using a precalibrated standard mercury sphygmomanometer. The mean of the two measurements was used in the analysis. Hypertension was defined as systolic blood pressure of 140 mmHg or over and/or diastolic blood pressure of 90 mmHg or over[9]. Patients who were treated with antihypertensive medication were also considered hypertensive.

Blood sampling was performed after 30 minutes rest in the supine position. In 122 diabetes patients (79 males, 43 females) blood sampling was performed under fasting conditions, before the morning dose of insulin was administered. As most participating patients lived at some distance from the hospital, the blood samples of the other 159 diabetes patients were collected after their usual morning insulin dose and normal breakfast. The blood samples to be used for LDL-oxidation and determination of malondialdehyde, were collected in EDTA Vacutainer tubes, placed on ice immediately and cooled to 4°C. Plasma, prepared for the determination of malondialdehyde, was protected against oxidation by addition of 2 mg/ml reduced glutathione and 1.2 mg/ml butylated hydroxytoluene (final concentrations) as well as by flushing the empty space of the tubes with nitrogen. Except for the routine clinical chemistry, all blood samples were stored at -80 °C. The entire blood processing procedure was completed within one hour.

The procedure for preparation and lipid peroxidation of LDL was adapted from the method as described by Esterbauer et al.[10] with modifications as described previously[11,12]. Briefly, from each subject 2 ml of frozen plasma was rapidly thawed and used for isolation of LDL by ultracentrifugation at 4 °C in the presence of 10 µM EDTA. To minimize the time between isolation and oxidation and to prevent the loss of lipophilic anti-oxidants[13], LDL was not

dialysed[11,12,14]. By omitting dialysis a more stable LDL-preparation is obtained, which can be stored in the dark at 4 °C under nitrogen for several days without affecting resistance time (lagtime) and maximum rate of oxidation (propagation rate)[15]. This improves the precision of the method, since each LDL-preparation could be oxidized consecutively in triplicate.
The kinetics of LDL-oxidation was followed by continuously monitoring the change of absorbance at 234 nm[10-12]. Absorbance curves of LDL-preparations obtained from six diabetes patients were determined in parallel. Each LDL-preparation was oxidized in three consecutive oxidation runs on the same day; the values shown for resistance time, maximum rate of oxidation and maximal diene production are means of these three values.
The intra-assay coefficients of variation for resistance time and maximum rate of oxidation were 2.6% and 3.1%, respectively, upon oxidation of the same LDL-samples in three consecutive runs on one day. The inter-assay coefficients were 4.9% and 7.4% respectively, and were obtained by determining the oxidation of LDL-samples from the same subjects prepared on different days. In every oxidation run one reference LDL-sample, prepared from a reference plasma stored at -80 °C, was used as a control. Oxidation runs with a deviation higher than 10% from the values of former reference measurements were omitted.
By using this highly standardized method, resistance time and maximum rate of oxidation do not differ between LDL prepared from plasma frozen in liquid nitrogen and that from freshly collected plasma from the same subject. In addition, no differences in these parameters were found upon storage of plasma at -80 °C up to 18 month[11, 12,15].

The procedure for measuring LDL-particle size was adapted from the method described by Mc Namara et al.[16], with the following modifications. Plasma from each subject was rapidly thawed, diluted ten times with a physiological salt solution and mixed with a solution containing 40% sucrose and 0.1% bromophenol blue. One microliter of this sample was loaded onto the applicator of the Pharmacia Phastsystem containing 4-15% nondenaturing polyacrylamide gradient gels and the appropriate buffer strips (Pharmacia) and was subjected to electrophoresis for 18 hours at 225 V and 15 °C. After electrophoresis the gels were silver stained using the Biorad Silver Stain kit. High molecular weight standards (thyroglobulin (size 17.0 nm) and ferritin (size 12.2 nm) (Pharmacia)) were used together with a reference serum containing alpha 2- macroglobulin (20.0 nm) and LDL-particles with a size of 26.0 nm.

After staining, the gels were scanned with an LKB Ultrascan 2202 laser densitometer (Pharmacia). In general two major LDL-subclasses were observed and in some cases a third subclass could be identified. The migration distances of the two major LDL-subclasses were compared with those of the standards (ferritin, thyroglobulin, alpha 2- macroglobulin) and with the upper band of the reference LDL. The migration distances of the two major LDL-bands were used to calculate the LDL-particle sizes. The inter-assay variation for the upper and lower bands of the reference LDL was 0.1 nm and 0.2 nm, respectively. The electrophoretic patterns were examined independently by two examiners to assign LDL-subclass phenotypes. There was never a disagreement between the two examiners assigning LDL-subclasses. LDL-subclass patterns were defined as phenotype A (major subclass >25.5 nm), phenotype B (major subclass <25.5 nm) or phenotype A/B (intermediate phenotype) according to Austin et al.[17,18]. Plasma malondialdehyde concentrations were determined in plasma lipid extracts, using 1,3-diethyl-2-thiobarbituric acid, exactly as described by Hoving et al[19].

Routine clinical chemistry

The clinical chemistry assays for total serum cholesterol, HDL-cholesterol and triglycerides were performed on a Hitachi 717 chemistry analyzer based on commercially available techniques (Boehringer Mannheim, Mannheim, Germany). Total serum cholesterol was determined by means of the CHOD-PAP method. HDL-cholesterol was measured after precipitation with sodium phosphotungstate-Mg^{2+}. Triglycerides were determined using the GPO-PAP method. All obtained from Boehringer Mannheim. LDL-cholesterol concentrations were calculated according the formula of Friedewald[20]. Phospholipid concentrations in LDL were determined using a commercially available colour reagent (Wako Chemicals, Neuss, Germany). One hundred microliter of LDL-sample (0.25 mg protein/ml) and 750 µl colour reagent were mixed for 10 minutes at 37 °C and the concentration was measured at 500 nm wavelength. The protein content of LDL-preparations was measured according to Lowry[21]. Apolipoprotein A-I, apolipoprotein B and lipoprotein[a] were determined by commercially available immunochemistry techniques on the Beckman Array Analyzer (Beckman, Fullerton, California).

Metabolic control was assessed by measuring glycosylated haemoglobin A1c (HbA1c) and glycosylated apolipoprotein B. HbA1c was measured by affinity chromatography (Pierce columns, Glyco test II) (upper limit of normal 6.0%) [22]. Glycosylated apolipoprotein B (as percentage of total apolipoprotein B) was

determined, according to Pantheghini[23].
Plasma vitamin A (retinol), beta-carotene and vitamin E (alpha-tocopherol and gamma-tocopherol) were determined according to Zaman et al.[24], with the following modifications: instead of a programmable UV-VIS detector, a 996 Photo Diode Array Detector (Waters Associates, Milford, USA) was used, and in the extraction procedure methanol and hexane were used instead of ethanol and hexane. To prevent oxidation of the tocopherols during the extraction procedure, butylated hydroxytoluene (BHT), pyrogallol, vitamin C and EDTA were added. Transferrin, uric acid and ceruloplasmin were determined according to routine procedure immunonephelometrically on an Array immune analyzer (Beckman, Fullerton, California). Ferritin was routinely analysed with the ferritin Enzymun test on the ES 607 analyzer (Boehringer Mannheim, Mannheim, Germany).

In twenty-four hour urine samples, collected by the patients the day before their outpatient visit, albumin was measured, using an immunonephelometric technique on the Array immune analyzer (Beckman, Fullerton, California). Urinary albumin excretion rate (UAER) was calculated and divided into three categories: normo-albuminuria: UAER <30 mg/24h; micro-albuminuria: 30 mg< UAER <300 mg/24h; macro-albuminuria: UAER >300 mg/24h. Seven patients with normo-albuminuria and fourteen patients with micro-albuminuria used ACE-inhibitors. The hospital files showed that in all patients the indication for this treatment was repeatedly proven albuminuria of over 100 mg/24h. Therefore, these patients were categorized as macro-albuminuric. Serum as well as urine creatinine concentrations were measured kinetically on the Hitachi 717, as described by Jaffé [25]. Creatinine clearance rate was calculated according to the Cockroft formula[26].

In order to assess the degree of diabetic retinopathy the examination included direct and indirect ophthalmoscopy through dilated pupils by an experienced ophthalmologist. Retinopathy was graded according to national standards in three categories: no retinopathy, background retinopathy and pre-/ proliferative retinopathy[27].
Neuropathy was assessed by vibration threshold measurement using a Somedic vibrameter type IV and by sensibility-testing using monofilaments according to Semmes-Weinstein (monofilaments by Gillis W Long, Hansons Disease Centre, Carreville, USA). The vibration threshold was determined according to a standardized procedure at six test sites: the dorsomedial aspect of the first

Table 1. Clinical characteristics of the study population

	Total group
Number of patients	281
Age (years)	38.2 (12.4)
Duration of diabetes (years)	17.2 (10.7)
Systolic blood pressure (mmHg)	139.1 (18.4)
Diastolic blood pressure (mmHg)	82.9 (8.3)
Hypertension	110 (39.1%)
Current smokers	91 (32.4%)
Number of cigarettes/ day	12.9 (9.0)
Frequency of insulin injections	
- 2 or 3 times a day	34 (12.1%)
- 4 times a day	175 (62.3%)
- CSII/ CIPII	72 (25.6%)
Creatinine clearance (ml/min)	107.0 (27.1)
HbA1c(%)	8.27 (1.90)
Glycosylated apolipoprotein B (%)	3.07 (0.91)
Total cholesterol (mmol/l)	4.98 (1.01)
HDL-cholesterol (mmol/l)	1.39 (0.37)
LDL-cholesterol (mmol/l)	3.06 (0.86)
Triglycerides (mmol/l)	1.18 (0.99)
Apolipoprotein A (mg/l)	1.38 (0.25)
Apolipoprotein B (mg/l)	0.89 (0.24)
Lipoprotein [a] (mg/l)	125.6 (179.4)
Uric Acid (mmol/l)	0.25 (0.06)
Alpha-tocopherol (µmol/l)	31.34 (9.37)
Gamma-tocopherol (µmol/l)	2.99 (1.80)
Beta- carotene (nmol/l)	324.8 266.9)
Retinol (µmol/l)	1.83 (0.69)
Ceruloplasmin (g/l)	0.38 (0.14)
Ferritin (µg/l)	92.7 (89.5)
Transferrin (g/l)	2.24 (0.40)

CSII= continuous subcutaneous insulin infusion. CIPII= continuous intra-peritoneal insulin infusion. Values are number of patients or means with percentage or standard deviations between parentheses.

metatarsal bone, the flat surface just above the medial malleolus and the dorsum of the metacarpal bone of the index finger for both the left and right side of the body. Vibration measurement was defined as abnormal when one of the measurements was two standard deviations above the reference value for age and localization. Sensibility-testing was performed at five regions on both legs and classified as abnormal when in one of the region pressure of monofilament 5.07 was not felt[28]. Peripheral neuropathy was defined as at least one abnormal vibration measurement or sensibility test-result. Severity of peripheral neuropathy was graded as the number of abnormal tests.

Data analysis

Table 2. Parameters of lipid peroxidation, LDL-phenotypes and LDL-particle size, specified by gender

	Men		Women		Total group	
Malondialdehyde (µmol/l)	1.54	(0.32)	1.50	(0.27)	1.52	(0.30)
LDL particle size						
- LDL 1 (nm)	26.6	(0.5)	26.6	(0.5)	26.6	(0.5)
- LDL 2 (nm)	24.4	(0.5)	24.3	(0.4)	24.4	(0.5)
LDL-phenotype						
- type A	121	(79.1%)	93	(72.7%)	214	(76.2%)
- type A/B (intermediate)	25	(16.3%)	31	(24.2%)	56	(19.9%)
- type B	7	(4.6%)	4	(3.1%)	11	(3.9%)
LDL-composition (percentage)						
- cholesterol ester	29.2	(3.6)	27.7	(3.4)***	28.5	(3.6)
- free cholesterol	9.6	(2.1)	9.8	(2.3)	9.7	(2.2)
- phospholipids	24.6	(3.0)	25.4	(2.3)*	25.0	(2.8)
- protein	29.3	(2.5)	28.9	(2.7)	29.1	(2.6)
- triglycerides	7.4	(1.9)	8.2	(2.1)***	7.8	(2.0)
LDL-oxidation resistance						
- lag time (min)	97.1	(8.6)	98.7	(9.3)	97.8	(9.0)
- propagation rate (nmol/mg/min)	10.6	(1.3)	10.5	(1.3)	10.6	(1.3)
- max diene production (nmol/mg)	449.9	(38.0)	452.4	(39.0)	451.1	(38.4)

Values are number of patients or means with percentage or standard deviations between parentheses. Difference between men and women:
* = $p \leq 0.05$, *** = $p \leq 0.001$

Table 3. Parameters of lipid peroxidation: the association with serum lipids and LDL-composition.

		LDL-oxidation		
	MDA	Lag time	Propagation rate	Maximal diene
Serum lipids				
- Total cholesterol	0.31*	-0.10	0.04	0.02
- HDL-cholesterol	0.16*	0.07	0.11	0.21*
- LDL-cholesterol	0.14*	-0.06	0.05	0.05
- Triglycerides	0.40*	-0.16*	-0.12*	-0.25*
- Apolipoprotein A	0.28*	0.01	0.01	0.06
- Apolipoprotein B	0.15*	-0.09	0	-0.07
- Lipoprotein [a]	0.02	-0.08	-0.03	-0.02
LDL-composition				
- cholesterol	-0.01	0.02	0.15*	0.21*
- free cholesterol	-0.05	0.11	0.05	0.06
- phospholipids	-0.01	-0.06	0.08	0.15*
- protein	-0.02	-0.18*	-0.17*	-0.37*
- triglycerides	0.12	0.16*	-0.20*	-0.16*

MDA= Malondialdehyde concentration. Values are partial correlation coefficients after adjustment for age, fasting conditions and gender. *= $p \leq 0.05$

Table 4. Parameters of lipid peroxidation: the association with anti-oxidants

		LDL-oxidation		
	MDA	Lag time	Propagation rate	Maximal diene
Anti-oxidants				
Uric Acid	0	-0.13*	-0.18*	-0.22*
Alpha-tocopherol	0.13*	0.11*	0.21*	0.32*
Gamma-tocopherol	-0.01	0.06	0	0.08
Beta-carotene	0.16*	-0.02	0.05	0.10
Retinol	0.12*	-0.11*	-0.14*	-0.16*
Ceruloplasmin	-0.004	-0.05	-0.08	-0.23*
Ferritin	0.04	-0.15*	-0.02	-0.03
Transferrin	0.11	-0.07	-0.01	-0.11

MDA= Malondialdehyde concentration. Values are partial correlation coefficients after adjustment for age, gender and triglyceride concentrations. *= $p \leq 0.05$

Table 5. Association between parameters of lipid peroxidation and diabetes complications

	Neuropathy		Nephropathy		Retinopathy		Any microvascular complication	
	absent	present	absent	present	absent	present	absent	present
Malondialdehyde (mmol/l)	1.51 (0.03)	1.54 (0.03)	1.52 (0.02)	1.54 (0.05)	1.53 (0.02)	1.51 (0.03)	1.51 (0.03)	1.53 (0.02)
LDL-oxidation								
lag time (min)	97.8 (0.7)	97.5 (0.8)	98.0 (0.6)	97.1 (1.3)	98.6 (0.6)	96.3 (1.0)	97.7 (0.9)	97.7 (0.7)
propagation rate (nmol/mg/min)	10.8 (0.1)	10.4 (0.1)	10.6 (0.1)	10.4 (0.2)	10.6 (0.1)	10.5 (0.1)	10.8 (0.1)	10.5 (0.1)
maximal diene (nmol/ml/mg)	454.5 (3.6)	448.7 (3.0)	453.6 (2.5)*	441.6 (5.7)*	451.8 (2.9)	449.6 (4.1)	454.4 (4.3)	450.1 (2.8)
LDL-composition (percentage)								
- cholesterol	28.3 (0.3)	28.7 (0.3)	28.4 (0.2)	28.9 (0.5)	28.4 (0.3)	28.6 (0.3)	28.2 (0.3)	28.7 (0.3)
- free cholesterol	9.8 (0.2)	9.5 (0.2)	9.7 (0.1)	9.6 (0.3)	9.8 (0.2)	9.6 (0.2)	10.0 (0.2)	9.5 (0.2)
- phospholipids	25.3 (0.2)	24.6 (0.2)	25.1 (0.2)	24.4 (0.5)	25.0 (0.2)	24.9 (0.3)	25.4 (0.2)	24.7 (0.2)*
- protein	28.9 (0.2)	29.3 (0.2)	29.0 (0.2)	29.3 (0.4)	29.1 (0.2)	29.1 (0.3)	28.9 (0.2)	29.2 (0.2)
- triglycerides	7.7 (0.2)	7.8 (0.2)	7.8 (0.1)	7.8 (0.3)	7.7 (0.2)	7.8 (0.2)	7.5 (0.2)	7.9 (0.2)

Values are means with standard errors between parentheses. Difference between patients with and without diabetic complication, after adjustment for age and gender: *= $p < 0.05$

The chi-square test was used to compare frequencies. Analysis of variance (ANOVA) was performed to test for differences between groups. In addition multiple regression analyses were performed to adjust for confounders. Partial correlation coefficients were calculated to describe the relations between parameters of lipid per oxidation and serum lipids or anti-oxidants. For serum triglyceride and LDL-cholesterol concentrations additional calculations were performed limiting the analyses to subjects of whom fasting blood samples were obtained. Two-sided P-values ≤ 0.05 were considered to be statistically significant. All analysis were performed using SPSS 6.1.3 for Windows.

RESULTS

The clinical characteristics of the study population are shown in Table 1. Table 2 summarizes the parameters of lipid peroxidation, serum lipid concentrations, LDL-phenotypes and LDL-composition. Malondialdehyde concentrations were significantly associated with the susceptibility of LDL for oxidation (regression coefficients for lag time -0.004 µmol/l per min (SEM 0.002) and for propagation rate -0.02 µmol/l per nmol/mg/min (0.01)). Lag time was associated with propagation rate (regression coefficient -1.59 min per nmol/mg/min (0.42)), as was propagation rate with the maximal diene production (regression coefficient 0.03 nmol/mg/min per nmol/mg (0); $p \leq 0.05$, both after adjustment for age, and gender).

No relationship was found between MDA levels or the susceptibility of LDL for oxidation and gender, fasting conditions, blood pressure or hypertension. Malondialdehyde concentrations were significantly associated with age (regression coefficient 0.003 µmol/l per year (0.001)). Smoking, duration of diabetes or frequency of insulin administration were also not associated with the parameters of lipid peroxidation.

Metabolic control, as assessed by HbA1c was not related to malondialdehyde concentrations nor to the oxidation resistance of LDL. Glycosylated apolipoprotein B was significantly associated with both lag time (regression coefficient 1.85 min per percent (0.59)) and maximal diene production (5.46 nmol/mg per percent (2.58), but not with malondialdehyde concentrations. Malondialdehyde concentrations were significantly associated with most serum

lipids, but not with LDL-composition (Table 3). Oxidation resistance was associated with serum triglyceride levels, the triglyceride fraction of the LDL-cholesterol as well as the protein fraction of the LDL-composition. Both malondialdehyde concentrations and oxidation resistance were not associated with particle size nor with LDL-phenotype. Table 4 summarizes the associations between serum anti-oxidants and the parameters of lipid peroxidation. Malondialdehyde was significantly correlated with alpha-tocopherol, beta-carotene as well as with retinol. Oxidation resistance was significantly correlated with alpha-tocopherol, retinol, ferritin and uric acid concentrations. These results were essentially the same after adjustment for body mass index, total cholesterol, HDL- or LDL- cholesterol or metabolic control.

Ninety-three patients were categorized as having diabetic retinopathy (of them 27 (29.3%) with pre- or proliferative retinopathy), 57 patients as having nephropathy (32 (56.1%) patients using an ACE inhibitor or having macro-albuminuria), and 146 patients as having diabetic neuropathy. Sixty three (43.2%) of these patients had 2 abnormal tests. No significant difference in malondialdehyde or oxidation resistance was found between patients with or without microvascular complications nor with the number of microvascular complications (Table 5). In addition, nephropathy, retinopathy and neuropathy were not associated with malondialdehyde level or the susceptibility of LDL for oxidation. No trend was found showing shorter lag time with increasing severity of nephropathy and neuropathy. These results were essentially the same after adjustment for serum anti-oxidants. Renal function as assessed by creatinine clearance was also not associated with any of the parameters of lipid peroxidation.

DISCUSSION

The results of this study show that nephropathy, neuropathy, retinopathy, as well as microvascular complications in general, were not associated with the susceptibility of LDL for oxidation or malondialdehyde concentrations. There was also no association with duration of diabetes or HbA1c. In contrast, glycosylated apolipoprotein B, was related to oxidation resistance. Most serum lipids were weakly correlated with malondialehyde concentrations. LDL-oxidation resistance was weakly correlated with serum triglyceride concentrations

as well as with the triglyceride and protein fraction of LDL-cholesterol.
The laboratory assays used in our study are according to international standards and applied to an unselected group of diabetic patients and control subjects. Although most blood samples were obtained in the non-fasting state, adjusting for the fasting condition or limiting the analysis to subjects with a fasting blood sample revealed the same associations.

Most of the results described in this article are in agreement with the results of other studies performed in diabetic patients, both for malondialdehyde[29-31] as well as for susceptibility of LDL oxidation[4,7,32-34], also not showing an association between lipid peroxidation parameters and diabetic complications. The different results as reported for malondialdehyde by other studies[35-37] regarding its relationship with HbA1c or the presence of albuminuria may be related to the small sample size in most of these studies (n<70), together with the fact that in some studies a combination of IDDM and NIDDM were compared with healthy controls. Also methodological differences of the oxidation procedure might play a role.

As reviewed by Guillano[3] there are many pathways by which hyperglycaemia could lead to an increased formation of free radicals. Proposed mechanisms include an increased influx of substrates through the polyol pathway, protein glycation products[38] and glucose itself by auto-oxidation[39,40]. The absence of an association in our study between the used parameters of lipid peroxidation and HbA1c, is in accordance with the findings of smaller studies[4,5,15]. In contrast, glycosylated apolipoprotein B, was found to be positively related with oxidation resistance, rather indicating that glycosylation protects LDL from in vitro oxidation. This finding was also reported in NIDDM patients[7] and might be explained by the fact that, free glucose molecules have besides the previously stated pro-oxidant properties, anti-oxidant properties by acting as a free radical scavenger[41]. It therefore likely that glucose or further glucose-metabolites covalently bound to the lipoprotein particles might also act as a free radical scavenger.

Although the pathophysiology of microvascular complications is probably multifactorial; results of in vitro[42], animal[43,44], and human studies[45] suggest that oxidative stress may be involved in their etiology. However, LDL-oxidation and malondialdehyde concentrations were not associated with the presence of microvascular complications.

Considering the limitations of a cross-sectional study, therapy directed against the progression of these microvascular complications may be an explanation for the absence of these relations. For example certain ACE-inhibitors have been shown to exhibit anti-oxidant properties. In addition patients with microvascular complications may be more likely to change their life style to a more 'healthier' way of living, e.g. using vitamin supplements. Although we can not exclude this effects, we found no difference in lipid peroxidation parameters comparing the patients with micro- or macro-albuminuria, using ACE-inhibitors and those without medication. The reported use of anti-oxidants supplements was found to be equally distributed across the different subgroups (data not shown). In addition, no difference was found for neuropathy, a complication most patients are unaware of its presence.

Given our results one could reject the hypothesis, that oxidative stress, induced by hyperglycaemia, could be the final common pathway in the development of microangiopathy. However, as reviewed by Guillano[3], other findings strongly support the role of this mechanism in the development of microvascular as well as macrovascular complications. Therefore it is more likely that the assays used in this study are not reliable estimates of oxidative stress in IDDM patients. This view is supported by the fact that in smokers oxidative stress was also not found to be increased, while there is strong evidence that smoking is associated with an increased oxidative modification of biological molecules in vivo[4-6].

In conclusion, the hypothesis that oxidative stress is the common pathway by which hyperglycaemia causes microvascular complications is not supported by the results of this cross-sectional study, using malondialdehyde-levels and the susceptibility of LDL to in vitro oxidation as parameters of oxidative stress. We could not disclose a direct relationship between metabolic control and oxidative stress, assessed by malondialdehyde concentration and susceptibility of LDL to in vitro oxidation. In addition, these two parameters appear not to differ between patients with and without microvascular complications.

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6

Health status in patients with insulin-dependent diabetes mellitus; relationship with metabolic control

SUMMARY

Given the importance of good glycaemic control in the prevention of diabetic complications, and the enormous interest in health status (HS), as one of the outcomes in studying effectiveness of medical care, the goal of this study was to examine the relationship between metabolic control and HS in patients with insulin-dependent diabetes mellitus (IDDM). The association between metabolic control (glycosylated haemoglobin, glycosylated apolipoprotein B and mean glucose values), diabetic (hypoglycaemic and hyperglycaemic) complaints and HS (Short Form 36 and Euroqol) were therefore studied in a group of 275 IDDM patients.

In general, metabolic control, was not or only weakly related with HS. In contrast, diabetic complaints (both hypo- and hyperglycaemic), showed a strong association with most of the dimensions of the SF-36 and Euroqol, with a highly negative impact on health status.

The results of this study indicate that attention should be paid to the different types of outcome of care in evaluation of the effects of care, in any case to clinical parameters and health status. Given the strong negative impact of diabetic complaints on health status, achieving strict metabolic control for the prevention of diabetic complications should not be the only goal in diabetes therapy. A daily life without hyperglycaemic complaints or hypoglycaemic events should be an equally important objective.

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Health status in patients with insulin-dependent diabetes mellitus; relationship with metabolic control

INTRODUCTION

Health status (HS) has become an important concept in studying the effect of care[1]. Because health status represents what a patient perceives as one of the most important outcomes of medical care, HS is increasingly regarded as a relevant adjuvant to traditional biological and clinical parameters of outcome such as metabolic control.

The importance of strict metabolic control for the prevention as well as the progression of long-term diabetic complications has been shown for patients with insulin-dependent (type 1) diabetes mellitus[2]. Because of this increased emphasis on better metabolic control, the burden on personal welfare is growing. Insulin therapy is commonly intensified by an increased frequency of injections or by administrating insulin by pump therapy with the obligation to increase the frequency of self-monitoring of blood glucose levels and an increased risk for hypoglycaemic events. The possible effects on health status to achieve this objective are mostly neglected or considered as less important.

Previous studies describing the relationship between metabolic control and health status have reported conflicting results[3-8]. In most of these studies this relationship was investigated in populations restricted to NIDDM patients[3-5] or consisting of both NIDDM and IDDM patients[7,8]. Although it seems reasonable to believe that the direct effect of metabolic control on health status will not be different for NIDDM and IDDM patients, differences in treatment regime as well as other parameters related to metabolic control, could be modifiers of these associations. Also in most of these studies not all aspects of metabolic control were assessed. In particular the presence of diabetic complaints, as parameter of metabolic control was left out of consideration.

We conducted a study to investigate the relationship between metabolic control, diabetic complaints and health status-variables in well defined group of patients with insulin-dependent diabetes mellitus .

PATIENTS AND METHODS

Patients

The present study was conducted at the outpatient clinic of the 'De Weezenlanden' hospital in Zwolle, a middle-sized town in the eastern part of the Netherlands. From January 1995 to January 1996, 293 consecutive patients with IDDM were invited to participate in the study. IDDM was defined as the start of insulin replacement therapy before the age of 30 years, or the absence of C-peptide secretion. Twelve patients refused to participate in the study. All patients applied self-control by blood glucose monitoring and administered their insulin by insulin-pen or pump (continuous subcutaneous insulin infusion (CSII)). The study protocol was approved by the hospital scientific and ethical committees and all patients gave their informed consent.

Study protocol

Patients were examined according to a standardized protocol by one trained physician. The schemes of insulin administration (2-3 or 4 times daily injections, and CSII) as well as co-morbidity were recorded. Co-morbidity consisted of the following diseases: chronic bronchitis, other lung diseases, serious back problems, rheumatism or articular complaints, cancer, diseases of the nervous system, and diseases of the thyroid gland. Metabolic control was assessed by haemoglobin A1c (HbA1c) and glycosylated apolipoprotein B. HbA1c was measured by affinity chromatography (Pierce columns, Glyco test II) (upper limit of normal 6.0%)[9]. Glycosylated apolipoprotein B, as percentage of total apolipoprotein B was determined according to Panteghini[10]. In all patients metabolic control was measured at the same day the health status questionnaires were filled out by the patients themselves.

Mean blood glucose values from day-curves, as measured by the patients at home were used as an additional parameter of metabolic control. For each of the three months preceding the outpatient visit the first reported day-curve of six or more measurements was recorded. This was done to prevent confounding by indication, because patients are normally instructed to perform a complete day-curve (more than six measurements per day) at least every two weeks or more often when necessary. From these day-curves mean glucose values were calculated for a period of one and three month preceding the outpatient visit.
In addition patients recorded all hypoglycaemic events and hyperglycaemic complaints (thirst, polyuria, polyphagia, fatigue, pruritus), during the preceding

three months. Hypoglycaemia was defined as an insulin reaction, which required immediate food intake by the patient himself or the assistance from a relative or physician, who injected glucose intravenously or glucagon intramuscularly, or for which hospital admission was necessary. In the latter three instances the hypoglycaemic event was graded as severe.
The presence of microvascular complications was defined as having nephropathy (albuminuria of more than 30 mg/ 24 hours or the use of an ACE-inhibitor), retinopathy (background or pre-/ proliferative assessed by fundoscopy in mydriasis classified according to national standards) or neuropathy (sensibility and vibration threshold testing: one or more abnormal test result) as described previously in more detail[Chapter 2].

Health status

Health status was measured by the SF-36 and the Euroqol, two generic instruments for quality of life assessment. The SF-36, the short-form version of the health survey used in the Medical Outcome Study[11,12] has been validated for the Dutch situation[13,14] as has the Euroqol, an instrument developed by the international Euroqol group[15]. The SF-36 encompasses eight dimensions of health: physical functioning, social functioning, functional role impairment (role functional), emotional role impairment (role emotional), mental health and general health perception, and one item measuring health change: health transition. For each of these dimensions, item scores were coded, summed and transformed to a scale from 0 (worst health) to 100 (best health)[11,16]. In addition to the scores for the eight dimensions of the SF-36 a physical and a mental component summary scale were calculated as described by Ware and Jenkinson using US-based population norms[17,18]. The Euroqol contains two dimensions: the thermometer and the weighted Euroqol score.
A supplement of the questionnaire contained questions regarding socio-economic characteristics (education and type of health insurance) of the patients. Education was graded by the highest finished schooling according to the Dutch educational system. High education was defined as 'Hoger Algemeen Voortgezet Onderwijs' or better. Patients were also classified by the kind of medical insurance (compulsory or private) as another parameter for socio-economic status. The income level when compulsory insurance has to be changed to private insurance is determined in the Netherlands at approximately 48.000 guilders (about 25.000 US dollars) before taxes.

Data analysis

The chi-square test was used to compare frequencies. Statistical differences between groups were calculated by ANOVA using Duncan's multiple range test (which corrects for multiple testing) as post hoc test. Multiple linear regression analyses were used to analyse the associations between continuous variables and to adjust for possible confounders.

The dimensions 'role physical' and 'role emotional' of the SF-36 were treated as a continuous variables, although they consist of only five respectively four outcome levels. This is usually done in studies with a large number of participants, for reasons of easiness of presentation and interpretation of differences. Because no change in health status was studied, health transition, the only single item scale of the SF-36 was not included in the analysis. Two sided P-values ≤ 0.05 were considered to be statistically significant. Analyses were performed using SPSS 6.1.3 for Windows.

RESULTS

Two hundred and seventy-five of the 281 patients completed the health status questionnaires (a response rate of more than 97 %). Table 1 summarizes the demographic and clinical characteristics of these patients. Metabolic control, as assessed by HbA1c and glycosylated apolipoprotein B did not significantly differ between men and women (8.07 % (SD 1.99) vs 8.49 % (1.77); 3.09 % (0.88) vs. 2.99% (0.92), respectively), although there appeared to be a tendency for worse metabolic control in females. Only glycosylated apolipoprotein B was associated with age (regression coefficient 0.01 per cent per year (SEM 0.01) $p \leq 0.05$ after adjustment for gender). No difference could be found regarding the various schemes of insulin administration (2-3, 4 times daily, or CSII). Duration of diabetes was also not related with any of the parameters of metabolic control. During the three months preceding the out-patient visit, 224 patients (81.5%) had hypoglycaemic events, of them 62 (27.7%) more than nine times. One or more severe hypoglycaemic events were reported by 48 patients (17.5%). One hundred and fifty patients (54.5%) suffered from one or more hyperglycaemic complaints. More women compared to men experienced hyperglycaemic complaints and hypoglycaemic events (83 (65.9%) vs 67 (45.0%) and 109 (86.5%) vs. 115 (77.2%), respectively, both $p \leq 0.05$). The occurrence of severe hypoglycaemic events did not differ between the sexes. Metabolic control, age

and duration of disease were not significantly associated with the presence of hypoglycaemic events or hyperglycaemic complaints.

Health status and metabolic control

The mean physical and mental component score of the SF-36 were 52 (SD 8)

Table 1. Demographic and clinical characteristics

Number of patients	275	
Gender (male)	149	(54.2%)
Age (years)	38.3	(12.5)
Duration of disease (years)	17.2	(10.8)
Smokers	89	(32.4%)
Alcohol users	167	(60.7%)
Level of metabolic control		
- HbA1c (%)	8.27	(1.90)
- glycosylated apolipoprotein B (%)	3.05	(0.90)
- mean glucose values 1 month (mmol/l)	7.27	(1.67)
- mean glucose values 3 month (mmol/l)	7.36	(2.20)
Socio-economic position		
- level of education (high)	90	(32.0%)
- health insurance (compulsory)	186	(66.2%)
Co-morbidity (one or more chronical medical conditions)	177	(63.0%)
Microvascular complications (one or more)	182	(66.2%)
- nephropathy	57	(20.3%)
- neuropathy	141	(51.3%)
- retinopathy	89	(32.4%)

Values are number of patients or means with percentage or standard deviations between parentheses.

and 51 (10), respectively. The scores for the Euroqol were 81 (15) for the thermometer and 90 (14) points for the weighted Euroqol score (Table 2). Metabolic control, as assessed by HbA1c was only weakly associated with the SF-36 dimensions; physical functioning, general health and the thermometer of the Euroqol (Table 2 en 3). After further adjustment for co-morbidity, type of insulin therapy, socio-economic position and duration of disease these associations lost their statistical significance. Except for the mean (3 month)

Table 2. Health status in IDDM patients specified by quartiles of HbA1c

	Total group	Quartiles of HbA1c			
		lowest (<6.9%)	second (6.9-8.2%)	third (8.2-9.3%)	highest (>9.3%)
SF-36					
Physical summary score	52 (8)	53 (6)	52 (8)	53 (8)	51 (9)
Mental summary score	51 (10)	52 (9)	52 (9)	50 (11)	49 (12)
Physical Functioning	90 (15)	94 (9)*#	88 (20)*	92 (11)	88 (16)#
Role Physical	83 (32)	83 (31)	88 (28)	82 (32)	77 (39)
Bodily Pain	87 (20)	88 (17)	87 (19)	88 (20)	84 (22)
General Health	70 (20)	70 (19)	73 (19)	70 (20)	65 (23)
Vitality	67 (20)	69 (19)	70 (19)	66 (20)	66 (23)
Social Functioning	87 (20)	90 (16)	87 (18)	86 (22)	84 (24)
Role Emotional	84 (32)	87 (29)	87 (29)	83 (33)	78 (36)
Mental Health	77 (17)	78 (16)	78 (17)	76 (16)	74 (20)
Euroqol					
Thermometer	81 (15)	81 (13)	82 (17)	81 (15)	79 (17)
Weighted Score	90 (14)	92(12)	90 (16)	90 (14)	87 (16)

Values are mean scores with standard deviations between parentheses.
[*] difference between lowest quartile and second quartile of HbA1c: $p \leq 0.05$
[#] difference between lowest quartile and highest quartile of HbA1c: $p \leq 0.05$

glucose values, which was related to weighted Euroqol score, the other parameters of metabolic control were not associated with health status. On the other hand, all dimensions of both SF-36 and Euroqol were inversely related to the number of hyperglycaemic complaints (Table 4). Also the presence of hypoglycaemic events in the past three months, in particular the occurrence of more than nine events, was strongly associated with most of the dimensions of the Euroqol and SF-36. Further adjustment for duration of diabetes, co-

Table 3. Health status and parameters of metabolic control

	HbA1c[1]	Glycosylated apolipoprotein B[2]	Mean glucose values 1 month[3]	3 month[4]
SF-36				
Physical summary score	-0.3 (0.2)	-0.2 (0.5)	-0.6 (0.5)	-0.4 (0.3)
Mental summary score	-0.4 (0.3)	-0.5 (0.7)	0 (0.7)	-0.5 (0.5)
Physical Functioning	-0.9 (0.4)*	-0.6 (1.0)	-1.2 (1.0)	-1.3 (0.7)
Role Physical	-0.9 (1.0)	-0.2 (2.2)	-0.1 (2.3)	-1.0 (1.6)
Bodily Pain	-0.6 (0.6)	-0.8 (1.3)	-0.4 (1.4)	-1.4 (0.9)
General Health	-1.3 (0.6)*	-0.5 (1.4)	-1.8 (1.3)	-1.2 (1.0)
Vitality	-0.8 (0.6)	-1.4 (1.4)	-1.4 (1.4)	-1.1 (1.0)
Social Functioning	-0.9 (0.6)	-1.1 (1.3)	-0.8 (1.5)	-1.6 (1.0)
Role Emotional	-1.3 (1.0)	-1.2 (2.2)	0.9 (2.4)	-1.1 (1.6)
Mental Health	-0.8 (0.5)	-0.6 (1.1)	0 (1.3)	-0.9 (0.9)
Euroqol				
Thermometer	-1.1 (0.5)*	0.6 (1.0)	-0.4 (1.0)	-0.8 (0.7)
Weighted Score	-0.7 (0.4)	-0.9 (0.9)	-1.4 (1.0)	-1.7 (0.7)*

Values are mean changes (estimated by linear regression coefficients) with standard errors between parentheses after adjustment for age and gender.
1 change per percent of HbA1c.
2 change per percent of glycosylated apolipoprotein B.
3 change per mmol/l glucose per 1 month period.
4 change per mmol/l glucose per 3 month period.
*= $p \leq 0.05$

Table 4. Health status and diabetes symptoms

	Hyperglycaemic complaints[1] A	Hypoglycaemic events[1] B	Severe hypoglycaemic events[1] C	Hyperglycaemic complaints[2] A	Hypoglycaemic events[2] B
SF-36					
Physical summary score	-1.8 (0.3)***	-2.4 (1.0)*	-1.7 (1.2)	-1.7 (0.3)***	-1.2 (1.0)
Mental summary score	-3.3 (0.4)***	-4.2 (1.4)**	0.6 (1.6)	-3.4 (0.4)***	-3.1 (1.3)*
Physical Functioning	-3.1 (0.6)***	-2.6 (2.0)	1.4 (2.2)	-3.4 (0.6)***	-0.7 (2.0)
Role Physical	-8.9 (1.4)***	-15.2 (4.6)**	-9.0 (5.3)	-8.9 (1.4)***	-10.3 (4.3)*
Bodily Pain	-4.5 (0.8)***	-3.7 (2.8)	-8.2 (3.2)*	-4.3 (0.8)***	-2.0 (2.7)
General Health	-5.8 (0.8)***	-9.2 (2.8)**	1.5 (3.2)	-5.7 (0.8)***	-5.7 (2.7)*
Vitality	-7.7 (0.8)***	-9.7 (2.8)***	0.4 (3.3)	-7.8 (0.8)***	-7.1 (2.4)**
Social Functioning	-6.5 (0.8)***	-7.9 (2.8)**	-1.6 (3.2)	-6.7 (0.8)***	-5.1 (2.5)*
Role Emotional	-9.5 (1.3)***	-9.4 (4.6)*	-1.9 (5.2)	-9.8 (1.3)***	-5.0 (4.2)
Mental Health	-4.6 (0.7)***	-7.2 (2.3)**	1.7 (2.7)	-4.9 (0.7)***	-6.1 (2.2)**
Euroqol					
Thermometer	-5.4 (0.6)***	-9.6 (2.1)***	0 (2.5)	-5.2 (0.6)***	-6.9 (1.9)***
Weighted Score	-4.5 (0.6)***	-7.0 (2.0)***	-1.6 (2.3)	-4.5 (0.6)***	-4.9 (1.9)**

[1] Values are mean changes (estimated by linear regression coefficients) with standard errors between parentheses after adjustment for age and gender.
[2] Values are mean changes (estimated by linear regression coefficients) with standard errors between parentheses after adjustment for respectively hypoglycaemic events and hyperglycaemic complaints.
A mean change per diabetic complaint.
B mean change for more than nine hypoglycaemic events during the last 3 month: present vs absent.
C mean change for severe hypoglycaemic events during the last 3 month in patients with hypoglycaemic events (n=224): present vs absent.
*= $p \leq 0.05$, **= $p \leq 0.01$, ***= $p \leq 0.001$

morbidity, socio-economic position (both education or type of insurance), type of insulin therapy, metabolic control or the presence of microvascular complications did not essentially change these relationships. Both hyperglycaemic complaints and hypoglycaemic events were independently from each other associated with most of the dimensions of the Euroqol and SF-36 (Table 4).

DISCUSSION

In this study, the relationship between metabolic control and health status in patients with insulin-dependent diabetes mellitus was examined. As indicators of metabolic control we used the biological parameters (HbA1c, glycosylated apolipoprotein B and self-recorded blood glucose levels) as well as clinically important parameters as hypoglycaemic events and hyperglycaemic complaints. Health status was measured by SF-36 as well as by the Euroqol.
The relationship between the biological parameters of metabolic control and health status was rather weak or absent. Both hypoglycaemic events and hyperglycaemic complaints were accompanied by a decrease of some health status dimensions.

Health status scores of the patients in our study were substantially higher compared to diabetes specific norms of the Medical Outcome Study[12] as well as to the patients of other the studies using the SF-36[4-5,7,19]. One possible explanation for the higher health status scores found in our study may be that the patients in these studies were older and therefore more likely to have severe complications and co-morbidity, two factors suggested to be strongly associated with a diminution of health status[4-20,21]. An other explanation could be found in the socio-economic and cultural differences of the study populations, which are also suggested to be strong determinants of health status[21].

The results concerning the absent or very weak relation between metabolic control and health status in diabetic patients are in agreement with other studies that also used the Short Form 36[3-5,22] as well with as other generic health status measuring instruments[6,23-25]. Studies that found stronger associations mostly used arbitrary cutoff points, instead of a continuous relationships[7,8,19,26-28].

In our study health status was measured by two generic instruments, the Short Form 36 and the Euroqol, because at the time of the study no diabetes specific instrument was validated for the Netherlands. Some health status questionnaires, conditional upon way the dimensions are assessed and the type of dimensions, are suggested to be more sensitive to detect a relationship between metabolic control and health status[8]. This might explain why Bardsley and colleagues found a significant but rather weak relationship (correlation coefficients lower 0.26) between dimensions of the Nottingham Health Profile, the Functional Limitations Profile and metabolic control, while no associations were found with a scale of the Positive Well-being questionnaire[6]. The same phenomenon was described recently by Anderson, who also suggested that a disease-specific questionnaire (in his study the Diabetes Care profile) would be a better instrument to study the relation between health status and metabolic control[1]. Given the absence of an association between metabolic control and health status in studies using the (disease-specific) Diabetes Health Profile (DHP) and the Diabetes Quality of Life (DQol)[19,29], it will at least not be valid for every diabetes specific questionnaire. In the other studies assessing this relationship which also used diabetes specific questionnaires, the association was also found to be weak[30-32], indicating that metabolic control, assessed by biological parameters, is at least from a heath status point of view not very important.

The rather strong relationship between health status scores and the number of hypoglycaemic events confirms the clinical experience that hypoglycaemia influences daily life of the diabetic patient in a substantial way. The same is true for hyperglycaemic complaints. The fact that both hypoglycaemic events and hyperglycaemic complaints are associated with some health dimensions independently of HbA1c, indicates that from a health status point of view it remains important in poor as well as in good regulated patients to optimise metabolic control without inducing hypoglycaemic events or hyperglycaemic complaints.

These findings are of importance for health services researchers as well as for clinicians. In the evaluation of the effect of medical care, attention should be paid to the different types of outcome of medical care, in any case to clinical parameters as well as health status.
Clinicians have to realise that for the patient, his health status is far more important than most clinical parameters are. Nevertheless, their treatment is based mostly on assessment and influencing clinical parameters such as HbA1c

or self reported glucose values. However, in a more patient-centred care, clinicians should also pay attention to the health status of their patients by assessing and trying to improve it by therapeutic modalities tailored towards the individual patient. Although it is considered that health status is partly beyond the control of medical providers, clinicians should make attempts to direct their treatment on this determinants as much as possible. The presence of hyperglycaemic or hypoglycaemic complaint can be considered as a useful indicator of decreased health status in clinical practice in diabetes mellitus.

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7

Health status in patients with insulin-dependent diabetes mellitus; the consequences of therapy

SUMMARY

The importance of strict metabolic control for the prevention as well as the progression of long-term diabetic complications has been proven for patients with insulin-dependent diabetes mellitus. Because of this increased desire for better metabolic control, the burden on personal welfare could also increase. Given the importance of good glycaemic control in the prevention of diabetic complications, and the growing interest in health status (HS) as one of the outcomes in studying effectiveness of medical care, the goal of this study was to examine the relationships between type of insulin replacement therapy, self-control and HS in patients with insulin-dependent diabetes mellitus (IDDM). The association between type of insulin replacement therapy (frequency of injections), self-control (number of day-curves/ measurements) and scores on Short Form 36 and Euroqol were therefore studied in a group of 275 IDDM patients. Metabolic control (HbA1c) and diabetic (hypoglycaemic and hyperglycaemic) complaints, were assessed as possible confounders.

The effect of treatment, as assessed by frequency of self-control was found to be strongly associated with the physical oriented dimensions of the SF-36 and both dimensions of the Euroqol. These associations were independent from metabolic control, diabetic complaints, or type of insulin replacement therapy. The lower dimension scores found for patients treated with continuous subcutaneous insulin infusion compared to patients with conventional treatment (daily 2-3 times injections) or four or more insulin injections per day, could be traced back to the fact that these patients performed self-control more frequently than their counterparts.

From the results of this study it seems important to realise that the frequency of self-control can have a negative impact on percieved health.

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INTRODUCTION

Health status (HS) has become an important concept in studying the effect of care[1]. Because health status represents what a patient perceives as one of the most important outcomes of medical care, HS is increasingly regarded as a relevant adjuvant to traditional biological and clinical parameters of outcome. The importance of strict metabolic control for the prevention as well as the progression of long-term diabetic complications has been proven for patients with insulin-dependent diabetes mellitus[2]. Because of this increased wish for better metabolic control, the burden on personal welfare is growing. Insulin therapy is commonly intensified by an increased frequency of injections or by administrating insulin by pump therapy with the obligation to increase the frequency of self-monitoring of blood glucose levels. The possible effects on health status to achieve this objective are mostly neglected or considered as less important.
We conducted a study to investigate the relationships between type of insulin replacement therapy and the frequency of self-control to health status-variables.

PATIENTS AND METHODS

Patients

The present study was conducted at the outpatient clinic of the 'De Weezenlanden' hospital in Zwolle, a middle-sized town in the eastern part of the Netherlands. From January 1995 to January 1996, 293 consecutive patients with IDDM were invited to participate in the study. IDDM was defined as the start of insulin replacement therapy before the age of 30 years, or the absence of C-peptide secretion. Twelve patients refused to participate in the study. All patients applied self-control by blood glucose monitoring and administered their insulin by insulin-pen or pump (subcutaneous insulin infusion (CSII). The study protocol was approved by the hospital scientific and ethical committees and all patients gave their informed consent.

Study protocol

Patients were examined according to a standardized protocol by one trained physician. The type of insulin replacement therapy (frequency of injections) as well as co-morbidity were recorded. Co-morbidity consisted of the following

diseases: chronic bronchitis, other lung diseases, serious back problems, rheumatism or articular complaints, cancer, diseases of the nervous system, and diseases of the thyroid gland. All blood glucose values from day-curves, as measured by the patient, were collected for the three months preceding the out-patient visit. Since patients of our outpatient department, treated with insulin-pen therapy, are normally instructed to perform a complete day-curve (more than six measurements per day) at least every two weeks or more often when necessary, and patients, administering their insulin by pump therapy (CSII) are advised to perform additional measurements every day before breakfast and before going to bed, a day-curve was therefore defined as two or more blood glucose measurements on the same day. Frequency of self-control was expressed as the number of day-curves or the number of glucose values measured per week.

Metabolic control was assessed by haemoglobin A1c (HbA1c), measured by affinity chromatography (Pierce columns, Glyco test II) (upper limit of normal 6.0%)[3]. Patients also recorded all hypoglycaemic events and hyperglycaemic complaints (thirst, polyuria, polyphagia, fatigue, pruritus), during the last three months. Hypoglycaemia was defined as an insulin reaction, which required immediate food intake by the patient himself or the assistance from a relative or physician, who injected glucose intravenously or glucagon intramuscularly, or for which hospital admission was necessary. In all patients metabolic control was measured at the same day the questionnaires were filled out by the patients themselves.

The presence of microvascular complications was defined as having nephropathy (albuminuria of more than 30 mg/ 24 hours or the use of an ACE-inhibitor), retinopathy (background or pre-/ proliferative assessed by fundoscopy in mydriasis classified according to national standards) or neuropathy (sensibility and vibration threshold testing: one or more abnormal test result) as described previously in more detail[Chapter 2].

Health status was measured by the SF-36 and the Euroqol, two generic instruments for quality of life assessment. The SF-36, the short-form version of the health survey used in the Medical Outcome Study[4,5] has been validated for the Dutch situation[6,7] as has the Euroqol, an instrument developed by the international Euroqol group[8]. The SF-36 encompasses eight dimensions of health: physical functioning, social functioning, functional role impairment

(role functional), emotional role impairment (role emotional), mental health and general health perception, and one item measuring health change: health transition. For each of these dimensions, item scores were coded, summed and transformed to a scale from 0 (worst health) to 100 (best health)[4-9]. In addition to the scores for the eight dimensions of the SF-36 a physical and a mental component summary scale were calculated as described by Ware and Jenkinson using US-based population norms[10,11]. The Euroqol contains two dimensions: the thermometer and the weighted Euroqol score.

A supplement of the questionnaire contained questions regarding socio-economic characteristics (education and type of health insurance) of the patients. Education was graded by the highest finished schooling according to the Dutch educational system. High education was defined as 'Hoger Algemeen Voortgezet Onderwijs' or better.

Patients were also classified by the kind of medical insurance (compulsory or private) as another parameter for socio-economic status. The income level when compulsory insurance has to be changed to private insurance is determined in the Netherlands at approximately 48.000 guilders (about 25.000 US dollars) before taxes.

Data analysis

The chi-square test was used to compare frequencies. Statistical differences between groups were calculated by ANOVA using Duncan's multiple range test (with correction for multiple testing) as post hoc test. Multiple linear regression analyses was used to analyse the associations between continuous variables and to adjusted for possible confounders. The dimensions role physical and role emotional of the SF-36 were treated as a continous variables, although they consist of only five respectively four outcome levels. This is usually done in studies with a large number of participants, for reasons of easiness of presentation and interpretation of differences. Because no change in health status was studied, health transition, the only single item scale of the SF-36 was not included in the analysis. Two sided P-values ≤ 0.05 were considered to be statistically significant. Analyses were performed using SPSS 6.1.3 for Windows.

RESULTS

Health status questionnaires were completed by 275 out of 281 patients (a response rate of more then 97%). The demographic and clinical characteristics of these patients specified by type of insulin replacement therapy are summarized in Table 1. Regarding the various schemes of insulin administration no significant difference could be found for metabolic control, socio-economic status, or co-morbidity. As expected patients administering their insulin by CSII had a significantly higher frequency of self-control. Although there appeared to be a tendency in patient treated by conventional therapy to have more microvascular complications compared to their counterparts, this difference was not statistically significant.

During the three months preceding the outpatient visit 150 (53.4%) patients suffered from one or more hyperglycaemic complaints and 224 patients (79.7%) had hypoglycaemic events, of them 63 (28.1%) more than nine times. More patients administering their insulin by CSII had hyperglycaemic complaints compared to those using an insulin-pen (55 (76.3%) vs 95 (46.8%); $p \leq 0.05$). The three treatment modalities did not differ for the presence of hypoglycaemic events. Patients experiencing hyperglycaemic complaints performed significantly more self-control measurements per week (3.4 day-curves per week (SEM 0.2) vs 2.1 day-curves per week (0.2) and 14.1 measurements per week (1.0) vs. 9.1 measurements per week (1.0)) compared to those not experiencing hyperglycaemic complaints. The same goes for patients having hypoglycaemic events ((3.0 day-curves per week (0.2) vs 2.1 day-curves per week (0.4) and 12.6 measurements per week (0.8) vs. 8.6 measurements per week (1.5)). Metabolic control, as assessed by HbA1c, was associated with both the number of day curves and control measurements (regression coefficients -0.11 % per day curve per week (0.5); -0.03 % per measurement per week (0.1), respectively $p \leq 0.05$ after adjustment for gender age and therapy).

Health status

Average scores for the SF-36 eight major dimensions ranged from a low of 67 (SD 20) for vitality to a high of 90 (SD 15) for physical functioning. The mean physical (PCS) and mental component score (MCS) of the SF-36 were 52 (SD 8) and 51 (10), respectively. The scores for the Euroqol were 81 (15) for the thermometer and 90 (14) points for the weighted Euroqol score.

Metabolic control, as assessed by HbA1c was only weakly associated with the

SF-36 dimensions; physical functioning, general health and the dimension of the Euroqol. In contrast, most of the dimensions of SF-36 and Euroqol were inversely related with the presence hypoglycaemic events as well as with the number of hyperglycaemic complaints (see Chapter 6).

Table 1. Demographic and clinical characteristics specified by type of treatment

	Conventional 2-3 times/day	Intensive treatment 4 times /day	Continuous
Number of patients	33 (12.0%)	170 (61.8%)	72 (26.2%)
Gender (male)	24 (72.7%)*#	99 (58.2%)*‡	26 (36.1%)#‡
Age (years)	46.6 (3.0)*#	36.4 (0.9)*	38.9 (1.1)#
Duration of disease (years)	26.3 (2.4)*#	14.6 (0.7)*‡	19.3 (1.1)#‡
Doses insulin per kg body weight (units per day)	0.71 (0.04)	0.75 (0.02)‡	0.61 (0.02)‡
Metabolic control (HbA1c (%))	8.6 (0.3)	8.2 (0.1)	8.4 (0.2)
Self-control: number of			
- day-curve (per week)	1.8 (0.4)#	2.1 (0.2)‡	5.2 (0.3)#‡
- control measurements (per week)	7.9 (1.7)#	8.6 (0.8)‡	21.6 (1.4)#‡
Socio-economic position			
- level of education (high)	7 (21.2%)	56 (32.9%)	27 (37.5%)
- health insurance (compulsory)	22 (66.7%)	113 (66.5%)	51 (70.8%)
Co-morbidity (one or more chronical medical condition)	20 (60.6%)	106 (62.4%)	51 (70.8%)
Microvascular complications (one or more)	27 (81.8%)	105 (61.8%)	50 (69.4%)

Values are number of patients or means with percentage or standard errors between parentheses.
[*] difference between conventional and 4 injections per day : $p \leq 0.05$
[#] difference between conventional and continuous insulin infusion: $p \leq 0.05$
[‡] difference between 4 injections per day and continuous insulin infusion: $p \leq 0.05$

Health status and therapy

The difference in health status scores between patients with conventional (2 or 3 times injections per day) and intensive insulin replacement therapy (4 or more injection per day or CSII) is summarized in Table 2. No difference in health status was found between patients with conventional and intensive therapy. However patients administering insulin by CSII had significantly lower health status scores compared those using an insulin-pen. These differences were essentially the same after adjustment for age, gender, HbA1c-levels, the presence of microvascular complications, co-morbidity, hypoglycaemic events

Table 2. Health status and insulin replacement therapy

	Conventional[1] 2-3 times/ day	Intensive treatment[1] 4 times /day	CSII	Insulin treatment[2] CSII versus IP
SF-36				
Physical summary score	50 (1)*	53 (0)*‡	50 (1)‡	-1.3 (1.2)
Mental summary score	55 (1)#	51 (1)‡	48 (1)#‡	-2.3 (1.7)
Physical Functioning	87 (3)*	93 (1)*‡	87 (2)‡	-2.4 (2.4)
Role Physical	82 (5)	88 (2)‡	72 (5)‡	-7.0 (5.6)
Bodily Pain	87 (3)*	89 (1)‡	81 (2)‡	-2.6 (3.3)
General Health	70 (4)*	72 (1)‡	63 (3)‡	-5.9 (3.4)
Vitality	70 (3)#	70 (1)‡	61 (3)#‡	-3.9 (3.4)
Social Functioning	89 (3)#	89 (1)‡	81 (3)#‡	-2.4 (3.3)
Role Emotional	96 (2)#	86 (2)‡	74 (4)#‡	-10.1 (5.5)
Mental Health	81 (3)#	78 (1)‡	72 (2)#‡	-3.4 (2.8)
Euroqol				
Thermometer	81 (2)	82 (1)‡	77 (2)‡	-0.5 (2.6)
Weighted Score	90 (3)	92 (1)‡	84 (2)‡	-3.9 (2.4)

1 Values are mean scores with standard errors between parentheses.
[*] difference between conventional and 4 injections per day: $p \leq 0.05$
[#] difference between conventional and continuous insulin infusion: $p \leq 0.05$
[‡] difference between 4 injections per day and continuous insulin infusion: $p \leq 0.05$

2 Values are mean changes (estimated by linear regression coefficient) with standard errors between parentheses after adjustment for age, gender and self-control. Mean change for therapy: continuous insulin therapy (CSII) versus insulin-pen (IP) therapy.

or hyperglycaemic complaints. Further adjustment for the number of blood glucose measurements showed that this difference between regimes of therapy could be attributed completely to the frequency of self-control. Duration of diabetes and total insulin doses were not associated with any of the dimensions of the Euroqol or SF-36.

The associations between the frequency of self-control, as assessed by the number of day-curves or measurements per week are shown in Table 3. Except for the mental component score and the dimensions role emotion and mental health of the SF-36, health status was strongly related with frequency of self-control. These associations were essentially the same after further adjustment for duration of diabetes, co-morbidity, microvascular complications, socio-

Table 3. Health status and self-control

	Number of day-curves[1]	Number of measurements[2]
SF-36		
Physical summary score	-0.7 (0.2)***	-0.2 (0)***
Mental summary score	-0.2 (0.2)	-0.1 (0.1)
Physical Functioning	-0.9 (0.3)**	-0.3 (0.8)**
Role Physical	-2.3 (0.8)**	-0.7 (0.2)***
Bodily Pain	-1.8 (0.4)**	-0.5 (0.1)***
General Health	-1.2 (0.5)**	-0.4 (0.1)***
Vitality	-1.2 (0.5)*	-0.3 (0.1)**
Social Functioning	-1.2 (0.5)**	-0.4 (0.1)***
Role Emotional	-1.2 (0.8)	-0.3 (0.2)
Mental Health	-0.2 (0.4)	-0.1 (0.1)
Euroqol		
Thermometer	-0.7 (0.4)*	-0.3 (0.9)**
Weighted Score	-0.7 (0.3)*	-0.3 (0.1)**

Values are mean changes (estimated by linear regression coefficients) with standard errors between parentheses after adjustment for age and gender.

[1] change per day-curve per week

[2] change per blood glucose measurement per week

* $p \leq 0.05$, ** = $p \leq 0.01$, *** = $p \leq 0.001$

economic position and/ or metabolic control (HbA1c, hyperglycaemic complaints and hypoglycaemic events). In addition, the same results were found when the analysis was limited to patients, treated with insulin-pen therapy.

DISCUSSION

In this investigation, the relationship between type of insulin replacement therapy and self-control to health status in patients with insulin-dependent diabetes mellitus was studied. Metabolic control as assessed by HbA1c, as well as hypoglycaemic events and hyperglycaemic complaints were assessed as possible confounders. Health status was measured by SF-36 as well as by Euroqol.
The effect of treatment as assessed by frequency of self-control was found to be strongly associated with the physical oriented dimensions of the SF-36 and both dimensions of the Euroqol. These associations were independent from metabolic control or diabetic complaints. The lower dimension scores found for patients treated with continuous subcutaneous insulin infusion compared to patients with conventional treatment (daily 2-3 times injections) or four or more insulin injections per day, could be traced back to the fact that these patients performed more frequently self-control than their counterparts.

Health status scores of the patients in our study were substantially higher compared to diabetes specific norms of the Medical Outcome Study[5] as well as to the patients of other the studies using the SF-36[12-15]. One possible explanation for the higher health status scores found in our study may be that the patients in these studies were older and therefore more likely to have severe complications and co-morbidity, both factors suggested to be strongly associated with a diminution of health status[14,16,17]. An other explanation could be found in the socio-economic and cultural differences of the study populations, which are also suggested to be strong determinants of health status[17].

Our findings that health status is strongly related with the frequency of self-control differ from other studies[18,19], which reported no significant association. It has to be mentioned that the absence of a strong correlation between self-control and HS as described in the article by Wredling[19], was only stated and no results were presented. In a recently performed study, assessing the determinants

of health status in 1800 diabetic patients, self-control was positively related with the dimension mental health of the SF-20, indicating better health status with a higher frequency of self-control[17]. In this study self-control was recorded indirectly using items drawn from the Summary of Diabetes Self-care activities questionnaire[17]. In the study performed by Gilden and his colleagues health status was found to be improved by introducing blood and urine glucose measurement in an group of older diabetes patients[18]. Given the fact that education was also part of the intervention this could be an explanation for the increase in health status.

In this study health status was measured by two general instruments, the Short Form 36 and the Euroqol, because at the time of the study no diabetes-specific instrument was validated for the Netherlands. However, when assessed by diabetes-specific questionnaires, being more sensitive towards clinical relevant issues[16] we would aspect to find even stronger association between self-control and health status.

Although the results of this investigation should be interpreted with caution, since it a cross-sectional study, the fact that self-control was associated with health status independently from type of insulin replacement therapy, metabolic control and co-morbidity leaves the suggestion that, an higher frequency of self-control per se could bring about a decrease in health status. In contrast to what we expected, self-control was associated only with the physical component of the SF-36, indicating that there was or is no mental burden from self-control. Even though it is obvious that the measurement of blood glucose values is painful and interfering with a patients daily living, we could not found an explanation why this is not affecting the mental component of the SF-36.

These findings are of importance for health services researchers as well as for clinicians. In the evaluation of the effect of medical care, attention should be paid to the different types of outcome of medical care, in any case to health status. Clinicians have to realise that for the patient, his health status is far more important than clinical parameters are. However medical treatment is based mostly on the assessment and control of clinical parameters such as HbA1c, among other ways by self-control. Clinicians have to be alert that a patients health status can be adversely influenced by the frequency of self-control.

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8

Management of patients with insulin-dependent diabetes mellitus

METABOLIC CONTROL

Insulin-dependent diabetes mellitus is a chronic disease requiring life-long treatment, including life-long drug use. The most important aim of the management of these patients is to prevent symptomatic hyperglycaemia and hypoglycaemia, which are related with morbidity and mortality. However, after the introduction of insulin treatment in 1923, it became apparent that diabetes mellitus is associated with a number of serious disorders, so called microvascular complications, which includes retinopathy, nephropathy, and neuropathy. Also the risk of cardiovascular disease is increased. Therefore, the prevention of diabetic complications has become an important aim of the management of IDDM patients.

Since diabetic complications also develop in patients with other types of diabetes, hyperglycaemia has long been suspected as the cause of these disorders. Indeed, a number of studies has shown an association between glycaemic control and the prevalence and incidence of complications[1-3]. Moreover, several studies have been published suggesting that strict metabolic control has a preventive effect on the development of microvascular complications[4-7]. The confirmation that strict metabolic control, achieved by an intensive insulin treatment regimen, effectively delays the onset and slows the progression of microvascular complications was given by the results of the Diabetes Control and Complications Trial (DCCT)[8-10].

The apparent association between glycaemia and complications, as well as between improved metabolic control and diminished occurrence of complications, has shifted the focus of the management of IDDM patients from the prevention of symptoms to reaching normo-glycaemia.

Still, even under near-normo-glycaemic circumstances, microvascular complications do develop[8,11]. The results in Chapter 2 show that in a population of adequately metabolic controlled patients there is no clear association between the level of HbA1c and the presence of complications. One explanation of this apparent discrepancy might be that HbA1c is not the optimal indicator of the effects of hyperglycaemia in these patients. Other glycosylated proteins such as apolipoprotein B or Advanced Glycosylation End products may be more appropriate measures. In our studies no association was found between glycosylated apolipoprotein B and complications (Chapter 2). However, more

studies on the (clinical) use of these new glycaemic measures are needed before a decision can be made on the clinical usefulness.

Other variables related to metabolic control, especially lipid concentrations or modified lipid particles may better reflect the risk of complications. Although there is a clear relation between dyslipidemia and the development of macrovascular (cardiovascular) complications[12], the association with microvascular complications is less clear (Chapters 2 and 5). Because of the aging of the IDDM population, assessment and treatment of cardiovascular risk factors becomes more important. Therefore, in clinical practice lipid profile should be assessed on an annual basis. This is in agreement with the recommendations by the National Cholesterol Consensus to screen high risk groups yearly[13].

OXIDATIVE STRESS

As discussed above, metabolic control alone does not fully explain the development of diabetic complications. A recently proposed mechanism, which may play a role in the development of microvascular complications, is based on tissue damage resulting from the formation and "action" of free radicals. Under physiological circumstances, these radicals are neutralized by various anti-oxidant systems. When the balance between these defence systems and the formation of free radicals is disturbed, oxidative stress emerges[14].

In diabetic patients, the formation of free radicals can be enhanced by increased glucose concentration through non-enzymatic glycation of protein substrates, auto-oxidative glycation, and activation of protein kinase C[14]. Under hyperglycaemic circumstances, increased activity of the polyol pathway may increase oxidative stress by depletion of the NADPH cell stores (pseudohypoxia)[15]. In addition, diabetes is known to induce changes in the content and activity of cellular anti-oxidant enzymes[16-18]. Therefore, it seems that oxidative stress is increased both by the presence of diabetes, as well as by the degree of metabolic control.

In vivo measurements of free radicals are virtually impossible given their reactivity, short half-life, and very low concentrations. Various methods have been developed to measure oxidative processes indirectly. One of these

proposed indirect indicators of oxidative stress is the level of (serum) anti-oxidants[19].
It is important to realise that the damage of free radicals could be local and that absolute levels of individual circulating anti-oxidants might not be the only factor determining the degree of anti-oxidant defence activity. Anti-oxidants are members of a rather large and actively cooperating family of chemical substances. Synergistic interactions between anti-oxidants are well known and may include anti-oxidant regeneration. As a result, when the levels of some anti-oxidants fall the protective effect of another anti-oxidant may become inadequate despite its apparently normal concentration[14]. As a result, it is of little use to measure single specific anti-oxidants in the management of IDDM patients. This is also suggested by the results in Chapter 3.

It would probably better to measure anti-oxidants as a whole. The use of the so-called total radical-trapping anti-oxidant parameter (TRAP) has recently been proposed to explore the anti-oxidant properties of a plasma sample[20,21]. This TRAP can be measured directly by an fluorescence-based method, but is occasionally calculated by a mathematical formula, including the serum levels of four natural anti-oxidants (protein bound SH groups, uric acid and vitamin E and vitamin C)[21]. Since direct TRAP measurement is a elaborative analysis it might not be used in routine clinical care. Recent studies, using the direct measurement indicated that TRAP activity is decreased in diabetic patients[20,21]. Further research, preferable prospective studies, has to be performed before the TRAP activity might be included in individual patient work-up in clinical practice.

Other possible indirect indicators of oxidative stress are malondialdehyde levels and the susceptibility of LDL to in vitro oxidation[14]. As for the measurement of anti-oxidants, the use of these two measures of lipid peroxidation is limited by the local activities of the free radicals and the interaction with other factors (notably lipid profile and anti-oxidants)[22-25]. In our studies, no relationship could be found between these two measures and the presence of diabetes (Chapter 4), the degree of metabolic control, or the presence of microvascular complications (Chapter 5). Given these results, it is clear that these measures of lipid peroxidation are not useful in clinical practice.

Although there seems currently no role for measures of oxidative stress in the management of IDDM patients, it could still be possible that free radicals

contribute to the development of diabetic complications. LDL-oxidation takes place in the vascular wall[26], and might therefore be related to endothelial activity. Formation of oxidized LDL is not always through metal-ion-dependent oxidation of lipids. For example, myeloperoxidase activity can lead to metal-ion-independent oxidation of lipids and proteins in LDL particles[27-29]. Moreover, phospholipase A2 activity has been suggested to be an alternative pathway to the formation of oxidized LDL[30]. As a consequence, an in vitro test of LDL-oxidation may not properly reflect oxidative stress in the vessels. It might be better to measure oxidized LDL directly in plasma samples. These assays are currently developed.

HEALTH STATUS

Apart from diabetic complications, perceived health is another important result of the care of IDDM patients. It is important to realise that in general, health status is far more important for patients than are clinical parameters, despite their knowledge about the risk of diabetic complications. Adding this dimension to the doctor-patient interaction should help to achieve more treatment goals and more satisfaction for both the physician and -most important- for the patient with insulin-dependent diabetes mellitus.

Given the importance of good glycaemic control in the prevention of diabetic complications, treatment of IDDM patients is mostly based on biochemical parameters such as HbA1c and (self-reported) glucose levels. Side effects of therapeutic measures to reach strict metabolic control are sometimes regarded as unavoidable by treating physicians. The results presented in Chapters 6 and 7 indicate that the most important determinants of health status are hypoglycaemic events and hyperglycaemic complaints, as well as the number of self blood glucose measurements.
Given the importance of health status for the patients, metabolic control should be optimised without inducing hypoglycaemic events or hyperglycaemic complaints. Moreover, physicians should be aware of the impact of the therapy described as well as of the frequency of self-control measurements on the perceived health of the patient, and incorporate this information in the treatment plan. The presence of hyperglycaemic or hypoglycaemic complaints may be considered as useful indicators of decreased health status in clinical practice in diabetes mellitus. Certainly, assessment of subjective complaints

should weigh as much as objective biochemical measurements in the approach to optimise care and health status in diabetic patients.

In conclusion, the presence of microvascular complications was not associated with increased HbA1c concentrations or parameters of oxidative stress. However, given the literature it remains important to improve glycaemic control. However, clinicians have to be alert that a patients' health status can be negativly influenced by intensive therapy and increased frequency of self-control.

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9

Summary

INTRODUCTION

The pathophysiological mechanisms by which hyperglycaemia leads to microvascular complications in diabetes mellitus are as yet poorly understood. Furthermore, even with intensive treatment and strict metabolic control, a number of patients still develops microvascular complications. Many hypotheses have been formulated and investigations performed, with a variable degree of success. Some of these hypotheses, in particular the hypothesis about oxidative stress, have been formulated and studied within the scope of this thesis. In addition, the impact of metabolic control and insulin replacement therapy on health status was investigated.

METABOLIC CONTROL, LIPID PROFILE DISTURBANCES

To assess one possible mechanism, we investigated in a cross-sectional study the association between metabolic control, as assessed by HbA1c, serum lipids, and the presence of microvascular complications (Chapter 2). This association was studied in a group of 281 consecutive patients with IDDM (153 men and 128 women) of the outpatient department of the 'De Weezenlanden Hospital' in Zwolle the Netherlands. IDDM was defined as starting insulin replacement therapy within 6 month after the first sign of diabetes mellitus and before the age of 30 years, or the absence of C-peptide secretion. Patients were generally well controlled (mean HbA1c 8.3% (SD 2.0)).
In these patients with IDDM there was no relationship between HbA1c and the presence of diabetic nephropathy and retinopathy. In contrast to this, HbA1c concentrations were found to be significantly increased in patients with an abnormal vibration threshold. The results also showed that the presence of diabetic neuropathy or retinopathy was not related to serum lipid concentrations. Only for diabetic nephropathy an association was found with triglyceride-concentrations.
Although the results of this a cross-sectional study should be interpreted with caution, the fact that this association was found to be independent from metabolic control leaves the suggestion that increased triglyceride levels might be an additional risk factor in the development of diabetic nephropathy. However, whether the rise in triglyceride levels has only a mere relationship with the actual presence of diabetic nephropathy or with the development of diabetic nephropathy has to be investigated by longitudinal studies.

OXIDATIVE STRESS

In diabetic patients, the formation of free radicals, highly reactive particles, which are also formed in under normo-glycaemic conditions, is suggested to be enhanced. Under normal physiological conditions the cell is protected by several anti-oxidants systems against tissue damage as the results of an overproduction of free radicals.
Increased oxidative stress, defined as a disturbed balance between the formation of free radicals and the anti-oxidant systems, could be a basic mechanism involved in the development of microvascular complications in patients with insulin-dependent diabetes mellitus. Since oxidative stress is not exclusively related to hyperglycaemia, but is also influenced by other factors like dietary factors (the intake of anti-oxidants) or hereditary factors (regulation of enzymatic anti-oxidants), it may also account for the development of microvascular complications in subjects with diabetes mellitus, despite strict metabolic control. In vivo measurements of free radicals is virtually impossible given their reactivity, short half-life, and very low concentrations. Therefore, various methods have been developed to investigate indirectly whether such oxidative processes occur. One of these proposed indirect indicators of oxidative stress is the content of anti-oxidants, indicating the total anti-oxidant status.

ANTI-OXIDANTS IN INSULIN-DEPENDENT DIABETES MELLITUS

In Chapter 3 we studied serum anti-oxidant concentrations, as parameter of oxidative stress, in the same 281 patients with IDDM as described in chapter 2 and in 98 non-diabetic control subjects, as well as the association between anti-oxidants and microvascular complications. These healthy controls (49 men and 49 women), were enrolled from a group of patients who were admitted for minor surgery at the department of anaesthesiology, in the same period as the IDDM patients were recruited. 'Healthy' was defined as 'no current diseases' except the indication for surgery (ASA classification category 1) and not using medication, except oral contraceptives.

In this study some of the anti-oxidants (retinol, transferrin, and uric acid) were indeed found to be decreased in patients with insulin-dependent diabetes mellitus compared to controls. In patients with diabetes, some of these anti-

oxidants (uric acid, alfa- and gamma-tocopherol and transferrin) were also associated with the degree of metabolic control. However, the presence of especially nephropathy, was found to be associated with increased levels of anti-oxidants (retinol, ferritin and uric acid), while no significant associations were found for the presence and severity of retinopathy and neuropathy alone.

These contrasting findings of decreased anti-oxidants levels in diabetes patients compared to control subjects and increased levels in diabetes patients especially with nephropathy may suggest the presence of a counteracting mechanism leading to increased levels of anti-oxidants when diabetic nephropathy develops or is present. To our knowledge there are no other reports on the existence of such a mechanism. Still, in recent studies there is evidence that enzymatic anti-oxidant systems are indeed modulated to meet the biological need imposed by oxidative stress.

MALONDIALDEHYDE AND LDL-OXIDATION

In Chapter 4 we studied the presence of oxidative stress by two other indirect parameters, malondialdehyde levels and the susceptibility of LDL to in vitro oxidation. The latter has recently been proposed as an appropriate measure of the response to in vivo oxidative stress, which is thought to occur within the matrix of the vessel wall close to the endothelium. Malondialdehyde is a degradation product of lipid peroxides, and the most commonly used marker of oxidative stress. When using these parameters, we could not confirm the existence of oxidative stress in patients with insulin-dependent diabetes mellitus when compared to control subjects. The results even indicated that patients with insulin-dependent diabetes mellitus were experiencing less oxidative stress than healthy controls.

As described in Chapter 5, oxidative stress, as measured by the susceptibility of LDL to in vitro oxidation and malondialdehyde concentrations, was not associated with the presence of microvascular complications. Also, no association was found between oxidative stress and the degree of metabolic control. In contrast, the extent of glycosylation of apolipoprotein B was found to be inversely correlated with in vitro oxidation of LDL. This finding could indicate that hyperglycaemia not only might cause oxidative stress, but plays a role in the defence against oxidative stress.

The (bio)chemical studies in this thesis were an attempt to identify a possible common pathophysiological mechanism, by which microvascular complications develop or progress in insulin-dependent diabetes mellitus. Despite the available evidence and suggestions in literature, the data in this thesis do not support either a different degree of oxidative stress in diabetic versus non-diabetic subjects, nor a definite influence of the degree of metabolic control (at least when assessed by HbA1c) on oxidative-anti-oxidative state. This can mean, that we did not study the 'appropriate' population with diabetes mellitus: studies in people with non-insulin dependent diabetes mellitus might yield different results. It is also possible, that the parameters employed are incorrect, and other methods to assess the true extent of oxidative stress should be used. As for the presented results, however, it only can be concluded, that the initial hypothesis regarding the influence of oxidative stress on the development and progression of diabetic complications is not supported by the results obtained.

HEALTH STATUS

Insulin-dependent diabetes mellitus is a chronic disease which can affect many aspects of everyday life. To maintain satisfactory blood glucose level in order to avoid hyperglycaemic complaints and hypoglycaemic events, food intake, exercise and insulin doses must be balanced. In addition, many patients are continuously aware of the risks of developing one or more microvascular complications. This ever-present responsibility for daily balance and worries about future health and well-being may represent an extra burden in the daily life of patients with insulin-dependent diabetes mellitus. Despite the excellent results in the DCCT, the main side effects of strict metabolic control from the patients side of view are considerable weight gain and a threefold increased risk of serious hypoglycaemia. To many patients, these side effects may seem an unacceptable price for strict metabolic control, because of both physical and emotional consequences. Furthermore, stricter metabolic control may require more intensive therapy, such as multiple daily doses of insulin (by manual injection or by insulin pump) and frequent measurements of blood glucose values. The latter regimen may as such already have negative effects on a patients health status.

In the second part of this thesis we presented the results of a study, which had as objective to investigate the possible association between metabolic control,

insulin replacement therapy and their consequences on health status. In general, metabolic control, as assessed by HbA1c, glycosylated apolipoprotein B, and mean self-reported glucose values, was not or only weakly related with health status. In contrast, diabetic complaints (both hypo- and hyperglycaemic) showed a strong association with most of the dimensions of the SF-36 and Euroqol, with a highly negative impact on health status (Chapter 6).

Assessing the effect of treatment on health status (Chapter 7), frequency of self-control was found to be strongly associated with the physical oriented dimensions of the SF-36 and both dimensions of the Euroqol. These associations were independent from metabolic control or diabetic complaints. The lower dimension scores found for patients treated with continuous subcutaneous insulin infusion (CSII) compared to patients with conventional treatment (daily 2-3 times injections) or four or more insulin injections per day, could be explained by the fact that these patients performed self-control more frequently. This finding confirms that treatment may indeed have its own negative effects on a patients health status.

In chapter 8 we discussed the consequences of oxidative stress and health status on the management of patients with IDDM. At this time there is no reason for measuring individual serum anti-oxidants or parameters of lipid peroxidation in clinical practice.
However, with regards to health status, physicians should be aware of the impact of intensive insulin therapy, as well as of the frequency in self-control measurements on the perceived health of the patient, and incorporate this information in the treatment plan. The presence of hyperglycaemic or hypoglycaemic complaints might be considered as an useful indicator of decreased health status in clinical practice in diabetes mellitus.

Samenvatting

INLEIDING

De pathofysiologische mechanismen waardoor hoge bloedglucosewaarden leiden tot het ontstaan en het voortschrijden van microvasculaire complicaties (diabetische nierschade, oogschade en schade aan het zenuwstelsel) zijn tot op dit moment nauwelijks bekend. Duidelijk is, dat centraal hierbij de verhoogde bloedglucosewaarden staan. Daarnaast ontstaan er echter bij een aantal patiënten microvasculaire complicaties ondanks intensieve behandeling en nauwgezette metabole regulatie (zeer lage HbA1c waarden). Om dit te kunnen verklaren zijn vele veronderstellingen onderzocht, echter geen enkele gaf een eenduidig antwoord. Een aantal van deze hypotheses, in het bijzonder met betrekking tot het vetspectrum en oxidatieve stress, zijn bestudeerd in het kader van dit proefschrift. Tevens werd er onderzoek verricht naar de gevolgen van het zo goed mogelijk regelen van de bloedglucosewaarden voor de gezondheidsbeleving van patiënten met insuline-afhankelijke diabetes mellitus (IADM).

METABOLE CONTROLE EN AFWIJKINGEN VAN HET LIPIDEN PROFIEL

Een van de veronderstellingen, zoals boven aangeduid, zou kunnen zijn dat afwijkingen van het vetspectrum verklaren waarom er toch complicaties ontstaan bij deze goed gereguleerde mensen met diabetes mellitus. Deze veronderstelling is onderzocht door te kijken of er een verband bestond tussen metabole controle, gemeten via het HbA1c, serum lipiden en het al dan niet bestaan van microvasculaire complicaties. Dit verband werd onderzocht in een groep van 281 patiënten met IADM (153 mannen en 128 vrouwen) die werden behandeld op de diabetes polikliniek van het Ziekenhuis 'De Weezenlanden' in Zwolle. Mensen werden beschouwd insuline-afhankelijke diabetes mellitus te hebben wanneer vóór het dertigste levens jaar en binnen zes maanden na de eerste diabetes klachten de noodzaak bestond om met insuline therapie te starten, of wanneer er geen C-peptide secretie meer in het bloed kon worden aangetoond (een bewijs dat de eigen alvleesklier geen insuline meer maakt).
Over het algemeen waren de IADM patiënten redelijk gereguleerd (gemiddeld HbA1c 8.3%).

Er kon geen verband worden aangetoond tussen het HbA1c en de aanwezigheid van diabetische nefropathie (nierschade) en retinopathie (oogschade). Wel waren de HbA1c waarden verhoogd bij patiënten met tekenen van neuropathie, met name bij het afwijkend zijn van gevoelssensaties voor trillingen. De resultaten van dit onderzoek toonden ook aan dat de aanwezigheid van diabetische neuropathie (schade aan het zenuwstelsel) of retinopathie niet in verband kon worden gebracht met de hoogte van serum lipiden concentraties. Alleen voor nefropathie kon er een significant verband worden aangetoond met de triglyceride concentraties.

Hoewel de resultaten uit een transversaal onderzoek (d.w.z. eenmalige meting in de tijd) altijd met de nodige voorzichtigheid dienen te worden geïnterpreteerd, blijft de suggestie bestaan, dat verhoogde triglyceriden concentraties een extra risico factor zouden kunnen zijn voor het ontstaan van diabetische nefropathie. Of echter een toename van triglyceriden concentraties inderdaad het ontstaan van nierschade kan bevorderen, kan alleen worden uitgezocht door mensen langere tijd te vervolgen.

OXIDATIEVE STRESS

Vrije radicalen zijn chemisch zeer reactieve deeltjes, die ook onder normale omstandigheden (b.v bij lage bloedglucosewaarden) worden gevormd. Bij patiënten met diabetes zou de vorming van vrije radicalen, zijn toegenomen. Onder normale omstandigheden wordt het lichaam beschermd tegen deze vrije radicalen door verscheidene anti-oxidant systemen. Dit systeem van chemische stoffen biedt bescherming tegen het optreden van weefselschade, welke zou kunnen ontstaan door een overmatige vorming van deze vrije radicalen, door ze te inactiveren of het ontstaan ervan te voorkómen.
Toegenomen oxidative stress, gedefinieerd als een verstoring van de balans tussen de vorming van vrije radicalen en de bescherming door deze anti-oxidant systemen, zou een verklaring kunnen zijn waardoor microvasculaire complicaties ontstaan bij patiënten met insuline-afhankelijke diabetes mellitus. Aangezien oxidatieve stress niet uitsluitend behoeft te worden veroorzaakt door hyperglycaemie (hoge bloedglucose waarden), maar ook door andere factoren

zoals dieetfactoren (inname van anti-oxidanten in de voeding) of erfelijke factoren (b.v de regulatie van enzymatische anti-oxidanten) wordt bepaald, zou deze stress ook het ontstaan van complicaties kunnen verklaren bij die diabetes patiënten, bij wie de bloedglucosewaarden zeer goed gereguleerd zijn.
Aangezien het meten van vrije radicalen in een levend organisme onmogelijk is, zijn er verscheidene methoden ontwikkeld om op een indirecte manier aan te tonen dat deze oxidatieve processen plaatsvinden. Eén van deze indirecte indicatoren voor de mate van oxidatieve stress is het meten van serum anti-oxidanten.

Anti-oxidanten bij patiënten met IADM

In Hoofdstuk 3 onderzochten we de serum anti-oxidant concentraties als maat voor oxidatieve stress, bij 281 mensen met IADM (dezelfde groep diabetes patiënten als beschreven in hoofdstuk 2) en in 98 niet-diabetes (controle) patiënten. Tevens onderzochten we het verband tussen anti-oxidanten bloedspiegels en het hebben van microvasculaire complicaties. De controle groep van mensen zonder diabetes (49 mannen en 49 vrouwen) werd willekeurig gekozen uit de groep patiënten, die de afdeling anesthesiologie bezochten voor een pre-operatief consult in verband met een kleine chirurgische ingreep. Als gezond werden beschouwd die personen, die geen andere ziekte of aandoening hadden dan die waarvoor zij de ingreep moesten ondergaan (ASA classificatie categorie 1) en die (behalve orale anti-conceptiva) geen andere medicatie gebruikten.

Uit dit onderzoek bleek dat de concentraties van sommige anti-oxidanten (retinol, transferrine en urinezuur) zijn verlaagd bij mensen met IADM, wanneer zij werden vergeleken met de gezonde controle personen. Binnen de groep diabetes patiënten bleken de concentraties van sommige anti-oxidanten (urinezuur, alfa- en gamma-tocopherol en transferrine) gerelateerd te zijn aan de mate van metabole regulatie. Verder bleek alleen de aanwezigheid van diabetische nefropathie geassocieerd te zijn met verhoogde concentraties van sommige anti-oxidanten (retinol, ferritine en urinezuur), terwijl er geen aantoonbaar verband bestond tussen concentraties van anti-oxidanten en de ernst en/of het hebben van retinopathie en neuropathie.

Deze tegenstrijdige bevindingen met enerzijds afgenomen anti-oxidant concentraties bij mensen met diabetes en anderzijds verhoogde concentraties bij diabetische nefropathie, kunnen wijzen op een mechanisme, waarbij

concentraties van anti-oxidanten toenemen, wanneer zich nierschade ontwikkelt of reeds bestaat. Voor zover wij weten is het bestaan van zo'n regulatie mechanisme voor anti-oxidant systemen in het bloed nooit aangetoond. In recente onderzoeken bestaan er echter wel aanwijzingen, dat enzymatische anti-oxidant systemen in de cellen zelf wel kunnen worden aangepast om weerstand tegen oxidatieve stress te bieden.

Malondialdehyde en LDL oxideerbaarheid

In Hoofdstuk 4 wordt het bestaan van oxidatieve stress verder onderzocht met behulp van twee andere indirecte parameters: malondialdehyde concentraties en de gevoeligheid van LDL-cholesterol deeltjes voor oxidatie buiten het lichaam. Van deze laatste parameter werd recent gesuggereerd dat het een geschikte maat zou zijn voor de reactie van het lichaam op oxidatieve stress, welke optreedt dicht bij de endotheelcellen (cellen die de wand van de bloedvaten bekleden). Malondialdehyde, de meest frequent gebruikte mate voor oxidatieve stress, is een afbraakprodukt van lipidenoxidatie (de chemische reactie van vetten met zuurstof).

Gebruik makend van deze parameters konden wij het bestaan van verhoogde oxidatieve stress bij mensen met diabetes ten opzichte van de controle groep niet bevestigen. De resultaten van deze onderzoeken gaven aan dat mensen met diabetes zelfs minder oxidatieve stress zouden hebben dan mensen zonder diabetes.

Zoals beschreven in Hoofdstuk 5 bestaat er ook geen verband tussen oxidatieve stress, gemeten middels LDL-oxidatie en malondialdehyde concentraties en het bestaan van microvasculaire complicaties. Verder bestaat er geen verband tussen oxidatieve stress en de mate van metabole controle.

Het biochemisch onderzoek in dit proefschrift vormde een poging een mogelijk mechanisme te ontdekken, waardoor microvasculaire complicaties ontstaan en voortschrijden bij mensen met insuline-afhankelijke diabetes mellitus. De bevindingen van dit proefschrift ondersteunen noch het bestaan van een verschil in oxidatieve stress tussen diabeten en niet diabeten, noch het bestaan van een duidelijke invloed van de mate van metabole controle (althans gemeten middels HbA1c) op de mate van oxidatieve stress. Dit kan komen omdat we deze verbanden niet bij de juiste patiënten groep hebben bestudeerd; mensen met niet-insuline-afhankelijke diabetes mellitus zouden andere

onderzoeksuitkomsten kunnen laten zien. Het is ook mogelijk, dat de gebruikte parameters hiervoor niet geschikt zijn en dat andere chemische methoden om oxidatieve stress vast te stellen hiervoor wel kunnen worden gebruikt. Voor de gepresenteerde resultaten kan men slechts concluderen dat de aanvankelijke hypothese met betrekking tot de invloed van oxidatieve stress op het ontstaan en het voortschrijden van diabetische complicaties niet wordt ondersteund door de verkregen resultaten.

GEZONDHEIDSBELEVING

Insuline-afhankelijke diabetes mellitus is een chronische aandoening, die gevolgen kan hebben voor een groot aantal facetten van het alledaagse leven. De inname van voedsel, lichaamsbeweging, en het aantal te spuiten eenheden insuline dienen telkens weer op elkaar te worden afgestemd om de bloedglucosewaarden op een acceptabel peil te houden en hyperglycaemische en hypoglycaemische klachten te vermijden. Daarnaast zijn sommige patiënten zich er terdege van bewust dat er altijd een kans blijft bestaan om een of meer complicaties te krijgen, hoe goed men de bloedglucosewaarden ook heeft gereguleerd.
Deze verantwoordelijkheid voor het onder alle omstandigheden goed regelen van de bloedglucosewaarden en de mogelijke zorgen over de toekomstige gezondheid en het welzijn, vormen een extra belasting voor het dagelijks leven van mensen met IADM. Hoewel uit de resultaten van de DCCT is gebleken dat middels het zeer goed reguleren van bloedglucosewaarden de kans op het ontwikkelen van complicaties kan worden verkleind, bleken belangrijke neveneffecten van deze regulatie onder andere een aanzienlijke gewichtstoename en een driemaal zo grote kans op het krijgen van een ernstige hypoglycaemie te zijn. Gezien de mogelijke lichamelijke maar ook emotionele gevolgen hiervan zouden deze neveneffecten voor vele patiënten een onacceptabele 'prijs' kunnen inhouden, die zij moeten betalen voor het verminderen van de kans op het krijgen van complicaties. Tevens is het noodzakelijk om hiervoor de insuline therapie verder te intensieveren, frequenter insuline injecties toe te dienen, over te gaan op insuline toediening middels een insulinepomp en vaker glucose-dagcurves te prikken (zelfcontrole). Dit laatste zou op zichzelf al een negatief effect kunnen hebben op hoe de mens met diabetes zijn gezondheid ervaart.

In het tweede gedeelte van dit proefschrift hebben we dan ook de resultaten beschreven van een onderzoek, met als doel de relatie te onderzoeken tussen metabole controle en de frequentie van insuline toediening enerzijds en zelf-controle, en de gezondheidsbeleving van mensen met diabetes anderzijds.
Hoewel het moeilijk lijkt om de ervaringen met betrekking tot het gevoel te meten, bestaan er sinds enkele jaren vragenlijsten, die de gezondheid en het gevoel van gezondheid en ziekte kunnen meten. Twee van deze meetinstrumenten (de SF-36 en Euroqol) zijn in dit proefschrift gebruikt als maat voor iemands gezondheidsbeleving.
Over het algemeen genomen was er geen of slechts een zeer zwak verband tussen metabole controle (o.a. HbA1c) en hoe een patiënt zijn gezondheid ervaart, gemeten met behulp van de SF-36 of Euroqol (vragenlijsten). Echter, het hebben van diabetische klachten (zowel hyperglycaemische als hypoglycaemische) bepaalde in sterke mate hoe diabetes patiënten hun gezondheid ervaarden, waarbij het hebben van meer klachten samen bleek te gaan met een verminderd 'gevoel van gezond zijn' (Hoofdstuk 6).

Wanneer men het effect van de behandeling op de gezondheidsbeleving onderzoekt, blijkt er een sterk negatief verband te bestaan tussen hoe vaak men zelfcontrole verricht en de op lichamelijke aspecten georiënteerde gezondheidsbeleving. Dit verband bleek niet te berusten op de mate van metabole controle of hoe vaak men diabetes klachten heeft. De bevinding dat patiënten, die worden behandeld middels een insulinepomp hun gezondheid als minder goed ervaren ten opzichte van patiënten die behandeld worden met insuline pennen, bleek te kunnen worden verklaard door het feit dat deze patiënten vaker zelfcontrole verrichtten. Tezamen genomen bevestigen deze bevindingen dat de behandeling van mensen met diabetes inderdaad negatieve gevolgen kan hebben op hoe iemand zijn/haar gezondheid ervaart.

In hoofdstuk 8 wordt beschreven wat de consequenties van dit onderzoek naar oxidatieve stress en gezondheidsbeleving voor de behandeling van mensen met IADM zouden kunnen zijn. Het belangrijkste is, dat het al dan niet aanwezig zijn van hypo-en hyperglycaemische klachten kan worden beschouwd als een bruikbare aanwijzing voor het verlaagd zijn van de gezondheidsbeleving van de diabetes patiënt. Op dit moment bestaan er voor de klinische pratijk geen redenen voor het meten van serum anti-oxidanten of parameters van vetzuuroxidatie. Echter, met betrekking tot de gezondheidsbeleving dienen behandelaars zich bewust te zijn van de gevolgen van intensieve

insulinebehandeling (met name van de frequentie van zelfcontrole) voor het 'gevoel van gezond zijn' bij individuele patiënt, en deze informatie mee te nemen in de adviezen naar de patiënt.

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MANUSCRIPTS BASED ON STUDIES DESCRIBED IN THIS THESIS

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