INSULIN TREATMENT IN TYPE 2 DIABETES

Leena Ryysy

Helsinki 2001
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Leena Ryysy

ACADEMIC DISSERTATION

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Navigare necesse est
vivere non est necesse

To our sons Ransu and Rieti
Abstract

Background and aims. Given that the pathogenesis of type 1 and 2 diabetes are markedly different, it is possible that patients with type 2 diabetes should not be treated with insulin following the principles used for treatment of type 1 diabetes. The present studies were undertaken to define 1) the optimal insulin treatment regimen for type 2 diabetic patients with secondary failure to oral anti-diabetic drugs (OAD), 2) causes of inter-individual variation in insulin requirements in type 2 diabetic patients and 3) how improvement of glycemic control by insulin therapy influences markers of endothelial activation as measured by serum concentrations of the soluble adhesion molecules sE-selectin and vascular cell adhesion molecule (sVCAM-1).

Subjects, study designs and methods. 153 poorly controlled type 2 diabetic patients who were treated with maximal doses of sulfonylurea alone or in combination with metformin were randomized to treatment for 3 months with either continued OAD and NPH insulin in the evening or continued OAD and NPH insulin in the morning, a 2 insulin injection regimen without OAD or a multiple insulin injection regimen without OAD. The goal was to achieve similar glycemic control with all insulin regimens and to compare effects of various regimens on insulin requirements, body weight, serum lipids and lipoproteins and episodes of hypoglycemia (study I). Study II was designed to determine what bedtime NPH insulin should be combined with, a sulfonylurea, metformin, both drugs or another injection of NPH insulin. Ninety-six type 2 diabetic patients who were treated with a maximal dose of sulfonylurea, were randomized to treatment for one year with bedtime NPH insulin and glibenclamide, or bedtime NPH insulin and metformin, or bedtime NPH insulin and glibenclamide and metformin, or bedtime NPH insulin and morning NPH insulin. The patients were taught to self adjust their insulin doses. In study III, 20 type 2 diabetic patients with stable glucose control and insulin dose, who were treated with combination therapy with bedtime NPH insulin and metformin for at least 1 year were studied. In each subject, the following measurements were performed: 1) measurement of action of intravenous insulin on endogenous glucose production (EGO) and utilization, (euglycemic insulin clamp combined with [3-3 H]glucose) 2) measurement of absorption (increase in free insulin and total insulin over 8 h after subcutaneous dose of regular insulin) and action of subcutaneous insulin (glucose infusion rate required to maintain euglycemia and suppress FFA) and 3) measurement of liver (proton spectroscopy) and intra-abdominal (magnetic resonance imaging) fat content and in addition, body weight, body composition, and the thickness of subcutaneous abdominal (ultrasound) fat were determined. In study IV, 81 type 2 diabetic patients, who participated in study II and 41 normal subjects were studied. In these groups, concentrations of serum sE-selectin and sVCAM-1 concentrations were determined. In the type 2 diabetic patients, the measurements were repeated at 3 and 12 months.

Results. Study I: The mean insulin doses of NPH insulin in the two groups receiving an OAD and NPH insulin were similar and 60 % lower than in the 2-injection or multiple injection group. The total doses of insulin were comparable in the 2- and multiple insulin injection groups. HbA1c concentrations decreased from approximately 8 to 10 % in all the insulin-treatment groups. All groups receiving insulin therapy gained weight. The smallest increment in body weight occurred in the OAD and evening NPH insulin group and the largest in the multiple-injection group (p < 0.05). The frequency of hypoglycemia was similar in all insulin treated groups. The concentration of serum VLDL triglycerides decreased by 13 to 28 % in the insulin treatment groups with no differences between the groups who used insulin. The concentrations of total, LDL, and HDL cholesterol remained unchanged. Study II: Patients receiving bedtime insulin and metformin showed a progressive decrease in HbA1c concentra-
tions over time. At 12 months, HbA1c values in this group averaged 7.2 % ± 0.2 %; which differed significantly from that in the other groups. Patients receiving bedtime insulin and metformin did not gain weight unlike the other groups. The frequency of hypoglycemic episodes in patients receiving bedtime insulin and metformin was significantly lower (p < 0.05) than that in patients receiving bedtime and morning insulin. Hypoglycemia limited adequate titration of the insulin dose in the group using NPH insulin and glibenclamide. Serum triglyceride concentrations decreased similarly in all groups. Study III: The amount of insulin absorbed was significantly correlated with glucose infusion rate required to maintain euglycemia (r = 0.74, p < 0.001) and suppression of FFA (r = -0.63, p < 0.005). On the other hand, the actions of intravenous and subcutaneous insulin were so closely correlated that the contribution of variation in insulin absorption to interindividual variation in insulin action was maximally 30 %. Of the relationships of measures of overall adiposity and fat distribution, the % liver fat was the best correlate of the % suppression of EGO by intravenous insulin. In multiple linear regression, 61.3% of variation in the daily insulin dose (units per day) could be explained by variation in the ability of subcutaneous insulin to suppress FFA (p < 0.001) and by insulin antibodies (p = 0.05). Of all measures of adiposity, the % liver fat was the parameter best correlated with the insulin dose. Study IV. Serum sE-selectin concentrations were 71 % higher in the type 2 diabetic patients than in the normal subjects before insulin therapy. During insulin therapy, sE-selectin concentrations decreased significantly compared to 0 month but the concentration at 12 months was still 55 % higher than in the normal subjects. Serum sVCAM-1 decreased transiently during the first 3 months and then increased back to baseline by 12 months. The change in HbA1c, both in diabetic men and women, was significantly correlated with the change in sE-selectin concentrations.

Conclusions. In poorly controlled type 2 diabetic patients receiving OAD therapy, the addition of NPH insulin in the evening improves glycemic control in a similar manner as a two-insulin-injection therapy regimen and multiple-insulin-therapy regimen, but induces less weight gain and hyperinsulinemia. Combination therapy with bedtime insulin and metformin prevents weight gain and seems superior to other bedtime insulin regimens with respect to improvement in glycemic control and frequency of hypoglycemia. Self-adjustment of the insulin dose is critically important to achieve glycemic targets. The major reason for interindividual variation in insulin requirements in type 2 diabetes is variation in insulin action. Variation in hepatic fat content may influence insulin requirements via an effect on the sensitivity of EGO to insulin. Improvement in glycemic control by insulin alone or insulin combined with either glibenclamide, metformin, or both agents induces a sustained decrease in sE-selectin, the magnitude of which seems to be dependent on the degree of improvement in glycemic control. Serum sE-selectin might provide a marker of effects of treatment of chronic hyperglycemia on endothelial activation.
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LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following publications, which are referred to in the text by their roman numerals:


ABBREVIATIONS

a.m. = ante meridium
AGE = advanced glucosylated end products
AIR = acute insulin secretory response
ALT = alanine aminotransferase
AMI = acute myocardial infarction
ATP = adenosine triphosphate
AVD = atherosclerotic vascular disease
BG = blood glucose
BMI = body mass index (kg/m²)
CHD = coronary heart disease
CVD = cardiovascular disease
DIGAMI = The Diabetes Insulin-Glucose in Acute Myocardial Infarction study
DCCT = Diabetes Control and Complications Trial
EGO = endogenous glucose output = hepatic and renal glucose production = Rₐ
FBG = fasting blood glucose
FFA = free fatty acids
FFM = fat free mass
Fig. = figure
FINFAT = original publication number II
FINMIS = original publication number I
FPG = fasting plasma glucose
GAD = glutamic acid decarboxylase
GINF = glucose infusion rate
GLN = gluconeogenesis
HbA₁c = glycated hemoglobin A₁c
HDL = high density lipoprotein cholesterol
h = hour(s)
e.g. = exempli gratia = for example
i.e. = id est = it is
i.v. = intravenous
ICA = islet-cell antibodies
ICAM-1 = intercellular adhesion molecule-1
ICAM-2 = intercellular adhesion molecule-2
IFG = impaired fasting glucose
IGT = impaired glucose tolerance
IIP = implantable insulin pump
K⁺ = potassium ion
KATP = potassium adenosine triphosphate
kJ = kilojoule
LADA = latent autoimmune diabetes in adults
LDL = low density lipoprotein cholesterol
LPL = lipoproteine lipase
M-value = amount of glucose infused to maintain euglycemia
MDI = multiple dose insulin
MI = myocardial infarction
min = minute(s)
MODY = maturity-onset diabetes in the young
NASH = nonalcoholic steatohepatitis
NGT = normal glucose tolerance
NMR = nuclear magnetic resonance
NPH = neutral protamine Hagedorn, NPH insulin
OAD = oral antidiabetic drugs
p.m. = post meridium
PAI-1 = plasminogen activator inhibitor-1
PECAM = platelet-endothelial adhesion molecule
PI3 = phosphatidylinositol 3
PPAR-γ = peroxisome proliferator-activated receptor gamma
PPG = postprandial blood glucose
R_a = glucose rate of appearance = EGO
R_d = glucose rate of disappearance
RIA = radioimmunoassay
s = second(s)
s.c. = subcutaneous
SA = specific activity
sE-selectin = soluble E-selectin
S_fat = methylene signal intensity
sICAM-1 = soluble intercellular adhesion molecule-1
SU = sulphonylureas
sVCAM-1 = soluble vascular cell adhesion molecule-1
S_water = water signal intensity
TE = echo time
TNF-α = tumor necrosis factor-α
t-PA = tissue plasminogen activator
TR = repetition time
UAER = urinary albumin excretion rate
UGDP = University Group Diabetes Program
UKPDS = United Kingdom Prospective Diabetes Study
VCAM-1 = vascular cell adhesion molecule-1
VLDL = very light density lipoprotein
vs = versus
vWF = von Willebrand factor
W/H = waist to hip ratio
WHO = World Health Organization
1. INTRODUCTION

It has been estimated that the global prevalence of type 2 diabetes will rise from about 160 million in the year 2000 to approximately 215 million in 2010 (29). The mortality from cardiovascular disease (CVD) and the incidence of non-fatal coronary heart disease (CHD) events is 2 to 4 times higher in patients with type 2 diabetes than in normal subjects and is the major cause of death of these patients (243,275,485). The overall objective of treatment of type 2 diabetes is to prevent acute and chronic complications while maintaining a high quality of life.

We know that the typical type 2 diabetic patient is obese and insulin resistant and has by definition residual insulin secretion (5,295,405). This knowledge raises the possibility that patients with type 2 diabetes may perhaps not benefit from the same type of insulin therapy than patients with type 1 diabetes, who are usually lean and often have hypoglycemic problems during insulin therapy. Until 1989, when the FINMIS (I) study was started, little was, however, known of whether and how patients with type 2 diabetes should be treated with insulin once oral antidiabetic drugs (OAD) no longer were able to maintain satisfactory glycemic control. Patients with type 2 diabetes were frequently admitted to the hospital, put on a diet meant for ‘insulin-treated diabetic patients’ which included 3 main meals and often even 3 snacks. The concept that C-peptide is a better marker of insulin resistance than secretion in type 2 diabetic patients was not commonly known. This sometimes resulted in abandoning insulin therapy in obese patients because “normal” or “elevated” C-peptide concentration was considered to imply sufficient insulin secretion despite simultaneous hyperglycemia. In the literature, there were very few randomized comparisons of different insulin treatment regimens in patients with type 2 diabetes. For example, in a meta-analysis published in 1991 (382), only 8 concurrent and 14 cross-over trials were identified with a mean of 11 and 14 patients per group. In these studies, glycemic control was concluded to be, on the average, better in patients treated with insulin and sulfonylureas than with insulin alone, and the dose of exogenous insulin was lower in those using insulin combination therapy compared to patients using insulin alone. These data documented that
oral agents do work even in poorly controlled patients with type 2 diabetes. On the other hand, these studies taught us little regarding the possible superiority of insulin combination therapy compared to insulin alone. Given that sulfonylureas still work even in advanced disease, their apparent superiority might simply have been explained by failure to decrease exogenous insulin doses sufficiently to match the potency of the sulfonylurea (531). Another issue that had not been tested was whether administration of insulin at bedtime might prevent weight gain compared to administration of the same dose of insulin in the morning or whether use of multiple insulin injections merely results in weight gain and no benefits with respect to glycemic control compared to simpler regimens. Another open question, still in 1994 when the FINFAT (II) study was started, was whether use of metformin might counteract weight gain which is inevitable when glycemic control is improved. There were also no data on what determines insulin requirements in patients with type 2 diabetes. This would seem of interest as type 2 diabetic patients vary greatly with respect to body weight and composition and insulin sensitivity. Finally, very little attention has been paid to effects of insulin therapy on parameters other than glycemic control and lipids and lipoproteins. These include effects of insulin therapy on various measures of vascular function such as on endothelial function, inflammatory markers and measures of coagulation and fibrinolysis.

The present studies were undertaken to define the optimal insulin treatment regimen for patients with type 2 diabetes. We also wished to search for causes of interindividual variation in insulin requirements in type 2 diabetes and were interested to determine effects of insulin therapy on markers of endothelial function.
2. REVIEW OF THE LITERATURE

2.1 PATHOGENESIS OF TYPE 2 DIABETES

2.1.1. Definition of type 2 diabetes

Diagnostic criteria
The diagnostic criteria of diabetes are similar regardless of the etiology of hyperglycemia. The first generally accepted criteria were published in 1979 by the National Diabetes Data Group classification (3). The recommendations of the National Diabetes Data Group were endorsed by the World Health Organisation (WHO) Expert Committee on Diabetes in 1980 and the World Organisation Study Group on Diabetes Mellitus in 1985 (5). These diagnostic criteria have recently been re-examined. The American Diabetes Association published new criteria in 1997 and the experts of WHO published highly similar criteria in 1998 (468) (Table 1).

The new aspects of these WHO criteria were: 1) the concentration of fasting plasma glucose, measured from venous blood, which establishes the diagnosis of diabetes is 7.1 mmol/l instead of 7.8 mmol/l, 2) a new category of altered glucose homeostasis called impaired plasma fasting glucose (IFG) was introduced, and defined as a fasting plasma glucose concentration between 6.1 and 7.0 mmol/l, 3) the terms insulin-dependent and non-insulin-dependent were discarded and replaced by the terms type 1 and type 2 diabetes.
Table 1. The new WHO diagnostic criteria for diabetes and other hyperglycaemic states (468). The oral glucose load used to determine oral glucose tolerance is 75g or 1.75g/kg for children. The diagnostic criteria are the same for adults and children.

<table>
<thead>
<tr>
<th></th>
<th>Glucose concentration in mmol/l (mg/dl)</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Plasma</td>
<td>Whole blood</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Venous</td>
<td>Capillary</td>
<td>Venous</td>
<td>Capillary</td>
<td></td>
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<tr>
<td>Diabetes mellitus</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting value</td>
<td>≥ 7.0 (126)</td>
<td>&gt; 7.0 (126)</td>
<td>≥ 6.1 (110)</td>
<td>≥ 6.1 (110)</td>
<td></td>
</tr>
<tr>
<td>or 2 h after 75 g</td>
<td>≥ 11.1 (200)</td>
<td>≥ 12.2 (220)</td>
<td>≥ 10.0 (180)</td>
<td>≥ 11.1 (200)</td>
<td></td>
</tr>
<tr>
<td>glucose load</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IGT</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fasting value</td>
<td>&lt; 7.0 (126)</td>
<td>&lt; 7.0 (126)</td>
<td>&lt; 6.1 (110)</td>
<td>&lt; 6.1 (110)</td>
<td></td>
</tr>
<tr>
<td>(if measured) and</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 h after 75 g</td>
<td>7.8-11.0 (140-199)</td>
<td>8.9-12.1 (160-219)</td>
<td>6.7-9.9 (120-179)</td>
<td>7.8-11.0 (140-199)</td>
<td></td>
</tr>
<tr>
<td>glucose load</td>
<td></td>
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</tr>
<tr>
<td>IFG</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Fasting value</td>
<td>≥ 6.1 (110)</td>
<td>≥ 6.1 (110)</td>
<td>≥ 5.6 (100)</td>
<td>≥ 5.6 (100)</td>
<td></td>
</tr>
<tr>
<td>and (if measured)</td>
<td>&lt; 7.0 (126)</td>
<td>&lt; 7.0 (126)</td>
<td>&lt; 6.1 (110)</td>
<td>&lt; 6.1 (110)</td>
<td></td>
</tr>
<tr>
<td>2 h after 75 g</td>
<td>&lt; 7.8 (140)</td>
<td>&lt; 8.9 (160)</td>
<td>&lt; 6.7 (120)</td>
<td>&lt; 7.8 (140)</td>
<td></td>
</tr>
</tbody>
</table>
Etiologic types and stages

The etiological types and stages of disorders of glycemia are described in Fig. 1.

<table>
<thead>
<tr>
<th>Types</th>
<th>Normoglycemia</th>
<th>Hyperglycemia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type 1*</td>
<td>Normal glucose regulation</td>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Type 2</td>
<td>Impaired glucose tolerance or</td>
<td>Not insulin requiring</td>
</tr>
<tr>
<td></td>
<td>impaired fasting glucose</td>
<td>Insulin requiring for control</td>
</tr>
<tr>
<td>Other specific types**</td>
<td></td>
<td>Insulin requiring for survival</td>
</tr>
<tr>
<td>Gestational diabetes**</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 1.** Disorders of glycemia: etiologic types and stages. * Even after presenting in ketoacidosis, these patients can briefly return to normoglycemia without requiring continuous insulin therapy (i.e., "honeymoon" remission). ** In rare instances, patients in these categories (e.g. type 1 diabetes presenting in pregnancy) may require insulin for survival. Adapted from Report of the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus (468)

Type 1 diabetes is characterized by beta-cell destruction usually via autoimmune mechanisms. The phenotype features of type 1 diabetes are early age at diagnosis, often but not always acute severe insulin deficiency accompanied by symptoms of hyperglycemia, ketoacidosis and subnormal body weight. GAD-antibodies can be detected in 65 to 90% of patients at diagnosis (291,390,427). Complete insulin deficiency usually develops during the few first years of the disease (34,434).

Type 2 diabetes is characterized by a combination of defects in insulin secretion and action (117,249,490). Diagnosis is usually made in adults, although the average age of onset is continuously decreasing perhaps as a consequence of increasing obesity in teenagers and children (18,139). At least 80 % of type 2 diabetes patients are overweight. Although the majority of type 2 diabetic patients are insulin resistant and obese, a subset is non-obese, relatively insulin-sensitive and insulin-deficient (5).
A subgroup of patients initially classified as having type 2 diabetes have a slowly evolving autoimmune type of diabetes, called latent autoimmune diabetes in adults (LADA) (472). The presence of autoantibodies, either islet-cell autoantibodies (ICA) and/or GAD- antibodies, is a marker for insulin dependency in patients with type 2 diabetes (178,471,474). At diagnosis, patients with LADA usually have clinical features of type 2 diabetes, but in such patients progressive autoimmune destruction of beta-cells occurs and may lead to absolute insulin deficiency and need for insulin treatment within few years (470,546). In unselected patients clinically classified as having at least initially type 2 diabetes approximately 10% have GAD-antibodies (355,474). These patients should, however, be classified as having type 1 diabetes according to the new classification criteria (17).

The genetics of type 2 diabetes in its most common type encountered in clinical practice are complex (329). It is clear that there is no single major gene of overwhelming importance in this type 2 diabetes (nothing akin to HLA in type 1). However, several genomic regions (e.g. on chromosomes 1, 7, 11 and 20) are showing interesting replications across datasets and thus represent foci for detailed exploration (329). Also, lifestyle-related factors such as physical activity levels and diet are, next to age, the most important determinants of the penetrance of a given set of diabetes-susceptibility genotypes. Although type 2 diabetes is generally a polygenic disorder (329) there are some rare forms such as maturity onset diabetes in young (MODY), which are monogenic autosomal dominantly inherited subtypes of type 2 diabetes. Mutations in five genes are currently known to cause MODY. These genes encode hepatocyte nuclear factor-4 alpha (MODY 1), glucokinase (MODY 2), hepatocyte nuclear factor-1 alpha (MODY 3), insulin promoter factor-1 (MODY 4), and hepatocyte nuclear factor-1 beta (MODY 5). The genes cause several types of abnormalities in insulin secretion. Patients with MODY can usually be treated with diet and OAD and do not usually require long-term insulin therapy for survival, although no systematic comparison between different treatment regimens has been performed (512).

Abnormalities in insulin action and secretion as causes of type 2 diabetes
The pathogenesis of abnormal glucose metabolism in type 2 diabetes involves abnormalities in insulin secretion and insulin action. The sequence in which these ab-
normalities develop and their relative contribution to the deterioration in glucose tolerance and the progression from normal glucose tolerance (NGT) to impaired glucose tolerance (IGT) and ultimately to type 2 diabetes is somewhat controversial. Current understanding of the pathogenesis of type 2 diabetes is based on a large number of cross-sectional (294,359,385,386) and prospective studies (78,79,197,294,295,324,500). Also an overview of the pathogenesis of type 2 diabetes has been given in review articles (114,143).

In prospective studies, in which non-diabetic individuals have been metabolically characterized, while still non-diabetic, and then followed for several years to determine who develops diabetes, have helped to identify metabolic abnormalities that predispose to diabetes. Lillioja S et al. (294,295) have shown that both a low early insulin response and impaired insulin action predict diabetes in Pima Indians. In Pima Indians (504), transition from NGT to IGT is also associated with an increase in body weight, a decline in insulin-stimulated glucose disposal and a decline in the acute insulin secretory response (AIR) to intravenous glucose. Progression from IGT to diabetes was accompanied by further increase in body weight, a decrease in insulin-stimulated glucose disposal and the AIR and an increase in basal endogenous glucose output (EGO). It has also been shown in other populations that insulin resistance predicts the development of type 2 diabetes (324,499).

Regarding the risk factors predisposing to type 2 diabetes, obesity is perhaps the most important one (79,261,362,506). The US National Commission on Diabetes reported that the risk of developing type 2 diabetes was about 2-fold increased in mildly obese, 5-fold in moderately obese and 10-fold in severely obese people (2). The relative risk imposed by obesity was highest among young people (20-45 years), whose risk was 3.8 times that of their non-overweight compatriots (489). Weight gain, in addition to body mass index kg/m² (BMI), is also a strong risk factor for diabetes (76,89,137), as is the duration of obesity (136). The risk of type 2 diabetes increases not only as a function of overall obesity, but also with increasing abdominal obesity, although there is increasing evidence that this applies more to some population than the others (132,363). People with upper body obesity are far more likely to develop type 2 diabetes than those with lower body obesity (55,72,132,199).
Lack of physical activity is another and perhaps the second most important risk factor for the development of type 2 diabetes. The British Regional Heart Study found that men who habitually were engaged in moderate levels of physical activity had a substantially reduced risk of diabetes compared with physically inactive men, even after adjustment for age, BMI and other risk factors (380). Physical training enhances insulin sensitivity (121), which could be a major mechanism, via which physical training prevents from diabetes. Tuomilehto et al. have recently shown, in their study addressing prevention of type 2 diabetes mellitus by changes in lifestyle (diet and exercise) among subjects with IGT, that the cumulative incidence of diabetes after four years was 11 % in the intervention group and 23 % in the control group. The risk of diabetes was reduced by 58 % (p < 0.001) in the intervention group. The reduction in the incidence of diabetes was directly associated with changes in lifestyle (473).

2.1.2 Regulation of fasting and postprandial glucose concentrations

What determines the fasting plasma glucose concentration?
The liver is the most important source of glucose after an overnight fast (368). It is responsible for the majority of postabsorptive glucose release, the rest being of renal origin. Glucose is produced every minute at a rate of approximately 11-12 µmol/kg of body weight (111). The brain is the major organ disposing glucose and accounts for ~50 - 60 % of total glucose disposal (221). The splanchnic bed, erythrocytes, and other parenchymal organs account for ~20 - 25 % of total glucose disposal (119). Some 10 - 20 % is disposed of by skeletal muscles and 1-5 % by adipose tissue (120). Only ~10 - 20 % of whole body glucose disposal is insulin dependent in the postabsorptive state (120). Thus only a small component of overall glucose disposal will be affected by conditions of insulin resistance and/or deficiency. On the other hand, glucose production is extremely sensitive to insulin (68). Glucose production is the sum of glucose produced via glycogenolysis and gluconeogenesis (GLN). Approximately 20 % of glucose production can be attributed to GLN, if calculated based on the extraction of gluconeogenic precursors by the splanchnic bed in non-diabetic subjects (498). When measured using [2,14 C]acetate, GLN accounts for ~30 % of EGO after an overnight fast (102). With this
method, GLN increases 2-fold in subjects fasted for 2.5 days and then accounts for > 97% of overall glucose production which now is of both hepatic and renal origin. When measured with [13C] NMR imaging after 23 hours of fasting, GLN accounts for as much as 70% of overall glucose production in normal subjects (424).

Regulation of the fasting glucose concentrations in type 2 diabetes
Several studies have shown that in patients with type 2 diabetes glucose production is increased in the postabsorptive state and directly correlated with the fasting plasma glucose concentration (68,117,411). Consoli et al. (103) and Magnusson et al. (311) have shown that the rate of glycogenolysis is unaltered in type 2 diabetes while GLN is increased and the rate of GLN in type 2 diabetes is significantly correlated with the fasting plasma glucose concentration. Using NMR techniques Magnusson et al. (311) have shown that hepatic glycogenolysis is even reduced in patients with type 2 diabetes. These results provide strong evidence that increased GLN is the main cause for increased EGO and fasting hyperglycemia in type 2 diabetes. Inhibition of GLN is not, however, sufficient to decrease fasting plasma glucose concentrations in patients with type 2 diabetes, unless glycogenolysis is also inhibited (395).

The mass-action effect of glucose. The rate of glucose uptake in muscle is influenced not only by insulin but also by the ambient glucose concentration. The ability of glucose to increase its own disposal is known as the mass-action effect of glucose (530). The ability of glucose per se to increase glucose uptake is dependent on the insulin concentration. The higher the insulin stimulated rate of glucose uptake, the greater the ability of glucose to increase glucose uptake (540). It has been shown that hyperglycemia in human diabetes increases glucose uptake in skeletal muscle both in vivo (27,150) and in vitro (545). The mechanism does not involve activation of PI 3-kinase, which is critical for normal action of insulin on glucose uptake (545). This implies that hyperglycemia induced glucose uptake is not necessarily impaired in insulin resistant individuals. However, in in vivo studies of patients with type 2 diabetes, the ability of glucose to stimulate glucose uptake was found normal in one study (27), while a concentration-dependent defect was found in another (352).
The ability of hyperglycemia to increase glucose uptake by pathways which are distinct from those used by insulin, explain why rates of glucose uptake are either normal or increased in type 2 diabetic patients under conditions of every-day life (338). Chronic hyperglycemia induces insulin resistance to glucose utilization, a phenomenon called glucose toxicity of insulin action (422,526).

Regulation of postprandial glucose concentrations in normal subjects and in patients with type 2 diabetes

Normally, glucose ingestion increases insulin and decreases glucagon secretion and suppresses EGO, allowing exogenous glucose to become the predominant source of glucose utilized (145,231,232,248,399). Approximately 30% is taken up by splanchnic tissues, i.e. the liver and the gut, (145,231,232,248,399), 30-40% is taken up by muscle (231,232,248,399), and the rest is primarily disposed of by the brain (248) over a 5-hour period. In muscle ~40% of the glucose taken up is oxidized, ~40% is stored as glycogen and the rest is released as lactate, alanine or pyruvate (248).

In type 2 diabetes, postprandial suppression of plasma glucagon concentrations and increase in plasma insulin by glucose are usually blunted (116,476). Again, because of the combined effects of glucose mass action and insulin resistance, postprandial forearm muscle glucose uptake in type 2 diabetes has constantly been found to be similar or even greater than in non-diabetic subjects (52,150,231,338). Mitrakou et al. have examined the contribution of altered muscle and liver glucose metabolism to postprandial hyperglycemia in type 2 diabetes (338). They administered an oral glucose load enriched with [14C]glucose to 10 type 2 diabetic patients and 10 normal subjects and measured muscle glucose disposal by determining forearm balances of glucose, lactate, alanine, O2 and CO2. In addition, a dual-label isotope method was used to compare overall rates of glucose appearance (Ra) and disappearance (Rd), suppression of EGO and splanchnic glucose sequestration. The increase in postprandial glucose concentrations (PPG) and of glucose Rd in patients with type 2 diabetes were due to impaired suppression of EGO (Fig. 2).
The amount of glucose taken up by muscle was not significantly different between patients with type 2 diabetes and normal subjects, although a greater amount of the glucose taken up by muscle in type 2 diabetes was released as lactate and alanine and less was stored (338). It was concluded that the ability of insulin to suppress EGO determines the degree of postprandial glycemia. Other studies have also found that insulin-induced suppression of hepatic glucose release (Fig. 3) and insulin stimulated muscle glucose uptake are the major factors regulating postprandial glucose metabolism (112,170,405).
Fig. 3. Mean ± SE plasma glucose (mmol/l) concentrations at hourly intervals from 0800 to 1600 (breakfast at 0800 and lunch at 1200) in normal individuals (cont) (●) and patients with type 2 diabetes (○), who are divided in mild and severe (sev) groups, in severe group insulin concentrations are low and hepatic glucose production is the main cause of elevated glucose concentrations. Modified from ref. (405).

2.1.3. Sensitivity of endogenous glucose production to insulin

Measurement of EGO

In humans, EGO has been studied using the hepatic venous catheterization technique (49,61,141), by tracer methodology (408), by combining both (116,428) and by NMR (311,424).

The most commonly used glucose tracer for measurement of hepatic glucose production is [3-3H]glucose. This tracer is handled by body tissues in a similar manner to cold glucose (115). The tritium from the 3-position in glucose is lost during glycolysis but not during glucose incorporation to and release from hepatic glycogen (514). The latter process is, however, of no concern when glucose production is measured under fasting conditions because, even after prolonged infusion of [3-3H]glucose, stimulation of glycogenolysis with glucagon does not increase the level of plasma radioactivity (175). Thus, if isotopic steady-state is achieved during infusion of [3-3H], i.e. glucose specific activity can be held constant, the rate of hepatic
glucose production, in fact EGO, can be accurately calculated by dividing the isotope infusion rate by the specific activity (SA) of glucose (514). However, quite frequently and especially when rapid fluctuations in the rate of entry of exogenous or endogenous glucose into the circulation occur, the specific activity of glucose changes (175). Calculation of the glucose kinetics now becomes dependent on the use of models that describe glucose kinetics under non-steady-state conditions. Of these the most commonly used is the modified one-compartment model described by Steele (447). Because of the inability of this model to describe whole body kinetics of glucose accurately, glucose production is underestimated when specific activity decreases; the greater the deviation from steady-state, the greater the underestimation (87,533). This phenomenon explains why physiologically impossible negative rates of glucose production can be observed, especially when the response of endogenous glucose production to insulin is determined using the insulin clamp technique (54,149). More reliable estimates of EGO can be obtained if changes in the SA of glucose can be minimized (104). Because the concentration of glucose in plasma and thereby the glucose pool is increased in patients with type 2 diabetes, the time to reach isotopic steady-state during tracer infusion is prolonged. This is why the infusion time of $[^3\text{H}]$glucose should be longer in type 2 diabetic patients than in normal subjects (87,533).

*Normal subjects*

Insulin plays a key role in the maintenance of normal glucose tolerance by suppressing EGO during a meal. Complete suppression of EGO by intravenous insulin has been found at peripheral insulin concentrations of 50-60 mU/l (116,417,540). Insulin is thought to suppress EGO by a direct effect on the liver rather than via an extrahepatic effect, although studies in conscious dogs have suggested that insulin action in the periphery may also be of importance (527). Insulin may also suppress EGO, at least in part, via a decrease in glucagon secretion (540). Under physiological conditions insulin does not inhibit gluconeogenesis but rather diverts gluconeogenic flux towards glycogen (81). Insulin effectively suppresses glycogenolysis (115). Insulin is less important in the regulation of splanchnic glucose uptake (45), which appears, based on studies performed in dogs, to be regulated primarily by the arterial-portal glucose gradient and to a smaller extent by glucose mass-action (116,347,371).
Obesity and fat distribution

Obesity is associated with decrease in the sensitivity of both EGO and peripheral glucose uptake to insulin (67,364,392). Hyperinsulinemia and insulin resistance are more pronounced in upper body obesity (378,379). Visceral adipose tissue has a higher turnover rate than other adipose tissue depots in both men and women. The visceral fat is drained via the portal vein to the liver, in contrast to periferal fat depots. The increased lipolytic activity of visceral fat, combined with its anatomical location, exposes the liver to higher concentrations of FFA than any other organ. FFA decrease insulin clearance by the liver and increase EGO (144,323). The causes for interindividual variation of hepatic sensitivity to insulin in obese subjects as well as in normal subjects are largely unknown.

Type 2 diabetes

Resistance of EGO to suppression by insulin has been a consistent finding in patients with type 2 diabetes (53,147). Plasma glucagon concentrations are inappropriately high in relation to prevailing hyperglycemia and hyperinsulinemia in type 2 diabetic patients in the postabsorptive state (477) and may therefore contribute to the increase in EGO. The contribution of excessive glucagon to the increase in GLN and EGO in type 2 diabetes, has not been examined directly. In type 2 diabetes EGO is increased despite hyperglycemia in the postabsorptive state and directly correlated with the fasting plasma glucose concentration (59,68,103,117,122,411). Fasting plasma FFA concentrations also correlate with EGO (189,463).

2.1.4. Cardiovascular disease - the major complication of type 2 diabetes

Epidemiology of coronary heart disease in type 2 diabetes

It has been shown that mortality from cardiovascular disease (CVD) and the incidence of the non-fatal coronary heart disease (CHD) events is 2 to 4 times higher in patients with type 2 diabetes than in non-diabetic subjects (69, 195, 243, 275, 276, 320, 396, 445, 480, 485, 493). CVD accounts for 70% of all deaths in people with diabetes (273). Diabetic patients without previous myocardial infarction have as high a risk of myocardial infarction as non-diabetic patients with previous myocardial infarction (195). Diabetic patients with myocardial infarction also have a worse
prognosis than non-diabetic patients with myocardial infarction (19,337). The risk of CHD is increased already in the prediabetic state (165,198). Diabetic women have even a higher risk for CHD than diabetic men when compared to non-diabetic counterparts (198,275).

**Classic cardiovascular risk factors**

In the 12 year prospective Multiple Risk Factor Intervention Trial (MRFIT) (445) CVD death rates were approximately 3 times higher in men with diabetes compared to men without diabetes (Fig. 4).

![Fig. 4. Age adjusted CVD death rates by the presence of number of risk factors for men screened for MRFIT, with and without diabetes at baseline. Modified from ref. (445)](image)

A significant positive relationship between serum cholesterol and CVD mortality was observed for both diabetic and non-diabetic men. However, at every concentration of serum cholesterol, the CVD death rate was several times higher in diabetic than in non-diabetic men. Results were similar for blood pressure and smoking. Thus, even when all classic risk factors were considered, CVD mortality still remained 3-fold higher in diabetic than in non-diabetic men. The inability of classic risk factors to explain increased CDH mortality has been confirmed in several other studies. Classic risk factors also do not explain the increased cardiovascular risk of IGT subjects (236). For example in the Whitehall study (166), almost three quarters of the increased relative risk of deaths from coronary heart disease and stroke in in-
individuals with IGT and type 2 diabetes were not explained with risk factors such as age, blood pressure, obesity, smoking or the serum cholesterol concentration.

Although classic risk factors do not explain excessive mortality for CVD in diabetic as compared to non-diabetic subjects, classic risk factors are important predictors of CVD death also in diabetic patients. This was also shown by the UKPDS (475), in which the major risk factors for CHD in type 2 diabetes were increased concentration of low density lipoprotein cholesterol (LDL), a decreased concentration of high density lipoprotein cholesterol (HDL), hyperglycemia, hypertension and smoking.

**Components of insulin resistance and chronic hyperglycemia as cardiovascular risk factors**

Several prospective epidemiological studies have shown that hyperinsulinemia predicts the risk of CHD in non-diabetic subjects (127,270,289,397). Some cross-sectional studies have shown that type 2 diabetes patients with atherosclerotic vascular disease (AVD) have higher concentrations of fasting plasma insulin or C-peptide or higher postglucose plasma insulin concentrations than those without AVD (419,431,446). In the UKPDS (475) fasting serum insulin concentrations were positively correlated with the risk of MI but this correlation became non-significant after adjustment for other risk factors. In contrast to these observations, in a 10-year follow-up of the "borderline" diabetic group of the Bedford Study, a low 2-h post-glucose plasma insulin was associated with an increased risk of CHD events (235). Lehto S et al. (288) have recently shown in a 7-year prospective study that the predictive value of hyperinsulinemia with respect to death from CHD was independent of conventional cardiovascular risk factors but not of risk factors clustering with hyperinsulinemia. By applying factor analysis and principal component analysis it was shown that the "hyperinsulinemia cluster" (a factor having high positive loading for BMI, triglycerides and insulin; and high negative loading for HDL cholesterol) was predictive of death from CHD in type 2 diabetic patients. Kaukua et al. got similar results (247). Other studies have also emphasized the association of hyperinsulinemia with several cardiovascular risk factors (194). These data suggest that causes of the insulin resistance syndrome, such as obesity and consequences of insulin resistance such as hypertriglyceridemia and a low HDL
cholesterol concentration are more important than hyperinsulinemia itself as risk factors of cardiovascular disease (192).

Many studies have indicated that hyperglycemia is an independent predictor for risk of CVD in type 2 diabetes (258,269,273,287). Many potential mechanisms may explain why poor metabolic control of diabetes predicts CHD events (63,396). Hyperglycemia is related to renal disease, microangiopathy and abnormalities in lipoprotein particle composition, which in turn are known to be atherogenic. Chronic hyperglycemia causes non-enzymatic glycosylation of all apolipoproteins (63), which may impede LDL recognition and consequent decreased clearance by the LDL receptor (513), and enhance LDL uptake by macrophages (63,326,513). Hyperglycemia may also accelerate trombus formation among diabetic patients (93) and it has been reported to cause irreversible glycation of proteins in the arterial wall, which may also contribute to the development of vascular complications (314).

**Dyslipidemia**

Two major abnormalities characterize lipoprotein metabolism in type 2 diabetes: fasting and postprandial concentrations of triglyceride-rich lipoproteins, especially very-low-density lipoproteins (VLDL) are higher and those of HDL cholesterol lower than in non-diabetic subjects (458,460).

In addition, an increase in atherogenic small dense LDL particles (lipid-poor and protein enriched) characterize dyslipidemia in type 2 diabetes (460). A preponderance of small dense LDL is associated with insulin resistance (406) and with the risk of myocardial infarction and CHD in non-diabetic subjects (35,105,167). In many prospective studies hypertriglyceridemia has been shown to be a predictor of CHD events and mortality (155,274,287). A meta-analysis of prospective studies suggested that hypertriglyceridemia is a risk factor of CHD even independent of HDL (212).

**Obesity and fat distribution**

Obesity is an important modifiable risk predictor for type 2 diabetes (254). Many cross-sectional studies have shown that excess abdominal fat in type 2 diabetic pa-
tients correlates with CHD events and other CHD risk factors (30,47,488). Prospective studies such as Quebec Cardiovascular Study have shown that the cluster of metabolic abnormalities found in patients with especially upper body obesity substantially increases the risk of CHD (126,281).

**Coagulation and fibrinolysis**

Fibrinogen has emerged as an important and independent predictor of cardiovascular disease and coronary events in a number of prospective studies in non-diabetic patients (203,244,333,524). Concentrations of factor VII, von Willebrand factor (vWF), tissue plasminogen activator (t-PA) antigen and plasminogen activator inhibitor-1 (PAI-1) have been identified in various studies as independent and significant predictors of myocardial infarction, fatal coronary events and severity of coronary disease in non-diabetic patients (6,201,333). Abnormalities of both coagulation and fibrinolysis have been described in type 2 diabetes with increased concentrations of fibrinogen (366), factor VII (164), vWF (101), t-PA antigen (179) and PAI-1 (148,179,184,330).

Many cross-sectional studies conducted in different populations have shown that PAI-1 and t-PA antigen, which represents t-PA/PAI-1 complexes, are strongly correlated with insulin, triglycerides, HDL cholesterol, BMI, W/H and blood pressure i.e. causes or consequences of the insulin resistance, and that the improvement of insulin resistance corrects metabolic abnormalities and concentrations of the fibrinolytic parameters (25, 33, 201, 241, 283, 439, 457, 486). These abnormalities in fibrinolytic and coagulation parameters could thus contribute to the increased risk of CHD in type 2 diabetes.

**Hypertension**

Hypertension is one of the classic risk factors for CHD and stroke (91,309), and is more common in people with type 2 diabetes than in the general population (7,43,243). Modan et al. initially, from a cross-sectional study, suggested that hyperinsulinemia is associated with hypertension independent of glucose tolerance or obesity (340). Some cross-sectional studies have supported the same association between insulin concentrations and hypertension (138,146,509). On the other hand, some epidemiological studies (65,131,345) have not found an independent relation-
ship between markers of insulin resistance and hypertension. In three prospective studies, insulin or insulin resistance predicted the incidence of hypertension (303,356,438) while in one insulin predicted the incidence of hypertension in lean but not in obese subjects (193).

In UKPDS there was an important association between CHD and blood pressure and this association persisted after adjustment for other risk factors of CHD: age, sex, ethnic group, glycemia, lipid concentrations, smoking and albuminuria (15,22). Taken together these data imply that hypertension is an important risk factor for CHD in type 2 diabetes, and that the mechanisms explaining the increased prevalence of hypertension in patients with type 2 diabetes are still unclear.

**Novel markers of cardiovascular risk in type 2 diabetes**

*Urinary albumin excretion*

Microalbuminuria is a risk predictor of CHD in patients with type 2 diabetes (129, 308, 354, 487), and in non-diabetic subjects (106, 229, 541). In many studies microalbuminuria and albuminuria have been shown to be associated with many other CHD risk factors (90, 210, 270, 328). Haffner et al. have shown that normotensive subjects with microalbuminuria had significantly higher triglyceride and insulin concentrations during an oral glucose tolerance test than normotensive subjects without microalbuminuria suggesting an increased atherogenic risk factor pattern in normotensive subjects with microalbuminuria (200). Hyperinsulinemia and microalbuminuria are also strong predictors for CHD events in elderly non-diabetic subjects (270).

A number of different mechanisms have been postulated to link microalbuminuria to CHD including insulin resistance (186, 353). The greater the albumin excretion rate or blood pressure, the greater the insulin resistance in type 2 diabetes (405). Subjects with microalbuminuria have significantly higher insulin concentrations than the normoalbuminemic subjects (186, 270), a generalized increase in vascular permeability (110), an atherogenic lipoprotein pattern (110, 328) and endothelial dysfunction (245, 332, 449). The causal relationship between microalbuminuria and vascular functions is not established. Possibly microalbuminuria is just a sensitive marker of vascular damage.
Changes in oxidative stress

Free radicals are continuously produced during aerobic metabolism (73). Incomplete scavenging of reactive radicals leads to oxidation of cellular lipids, proteins, nucleic acids and glycoconjugates (48). An imbalance between protective antioxidants and increased free radical production results in oxidative stress. Oxidation of circulating LDL has been linked to the initiation and progression of atherosclerosis and ultimately to the pathogenesis of CHD (450).

Many studies have suggested that oxidative stress is increased in type 2 diabetes (20, 73, 74, 90, 342, 376, 456, 467) and contribute to the increased incidence of CHD in type 2 diabetes.

Endothelial dysfunction

Vascular endothelium has a central and primary role in the atherogenic process (239, 326, 327). Normally endothelium actively regulates vascular tone and permeability, the balance between coagulation and fibrinolysis, the composition of the subendothelial matrix, the extravasation of leukocytes and the proliferation of vascular smooth muscle and renal mesangial cells. To carry out these functions, the endothelium produces a variety of regulatory mediators, such as nitric oxide, prostanoids, endothelin, angiotensin II, t-PA, PAI-1, vWF, adhesion molecules and cytokine (388). At the in vivo level, endothelial cell products can be measured in the circulation, with altered concentrations potentially reflecting endothelial activation and dysfunction (448). Endothelial dysfunction can also be measured by measuring the ability of endothelium dependent agents to reduce vasodilatation (84, 88, 375).

Endothelial dysfunction has been found to characterize non-diabetic insulin resistant obese subjects (277), patients with IFG (492) and in young normotensive first-degree relatives of type 2 diabetic patients in association with insulin resistance (41). Multiple factors could contribute to endothelial dysfunction in type 2 diabetic subjects compared with non-diabetic subjects matched for traditional causes of endothelial dysfunction, such as smoking habits, LDL cholesterol concentrations and blood pressure. Such factors include those known to be associated with increased
cardiovascular risk, such as chronic hyperglycemia (258, 501), hyperinsulinemia independent of insulin resistance (426), insulin resistance and its consequences (405).

Adhesion molecules as markers of endothelial activation

Cellular adhesion molecules have been shown to participate in cell emigration, signaling functions and other vascular physiological responses (159, 160). Shedding of cellular adhesion molecules from the surface of activated endothelium and macrophages results in measurable plasma concentrations of soluble cellular adhesion molecules (384), which are increased in many inflammatory and immunological conditions characterized by endothelial dysfunction (256, 358, 523, 543).

There are four classes of adhesion molecules (160): 1) Integrins; 2) The immunoglobulin gene superfamily, the prototypic members of which are vascular cell adhesion molecule-1 (VCAM-1), intercellular adhesion molecule-1 and -2 (ICAM-1, -2) and platelet -endothelial adhesion molecule (PECAM), which all have been implicated in atherogenesis (393); 3) Cadherins; 4) Selectins, of which three types have been identified: E-, P- and L-selectin. E-selectin is expressed exclusively on endothelial cells (159).

In vitro, hyperglycemia promotes leukocyte adhesion to endothelial cells through up-regulation of cell-surface expression of E-selectin, ICAM-1 and VCAM-1 (341). Stimulation of endothelial cells with AGE also increases expression of these adhesion molecules (268). It has been shown that in non-diabetic subjects the degree of insulin resistance significantly correlates with concentrations of sE-selectin (80). In type 2 diabetic patients, concentrations of serum sE-selectin have been increased in most (23, 95, 97, 125, 451), although not all (128, 242) comparisons with normal subjects, and have correlated with glycemia in five studies (23, 95, 97, 125, 451). Most comparisons between type 2 diabetic patients and normal subjects have shown increased serum sICAM-1 concentrations in type 2 diabetic patients (97, 125, 128, 242, 271). In seven studies (23, 42, 108, 125, 242, 297, 451) serum sVCAM-1 concentrations have been increased in type 2 diabetic patients compared with normal subjects, while no difference was found in four studies (42, 95, 128, 242). A correlation with glycemic control and sVCAM-1 was found in one study.
These cross-sectional data suggest that sE-selectin may be a more sensitive indicator of hyperglycemia-induced endothelial activation \textit{in vivo} than sVCAM-1 or sICAM-1, although the expression of all three is regulated by glucose \textit{in vitro}.

Data on the effects of various treatments on circulating adhesion molecules in type 2 diabetes are very limited. A positive correlation with glycemic control was reported in two short-term studies (23, 94), which, respectively, included 16 and 34 patients with type 2 diabetes and lasted 2 and 12 weeks. There are, however, no data on long-term effects of insulin therapy on these markers of vascular inflammation or endothelial activation.

Regarding the relationship between circulating adhesion molecules and CHD, sVCAM-1 has been reported to be increased in patients with type 2 diabetes with overt macrovascular disease compared with patients who are clinically free of CHD (242, 367) and its concentration have been reported to correlate with intima-media thickness (367). sVCAM-1 concentrations have also been shown to correlate with the extent of atherosclerosis in non-diabetic subjects (381). sE-selectin concentrations have been shown to predict restenosis after angioplasty (51) and sICAM-1 concentrations predict the risk of future myocardial infarction in apparently healthy men (416).
2.2 GENERAL PRINCIPLES IN THE TREATMENT OF TYPE 2 DIABETES

The overall objective of treatment for type 2 diabetes is to prevent acute and chronic complications of diabetes while maintaining high quality of life. Because patients with type 2 diabetes have a 2 to 4-fold increased mortality from CVD, the ultimate goal of all therapies in type 2 diabetes is to reduce this burden.

2.2.1. Diet and exercise

Lifestyle modifications are important components of the treatment of type 2 diabetes.

Diet
There are at present no data which would have documented beneficial effects of diet therapy on cardiovascular outcome in patients with type 2 diabetes, on the other hand for IGT patients such data exists (473). Weight loss improves insulin sensitivity in obese non-diabetic subjects (177). Weight reduction in obese type 2 diabetic subjects has also been shown to ameliorate insulin resistance, but may not restore \( \beta \)-cell dysfunction (39,158). Although weight loss theoretically is the best treatment for type 2 diabetes, results obtained so far have not been particularly convincing. The largest study examining effects of diet intervention on glucose control and other parameters is the UKPDS, which was a randomized, controlled 11-year trial on the effects of improved metabolic control on complications in type 2 diabetes. After initial diet therapy, 4209 asymptomatic patients who remained hyperglycemic were assigned to either a conventional therapy policy, which primarily consisted of diet alone, or to an intensive therapy policy, which aimed at maintaining fasting plasma glucose concentrations less than 6.0 mmol/l, with assignment to primary therapy with sulphonylurea or insulin or metformin. The initial diet therapy substantially reduced plasma glucose concentrations and serum triglycerides, marginally decreased total cholesterol and increased HDL cholesterol concentrations (319). However, the effect of the diet was transient since glycemic control progressively deteriorated during the study, even in the individuals who were using
antihyperglycemic therapies (10). In addition to the UKPDS, data are sparse regarding long-term effects of weight loss. In the study of Uusitupa et al., 12 months intensiv diet education of recently diagnosed patients with type 2 diabetes resulted in improved glycemic control compared to a conventional treatment group. Only the intensively advised group lost weight and showed beneficial changes in serum lipids and lipoproteins (479,483). Weight reduction and a decrease in saturated fat intake appeared to be the main determinants of successful treatment results. During the second year of observation, both groups gained weight and glycemic control deteriorated. Despite this, a greater proportion of patients in the intensive as compared to the control group still was in good metabolic control. The intensively treated group also used less frequently antihyperglycemic agents than the conventional group (479,483). Similar results have been found also in other, usually more short-term, studies (251,372,484,505,507,508,510,511). A high intake of dietary fiber may also improve glycemic control, and lower plasma lipid concentrations and systolic or diastolic blood pressure in patients with type 2 diabetes (77,482). These effects are quite weak, however. Use of pharmacological agents such as orlistat may help to sustain weight loss (vide infra).

Cessation of smoking is also an important component in all lifestyle intervention programs.

**Exercise**

Prospective epidemiological data suggest that physical activity can reduce mortality from cardiovascular disease in patients with type 2 diabetes (502). In the Aerobic Center Longitudinal Study the association between low cardiorespiratory fitness and physical activity with total mortality in 1263 men with type 2 diabetes was studied. After adjustment for age, pre-existing and family history of cardiovascular disease, fasting glucose and cholesterol concentrations, overweight and hypertension, type 2 diabetic men in the low-fitne ss group had a risk for all-cause mortality of 2.1 compared to those in the high-fitne ss group. The majority of deaths were attributable to cardiovascular disease (502). Intervention studies which would have documented physical activity to reduce cardiovascular disease in patients with type 2 diabetes are currently lacking.
Regarding the mechanisms which might underlie the seemingly beneficial cardiovascular effects, physical training is known to improve insulin sensitivity of glucose uptake in skeletal muscle (263), and in many but not all of short-term training studies, potentially beneficial effects on serum lipids and blood pressure have been observed (525). In non-diabetic subjects, physical training has been consistently shown to enhance fibrinolysis by decreasing concentrations of PAI-1 (525). Data on training effects on such parameters are limited in type 2 diabetes.

2.2.2. Mechanism of action of oral agents

Fig. 5. shows mechanisms of action of OAD and Table 2 compares properties of various OAD.
## Table 2. Properties of various OAD

|                               | Dose range\(^1\) (mg) | Doses per day/dose increase (mg) | HbA1c - reduction (%)
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<td><strong>Liver glucose production inhibitors</strong></td>
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<td>Metformin</td>
<td>500-2500</td>
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<td>1.5-2.0</td>
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<td><strong>Insulin secretion enhancers</strong></td>
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<td>Sulphonylureas 1(^{st}) generation</td>
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<td>Tolbutamide</td>
<td>500-2000</td>
<td>2/500</td>
<td>↑↑</td>
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<tr>
<td>Chloropropamide</td>
<td>125-500</td>
<td>1/125</td>
<td>↑↑</td>
</tr>
<tr>
<td><strong>2(^{nd}) generation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glibenclamide</td>
<td>1.75-14</td>
<td>2/1.75</td>
<td>↑↑</td>
</tr>
<tr>
<td>Glipizide</td>
<td>2.5-15</td>
<td>1/2.5</td>
<td>↑↑</td>
</tr>
<tr>
<td>Gliclazide</td>
<td>80-320</td>
<td>2/80</td>
<td>↑↑</td>
</tr>
<tr>
<td><strong>3(^{rd}) generation</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glimepiride</td>
<td>1-8</td>
<td>1/2</td>
<td>↑↑</td>
</tr>
<tr>
<td>Glinides</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Repaglinide</td>
<td>0.5-4.0(^2) (12)</td>
<td>3-4/1</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>Nateglinide</td>
<td>120-360</td>
<td>3/30-120</td>
<td>↑↑</td>
</tr>
<tr>
<td><strong>Insulin sensitizers</strong></td>
<td></td>
<td></td>
<td></td>
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<td>Glitazones</td>
<td></td>
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<td></td>
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<tr>
<td>Pioglitazone</td>
<td>7.5-45</td>
<td>1/15</td>
<td>↑↑↑</td>
</tr>
<tr>
<td>Rosiglitazone</td>
<td>2-8</td>
<td>2/2</td>
<td>↑↑</td>
</tr>
<tr>
<td><strong>Carbohydrate absorption affecting Alpha glucosidase blockers</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Acarbose</td>
<td>100-300</td>
<td>3/50</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td>Miglitol</td>
<td>100-300</td>
<td>3/50</td>
<td>0.5-1.0</td>
</tr>
<tr>
<td><strong>Absorption inhibitors</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Guar gum</td>
<td>5-15 g</td>
<td>3/2.5 g</td>
<td>0.5</td>
</tr>
</tbody>
</table>

\(^1\) Recommended maximum dose in bold  
\(^2\) With maximum dose  
ND = not exactly known
**Metformin**

Metformin lowers blood glucose by inhibiting EGO (222). About 50 - 60 % of ingested metformin is absorbed. It is not bound to plasma proteins and is very little metabolized (38). Over 90 % is eliminated rapidly by the kidneys (38). Metformin induces less weight gain than other oral agents (118). Side effects of metformin include slight gastrointestinal dysfunction and slight danger of lactic acidosis (38), but it can cause vitamin B₁₂ malabsorption, which can be reversed by increased intake of calcium (46).

**Sulphonylureas**

Sulphonylureas (SU) stimulate insulin secretion by closing the ATP dependent K⁺-channels in β-cells (315,440). Differences between various sulphonylureas are shown in Table 2. Of the 2nd generation sulphonylureas glibenclamide is long-acting and the absorption of glibenclamide is rather slow and varies between individuals (225). It is completely metabolized (142) and half of a given dose of glibenclamide is eliminated by renal excretion of metabolites and a significant portion is excreted through bile. Although renal insufficiency should not alter the elimination rate of this very lipophilic drug, it may nevertheless increase the risk of hypoglycemia (142). Glipizide is also completely metabolized and the metabolites are inactive (142). It is relatively rapidly acting and also one of the most short-acting (142,335). Renal insufficiency does not seem to alter the elimination rate of glipizide.

Gliclazide is also a rapid- and short-acting sulphonylurea. Most of the gliclazide is eliminated by excretion of inactive metabolites through the kidneys, and a small fraction is eliminated by the bile (142). Gliclazide differs from other sulphonylureas in that it has antioxidant properties, the clinical significance of which is uncertain (92,180,237,350).

Glimepiride is a 3rd generation sulphonylurea which can be taken once per day (423). Glimepiride does not bind to ATP dependent K⁺- channels outside β-cells, such as those of the myocardial or vascular smooth muscle cells (443). Hypoglycemia may be a smaller treatment problem with glimepiride compared to gliben-
clamide (133). Main side effects of SU are hypoglycemia and gastrointestinal troubles and very seldom cholestatic hepatitis (335).

Glinides

Repaglinide and nateglinide are novel insulin secretagogues, which differ from the sulphonylureas in their molecular structure, time action profile and excretion mechanisms (40,134). They stimulate the prandial release of insulin by inhibiting the ATP dependent K⁺- channels in β-cells, and appear to bind to a different receptor site from sulphonylureas (40,134). They are rapidly absorbed and eliminated, which contributes to a fast onset and offset of action (40,134). Glinides are almost entirely metabolized. Metabolites of repaglinide are excreted mostly in the bile and those of nateglinide in the urine. In comparative clinical trials in patients with type 2 diabetes mellitus, nateglinide 120 mg and repaglinide 4 mg three times daily before meals provided similar glycaemic control as glibenclamide 10 mg/day (213).

Glitazones

Glitazones are peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists. PPAR-γ is a nuclear receptor that regulates the expression of several genes involved in metabolism. This receptor controls adipocyte differentiation, lipid storage, and insulin sensitisation (436). Besides metabolic activities, glitazones have effects as diverse as the control of host defense, cell proliferation, and tumorigenesis (228,360,436). The reduction of hyperglycemia is associated with a near normalization of the rate of EGO and a 40-60% increase in insulin mediated glucose disposal as measured by the glucose clamp technique (357). There are now two glitazones available, rosiglitazone and pioglitazone. When compared 8 mg rosiglitazone (twice a day for monotherapy) and 45 mg pioglitazone once daily the decrease in HbA₁c was similar (near 2 %). Ppioglitazone reduces LDL and triglycerides whereas rosiglitazone slightly increases them (252). HDL increased with pioglitazone treatment whereas there was no change with rosiglitazone (252). Weight gain was greater with pioglitazone and oedema was the same with both medications (252). Adverse effects are weight gain, edema and lowering of hemoglobin (163). This prohibits the use of these medications in patients with cardiac insufficiency.
Acarbose and guar gum

Acarbose inhibits in a dose dependent manner, competitively and reversibly, intestinal α-glucosidases and in addition displays an inhibitory effect on pancreatic α-amylase (265). Consequently, acarbose inhibits intestinal digestion of carbohydrates, especially of starch and sucrose. This effect leads to a delayed absorption of monosaccharides in distal parts of the small intestine (265) and results to a decrease in PPG and insulin concentrations, FBG is decreased only to a minor degree (265). Less than 2% of acarbose is absorbed from the gut. Acarbose reduces HbA1c about 0.5% (216,280) and HbA1 1.4% (220). Gastrointestinal side effects are the most common and for example occurred in UKPDS 8 to 30% (216).

Guar gum is a soluble dietary fiber which is used for lowering serum total cholesterol in patients with hypercholesterolemia and for lowering blood glucose in type 2 diabetic patients (481,482,494).

Orlistat

Orlistat is a powerful inhibitor of gastrointestinal lipase and as such, reduces fat absorption by approximately 30% (307). Orlistat is minimally absorbed. Adverse effects were primarily, and almost exclusively, gastrointestinal. Due to ability to block fat absorption, orlistat also has the capability to inhibit absorption of fatsoluble vitamins (307). The ability of orlistat to improve glycemic control is strictly proportional to the amount of weight loss achieved. In studies with orlistat 5 - 10% and >10% changes in body weight were associated with 1 and 1.5 - 2% decrease in HbA1c (214,298).

2.2.3. Effect of oral agents on risk factors and markers of micro- and macrovascular complications and hypoglycemia

Effects of OAD on various risk factors and markers of complications have been included in Tables 3 - 6. These Tables include studies after 1980, which have been either placebo controlled or have had an active comparative drug, contain data as HbA1 or HbA1c, and have lasted at least 3 months.
Table 3. Effects of metformin containing regimens on various markers of cardiovascular risk. Studies, performed after 1980 and which have been either placebo controlled or have had an active comparator drug, contain data as HbA1c or HbA1c, and have lasted at least 3 months, are included.

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental Comparator drug</th>
<th>Concomitant drug</th>
<th>HbA1c change %</th>
<th>FBG change mmol/l</th>
<th>S-FFA</th>
<th>S-Tg</th>
<th>S-Chol</th>
<th>S-HDL</th>
<th>S-LDL</th>
<th>Blood Pressure</th>
<th>Weight</th>
<th>S-ins</th>
</tr>
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<tr>
<td>deFronzo et al. (118)</td>
<td>Protocol 1</td>
<td>Metformin</td>
<td>↓1.5</td>
<td>↓3.1</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
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<tr>
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<td>0 *</td>
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<td></td>
<td>Protocol 2</td>
<td>Metformin</td>
<td>↓0.4</td>
<td>↓0.1</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td></td>
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<td></td>
<td>↓1.7</td>
<td>↓3.5</td>
<td>NS</td>
<td>↓</td>
<td>NS</td>
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<td>↓</td>
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<td></td>
<td>Glibencl.</td>
<td></td>
<td>↑0.2</td>
<td>↑0.8</td>
<td>NS</td>
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<td>Metformin</td>
<td>Metformin</td>
<td>↓1.4</td>
<td>↓3.3</td>
<td>NS</td>
<td>↓</td>
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<td>Metformin</td>
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<td>↑2.6</td>
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<td>NS</td>
<td>↓</td>
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<td>Hermann et al. (205)</td>
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<td>NS</td>
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<td></td>
<td>Placebo</td>
<td>Metformin</td>
<td>↓1.3</td>
<td>↓2.1</td>
<td>NS</td>
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<td>Josephkutty et al. (240)</td>
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<td>Troglitazone</td>
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<tr>
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<td>Metformin</td>
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<td>↑1.1</td>
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<td>Lalor et al. (279)</td>
<td>Metformin</td>
<td>Guar gum</td>
<td>↓2.8</td>
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<td>NS</td>
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<td>Metformin</td>
<td>↓1.9</td>
<td>NS</td>
<td>↓</td>
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<td>↓</td>
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<tr>
<td>Nagi et al. (349)</td>
<td>Metformin</td>
<td>Placebo</td>
<td>↓1.5</td>
<td>↓3.1</td>
<td>↓</td>
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<td>↓</td>
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<td>Metformin</td>
<td>↓0.7</td>
<td>↑</td>
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<td>↑</td>
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<tr>
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<td>Placebo</td>
<td>↓0.7</td>
<td>↑</td>
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</table>

* Mean change during placebo set as 0%.
↓ Significant decrease.
↑ Significant increase.
NS No significant change.
Glibencl. = glibenclamide
Table 4. Effects of regimens containing sulphonylureas or glinides on various markers of cardiovascular risk. Studies performed after 1980 and which have been either placebo controlled or have had an active comparator drug, contain data as HbA1c or HbA1c, and have lasted at least 3 months, are included.

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental Comparator drug</th>
<th>Concomitant drug</th>
<th>HbA1c change%</th>
<th>FBG change mmol/l</th>
<th>S-FFA</th>
<th>S-Tg</th>
<th>S-Chol</th>
<th>S-HDL</th>
<th>S-LDL</th>
<th>Blood Pressure</th>
<th>Weight</th>
<th>S-ins</th>
</tr>
</thead>
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<tr>
<td>Amador-Licona et al. (28)</td>
<td>Glibenclamide</td>
<td>Metformin</td>
<td>↓0.8</td>
<td>NS</td>
<td>↑</td>
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<td>↑</td>
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<td>Pagano et al. (370)</td>
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<td>Miglitol</td>
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<td></td>
<td>↓</td>
<td>↑</td>
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<td>↑</td>
<td>NS</td>
<td>↑</td>
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<tr>
<td>Salman et al. (429)</td>
<td>Gliclazide</td>
<td>Acarbose</td>
<td>↓1.8</td>
<td>↓2.0</td>
<td>NS</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓</td>
<td>NS</td>
<td>→</td>
<td>↑</td>
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<tr>
<td>Birkeland et al. (57)</td>
<td>Glibenclamide</td>
<td>Placebo</td>
<td>↑0.2</td>
<td>↑1.1</td>
<td>NS</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>NS</td>
<td>↑</td>
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<td>Horton et al. (218)</td>
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<td>Metformin</td>
<td>↓0.5</td>
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<td>NS</td>
<td>↑</td>
<td>↑</td>
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<td>NS</td>
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<td>Placebo</td>
<td>↑1.1</td>
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<td>NS</td>
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<td>Placebo</td>
<td>↑1.5*</td>
<td>↑1.9*</td>
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<td>NS</td>
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<td>Moses et al. (343)</td>
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<td>Metformin</td>
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<td>↑</td>
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<td>↑</td>
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<td>Placebo</td>
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<td>NS</td>
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<td>Raskin et al. (403)</td>
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<td>Troglitazone</td>
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<td>Wolffensbuttel et al. (517)</td>
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<td>Troglitazone</td>
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<td>Placebo</td>
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<td>↑</td>
<td>↑</td>
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<td>NS</td>
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<td>Metformin</td>
<td>↓0.5</td>
<td>↓0.2</td>
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<td>Glibenclamide</td>
<td>Metformin</td>
<td>↓1.0</td>
<td>↓2.4</td>
<td></td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>NS</td>
<td>↑</td>
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</tbody>
</table>

* Pharmacotherapy-naive patients, ↓ significant decrease, ↑ significant increase, NS no significant change
Table 5. Effects of glitazones containing regimens on various markers of cardiovascular risk. Studies, performed after 1980 and which have been either placebo controlled or have had an active comparator drug, contain data as HbA1c or HbA1c, and have lasted at least 3 months, are included.

<table>
<thead>
<tr>
<th>Study</th>
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<th>HbA1c change %</th>
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<th>S-Chol</th>
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<th>Blood Pressure</th>
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<tr>
<td>Fonseca et al. (152)</td>
<td>Rosiglitazone 4mg</td>
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<td>↑</td>
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<td>Comparator drug</td>
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<td>FBG change mmol/l</td>
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<td>S-Chol</td>
<td>S-HDL</td>
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<td>↓1.3</td>
<td>↓3.2</td>
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<td>SU</td>
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<td>600mg</td>
<td>SU</td>
<td>↓1.1</td>
<td>↓</td>
<td>↑</td>
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<td>SU</td>
<td>↑1.9</td>
<td>↑</td>
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<td>600mg</td>
<td>SU</td>
<td>↑0.9</td>
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<td>SU</td>
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<td>SU</td>
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<td>600mg</td>
<td>SU</td>
<td>↓0.8</td>
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<td>Yale et al. (522)</td>
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<td>Gliben.+met.</td>
<td>SU</td>
<td>↓1.4</td>
<td>↓</td>
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↓ Significant decrease.
↑ Significant increase.
NS No significant change.
SU sulphonylurea
Glibencl.=Gliben.=glibenclamide
Met.=metformin
Table 6. Effects of acarbose and orlistat containing regimens on various markers of cardiovascular risk. Studies, performed after 1980 and which have been either placebo controlled or have had an active comparator drug, contain data as HbA1c or HbA1c, and have lasted at least 3 months, are included.

<table>
<thead>
<tr>
<th>Study</th>
<th>Experimental drug</th>
<th>Concomitant drug</th>
<th>HbA1c change %</th>
<th>FBG change mmol/l</th>
<th>S-FFA</th>
<th>S-Tg</th>
<th>S-Chol</th>
<th>S-HDL</th>
<th>S-LDL</th>
<th>Blood Pressure</th>
<th>Weight</th>
<th>S-ins</th>
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<td>Placebo</td>
<td>↓0.6</td>
<td>↓0.9</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
<td>↓</td>
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<td>Coniff et al. (99)</td>
<td>Acarbose</td>
<td>Placebo</td>
<td>↓0.8</td>
<td>↓0.4</td>
<td>NS</td>
<td>NS</td>
<td>↓</td>
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<td>Acarbose 100mg x 3 t.i.d.</td>
<td>Placebo</td>
<td>↓0.7</td>
<td>↓1.1</td>
<td>↓</td>
<td>NS</td>
<td>↓</td>
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<tr>
<td></td>
<td>Acarbose 200mg x 3 t.i.d.</td>
<td>Placebo</td>
<td>↓1.1</td>
<td>↓0.9</td>
<td>NS</td>
<td>NS</td>
<td>↓</td>
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<td>Hanefeld (202)</td>
<td>Acarbose</td>
<td>Placebo</td>
<td>↓0.7</td>
<td>↓1.4</td>
<td>↓</td>
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<td>Placebo</td>
<td>↓1.4</td>
<td>↓0.8</td>
<td>↓</td>
<td>NS</td>
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<td>Hofman et al. (216)</td>
<td>Acarbose</td>
<td>Placebo</td>
<td>↓0.5</td>
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<td>Hollander et al. (214)</td>
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<td>SU</td>
<td>↓0.3</td>
<td>↑1.0</td>
<td>↓</td>
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<td>SU</td>
<td>↑0.2</td>
<td>↑1.2</td>
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<td>Lindgarde et al. (298)</td>
<td>Orlistat</td>
<td>Placebo</td>
<td>↓2.7</td>
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<tr>
<td>Lam et al. (280)</td>
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<td>SU &amp; Metformin</td>
<td>↓0.5</td>
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<td>↓</td>
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<tr>
<td></td>
<td>Placebo</td>
<td>SU &amp; Metformin</td>
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</table>

↓ Significant decrease.
↑ Significant increase.
NS No significant change.
* Mean change during placebo set as 0%.
SU sulphonylurea
**Glycemic control**

Chronic hyperglycemia is a significant predictor of both micro- and macrovascular complications (12,13,14), without any glycemic threshold for either type of complication (273). In the Wisconsin Epidemiologic Study of Diabetic Retinopathy (258) a 1 % increase in HbA1c increased the risk of developing retinopathy over 10 years by 60 to 70 % and the risk of death from ischaemic heart disease by 10 %. In the UKPDS, an increase of HbA1c from 6 to 10 % increased the risk of retinopathy 10-fold but the risk of myocardial infarction only 2-fold. This suggests that chronic hyperglycemia is a weak risk predictor of macrovascular disease compared to microvascular disease. On the other hand, a retinal photography is a far more sensitive tool to detect harmful effects of chronic hyperglycemia than is myocardial infarction, which represents the end point of a long silent atherothrombotic process.

**Serum free fatty acids, lipids and lipoproteins**

In many (75,113,118,174,259,348,373,435) although not all (66,154) studies metformin slightly has decreased triglycerides and serum cholesterol concentrations and increased HDL cholesterol. In some studies metformin has been shown to decrease also LDL cholesterol (113,215,344,400,521).

Improved glycemic control with SU treatment slightly lowers plasma total cholesterol, total triglyceride, VLDL cholesterol and triglycerides, and LDL cholesterol and apolipoprotein B (462). Improved blood glucose control in SU-treated type 2 diabetic patients is associated with either moderate increase (442) or no change (462) in HDL levels; however, HDL was not normalized even when plasma cholesterol and triglycerides were normal (461). In some (64,302) but not all (185,223) studies HDL cholesterol was lower in patients treated with SU rather than with insulin, despite comparable glycemic control.

Glitazones also induce changes in serum lipids and lipoproteins (98,224). The effects appear to be drug rather than class-specific. This conclusion is weakened by lack of data directly comparing different drugs in the same study. In pioglitazone studies (32, 135, 253, 339), pioglitazone reduced FFA and triglycerides and increased HDL, in LDL there was a little increase (135) or no significant change. Rosiglitazone has been shown to increase total serum cholesterol, LDL and HDL and
to decrease triglycerides (152, 377, 383, 516), but the decrease is less than with pioglitazone. All glitazones decrease serum FFA concentrations (135, 153, 310, 339, 437, 516). Orlistat, by inhibiting pancreas lipase, inhibit fat absorption and reduces significantly total cholesterol and especially LDL cholesterol (214, 298).

**Blood pressure**

Data on the effects of antihyperglycemic therapies on blood pressure are sparse (Tables 3 - 6) and do not allow any firm conclusions to be drawn.

**Coagulation and fibrinolysis**

Data are also sparse regarding effects of antihyperglycemic therapies on markers of coagulation and fibrinolysis. A decrease in PAI-1 antigen concentrations and PAI-1 activity with metformin treatment has been shown in many studies (154, 181, 182, 183, 373). A 24 - 48-month treatment with gliclazide has been shown to improve fibrinolytic activity, increase tissue plasminogen activator (t-PA) concentrations, and decreased PAI-1 activity (26). Gliclazide may also have an effect on fibrin network structure, rendering it more amenable to fibrinolysis, and may exert a potent free radical scavenging activity (237, 350). Inhibition of platelet adhesiveness and aggregability has also been shown (257). A potentially beneficial effect on prostacyclin/thromboxane ratios was also found (162).

**Urinary albumin excretion**

There are few data on effects of antihyperglycemic therapies on urinary albumin excretion. These have been summarized in Tables 3 - 6.
**Serum insulin concentrations**

Metformin generally decreases serum insulin concentrations (113,154,182,373), with a few exceptions, in two studies the insulin concentration did not change (66,391). Sulphonylureas generally increase insulin (57,190,413). Glitazones improve glucose tolerance, and decrease plasma insulin concentrations in a dose-dependent manner (219,224,516,522). Acarbose decreases postprandial insulin concentration compared with placebo or glibenclamide treatment (211). In the UKPDS no significant effect on insulin sensitivity or β-cell function were seen with acarbose treatment (216).

**Markers of endothelial activation**

Data are limited with respect to the effect of various oral antidiabetic agents on circulating levels of adhesion molecules. Treatment with sulphonylurea, sulphonylurea and metformin, and troglitazone for 2-3 months has been reported to decrease sE-selectin concentrations, possibly because of reduced oxidative stress (96,97). Gliclazide administration to type 2 diabetic patients may inhibit the increased adhesiveness of monocytes to endothelial cells and reduces the production of tumor necrosis factor-α (TNF-α) by these cells (125,410).

**Body weight**

Metformin generally does not change or reduces body weight, while sulphonylureas and especially glitazones increase weight. Acarbose does not greatly change body weight while orlistat decreases it (Tables 3 - 6).

**Hypoglycemia**

Metformin does not cause hypoglycemia (113) while hypoglycamia is a problem of sulphonylurea therapy. Glitazones or acarbose do not cause hypoglycemia (216,413) (Table 2).
2.2.4. Effect of oral agents on diabetic long-term complications

Macrovascular disease
So far there are limited data with respect to oral agents and macrovascular disease. A detrimental effect of tolbutamide on CVD was reported in a study published in 1970 by the University Group Diabetes Program (UGDP) (1,260), which found that CVD mortality was higher in patients given tolbutamide than in those given insulin (12.7 % vs 6.2 %) (334). This study initiated the controversy of SU and cardiac events. The UGDP results were widely debated and subsequently questioned because of a number of apparent problems with the study itself (107). In contrast, Paasikivi found that treatment with tolbutamide, after an acute myocardial infarction was associated with decreased mortality after 18 months compared with those treated with insulin (369). In the Secondary Prevention Reinfarction Israel Nifedipine Trial (SPRINT) (50), SU treatment after a cardiac infarction was associated with lower mortality rates than was insulin treatment.
By far the most extensive study examining effects of intensive therapy with either sulphonylureas, insulin or metformin on macrovascular disease has been the UKPDS. Intensive glycemic control, which was achieved using insulin or sulphonylureas (glibenclamide or chlorpropamide) decreased HbA1c by 0.9% and reduced macrovascular disease, as determined from the occurrence of myocardial infarction, by 16%, which was marginally significant (p=0.052) (Fig. 6).

**Fig. 6.** Adapted from UKPDS (14). Kaplan-Meier plots of aggregate endpoints: myocardial infarction for intensive and conventional treatment.
Importantly, this risk reduction for MI: 14 % for a 1 % reduction in HbA1c (454) corresponded closely to epidemiological predictions: 16 % lowering of MI for a 0.9 % decrease in HbA1c. This was perhaps the most important result of the UKPDS i.e. that it proved insulin and sulphonylureas not to have any harmful effects on macrovascular disease, at least in newly-diagnosed patients who were relatively young and free of coronary artery disease when the study started. In the UKPDS, in the overweight subset of patients randomized to metformin, the incidence of myocardial infarction decreased by 39 % compared with patients treated with diet alone or insulin or sulphonylurea (13) (Fig. 7).

Fig. 7. Adapted from UKPDS (13) Kaplan-Meier plots of aggregate endpoints: myocardial infarction for conventional (C), intensive (I) and metformin (M) treatment. Metformin vs conventional p < 0.01 and metformin vs intensive p < 0.12.
Microvascular disease

In the UKPDS, intensive glucose control with either sulphonylurea or insulin significantly (by 25%) decreased the risk of microvascular complications (renal failure or death, photocoagulation, vitreous haemorrhage) (14,454) (Fig. 8).

Fig. 8. Adapted from UKPDS (14) Kaplan-Meier plots of aggregate endpoints: microvascular disease, for intensive and conventional treatment.

In addition, there were significant decreases in cataract extraction (by 24%), retinopathy (by 21%), and albuminuria (by 33%) at twelve years (454).
2.3 INSULIN TREATMENT IN TYPE 2 DIABETES

The major indication for insulin treatment in type 2 diabetes is chronic hyperglycemia which cannot be adequately controlled with OAD.

2.3.1. Effect of different insulin treatment regimens on risk factors and markers of micro- and macrovascular complications and hypoglycemia

Effects of different combination therapies with insulin or insulin alone on various risk factors and markers of complications have been included in Tables 7 and 8. These tables include placebo controlled or parallel designed studies from 1990 and have lasted at least 3 months. The tables are separated to insulin-naive patients and previously insulin treated patients. Most of same kind studies before 1990 consisted only 9 to 22 patients and lasted from 1 to 4 months (37, 187, 188, 255, 272, 293, 304, 365, 407, 414, 433, 452), two lasted one year and consisted 64 and 30 patients respectively (70, 398) and one with 20 patients lasted 325 days (191).
Table 7. Effects of insulin containing regimens on various factors and markers of cardiovascular risk. Studies in insulin-naive type 2 diabetes patients. Table includes studies, performed after 1990 and which have been either placebo controlled or parallel designed and lasted at least 3 months

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>duration of treatment, months</th>
<th>insulin</th>
<th>concomitant drug</th>
<th>HbA1c or change %</th>
<th>FBG or change mmol/l</th>
<th>S-Tg</th>
<th>S-Chol</th>
<th>S-HDL</th>
<th>S-LDL</th>
<th>insulin dose compared to the other group</th>
<th>weight</th>
<th>blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chow et al. (83)</td>
<td>53</td>
<td>6</td>
<td>bedtime insulin b.i.d</td>
<td>SU and/or MET</td>
<td>↓ 1.5</td>
<td>↓ 4.9</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↑ 380 % (57 IU)</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Riddle et al. (412)</td>
<td>21</td>
<td>4</td>
<td>premix 30/70 b.s.</td>
<td>glibenclamide</td>
<td>↓ 1.3</td>
<td>5.9</td>
<td>↓</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↑ 58 %</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Riddle et al. (415)</td>
<td>145</td>
<td>6</td>
<td>premix 30/70 b.s.</td>
<td>glimepiride</td>
<td>↓ 0.8</td>
<td>7.5</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↑</td>
<td>↑</td>
<td>↔</td>
</tr>
<tr>
<td>Wolffenbuttel et al. (519)</td>
<td>95</td>
<td>6</td>
<td>bedtime insulin breakfast insulin b.i.d.</td>
<td>glibenclamide</td>
<td>↓ 2.4</td>
<td>6.0</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Karlander et al. (246)</td>
<td>20</td>
<td>11</td>
<td>premix b.i.d.</td>
<td>glibenclamide</td>
<td>↓ 2.0</td>
<td>6.0</td>
<td>↓</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
<tr>
<td>Landstedt-Hullin et al. (284)</td>
<td>80</td>
<td>4</td>
<td>preprandial regular</td>
<td>glibenclamide</td>
<td>↓ 1.2</td>
<td>2.8</td>
<td>↓</td>
<td>↓</td>
<td>↔</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
</tr>
</tbody>
</table>

n = number of patients, b.s. = before supper, b.i.d. = insulin twice daily, SU = glibenclamide or glipizide or gliclazide, ↓ = significant decrease or decrease in numbers, ↑ = significant increase or increase in numbers, ↔ = not significant change
Table 8. Effects of insulin containing regimens on various factors and markers of cardiovascular risk. Studies in patients treated previously with insulin. Table includes studies, performed after 1990 and which have been either placebo controlled or parallel designed.

<table>
<thead>
<tr>
<th>Study</th>
<th>n duration of treatment, months</th>
<th>insulin or concomitant drug</th>
<th>HbA1c or change %</th>
<th>FBG or change mmol/l</th>
<th>S-Tg</th>
<th>S-Chol</th>
<th>S-HDL</th>
<th>S-LDL</th>
<th>insulin dose compared to the other group</th>
<th>weight</th>
<th>blood pressure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relimpio et al.</td>
<td>47 4</td>
<td>previous insulin</td>
<td>metformin</td>
<td>↓1.9</td>
<td>↓2.9</td>
<td>↓</td>
<td>↓</td>
<td>↔</td>
<td>↔</td>
<td>↑20 %</td>
<td>↔</td>
</tr>
<tr>
<td>(409)</td>
<td></td>
<td>insulin increased</td>
<td>metformin</td>
<td>↓0.03</td>
<td>↓1.4</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↑29 %</td>
<td>↔</td>
</tr>
<tr>
<td>Aviles-Santa et al.</td>
<td>43 6</td>
<td>previous insulin</td>
<td>metformin</td>
<td>↓2.5</td>
<td>↓3.5</td>
<td>↔</td>
<td>↔</td>
<td>↓</td>
<td>↓</td>
<td>↑21 %</td>
<td>↔</td>
</tr>
<tr>
<td>(36)</td>
<td></td>
<td>insulin increased</td>
<td>placebo</td>
<td>↑1.6</td>
<td>↓3.5</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↑21 %</td>
<td>↔</td>
</tr>
<tr>
<td>Feinglos et al.</td>
<td>37 3</td>
<td>previous insulin</td>
<td>glipizide</td>
<td>9.8*</td>
<td>6.8</td>
<td>↔</td>
<td>↔</td>
<td>↓</td>
<td>↓</td>
<td>↑21%</td>
<td>↔</td>
</tr>
<tr>
<td>(140)</td>
<td></td>
<td>insulin increased</td>
<td>placebo</td>
<td>11.4*</td>
<td>8.7</td>
<td>↔</td>
<td>↔</td>
<td>↓</td>
<td>↔</td>
<td>↑21%</td>
<td>↔</td>
</tr>
<tr>
<td>Kelley et al.</td>
<td>145 6</td>
<td>previous insulin</td>
<td>acarbose</td>
<td>↓0.7</td>
<td>↓2.8</td>
<td>⊥</td>
<td>↔</td>
<td>↔</td>
<td>↑</td>
<td>↔8 %</td>
<td>↔</td>
</tr>
<tr>
<td>(250)</td>
<td></td>
<td>previous insulin</td>
<td>placebo</td>
<td>↑0.1</td>
<td></td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔8 %</td>
<td>↔</td>
</tr>
<tr>
<td>Robinson et al.</td>
<td>19 3</td>
<td>previous insulin</td>
<td>metformin</td>
<td>↓1.1</td>
<td>↓3.8</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔6 %</td>
<td>↔</td>
</tr>
<tr>
<td>study I (418)</td>
<td></td>
<td>replaced by placebo</td>
<td>placebo</td>
<td>↑0.5</td>
<td>↑1.9</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔6 %</td>
<td>↔</td>
</tr>
<tr>
<td>Robinson et al.</td>
<td>14 3</td>
<td>previous insulin</td>
<td>metformin</td>
<td>↑1.4</td>
<td>↑2.5</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↑44%</td>
<td>↔</td>
</tr>
<tr>
<td>study II (418)</td>
<td></td>
<td>replaced by previous metformin restarted</td>
<td></td>
<td></td>
<td></td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔6 %</td>
<td>↔</td>
</tr>
<tr>
<td>Lindström et al.</td>
<td>15 3</td>
<td>MDI</td>
<td>glibenclamid</td>
<td>6.0</td>
<td>7.0</td>
<td>↑</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↓25%</td>
<td>↔</td>
</tr>
<tr>
<td>(299)</td>
<td></td>
<td>MDI</td>
<td>placebo</td>
<td>6.3</td>
<td>7.8</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔25%</td>
<td>↔</td>
</tr>
<tr>
<td>Schwartz et al.</td>
<td>350 6</td>
<td>previous insulin</td>
<td>troglitazone</td>
<td>↓0.8</td>
<td>↓1.9</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑11%</td>
<td>↑</td>
</tr>
<tr>
<td>(437)</td>
<td></td>
<td>previous insulin</td>
<td>200 mg</td>
<td>↓1.4</td>
<td>↓2.7</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑</td>
<td>↑29%</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td>placebo</td>
<td>600 mg</td>
<td>↑0.1</td>
<td></td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↑29%</td>
<td>↑</td>
</tr>
<tr>
<td>Giugliano et al.</td>
<td>50 6</td>
<td>previous insulin</td>
<td>metformin</td>
<td>↓1.8</td>
<td>↓4.1</td>
<td>↓</td>
<td>↓</td>
<td>↑</td>
<td>↓</td>
<td>↓25%</td>
<td>↔</td>
</tr>
<tr>
<td>(174)</td>
<td></td>
<td>previous insulin</td>
<td>placebo</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔25%</td>
<td>↔</td>
</tr>
<tr>
<td>Chiasson et al.</td>
<td>91 12</td>
<td>previous insulin</td>
<td>Acarbose</td>
<td>↓0.4</td>
<td>↑0.1</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↓30%</td>
<td>↔</td>
</tr>
<tr>
<td>(82)</td>
<td></td>
<td>previous insulin</td>
<td>placebo</td>
<td>↓0.01</td>
<td>↑0.1</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↔</td>
<td>↓30%</td>
<td>↔</td>
</tr>
</tbody>
</table>

n = number of patients, * = HbA1c, MDI = multiple dose insulin, ↓ = significant decrease or decrease in numbers, ↑ = significant increase or increase in numbers, ↔ = not significant change
Glycemic control

According to a recent review (531), a total of 36 prospective comparisons (excludes the FINMIS and FINFAT studies), each lasting 2 months or longer, between various insulin-combination therapy regimens and insulin alone for the treatment of type 2 diabetic patients have been performed. Of the 9 studies carried out in insulin-naive patients, one compared insulin combined with sulphonylurea and metformin to insulin alone (83) and 8 compared sulphonylurea and insulin to insulin alone (37, 44, 284, 398, 412, 414, 415, 519). Better glycemic control was achieved with combination therapy in some studies (398, 412, 414) and similar improvement in others (37, 44, 83, 284, 519). Reduction of the insulin dose averaged 60% when both sulphonylurea and metformin were used in addition to insulin and 35% when sulphonylurea alone was used.

In studies in previously insulin treated patients treated with insulin plus metformin (36, 174, 409, 418), glycemic control was better compared with patients treated with insulin only. In studies patients treated with insulin and sulphonylurea after previous insulin therapy, glycemic control was better (191, 255, 272, 293, 301, 365, 407, 433, 452) or similar (70, 85, 140, 188, 299, 304) with insulin-combination therapy than with insulin alone. In all studies comparing insulin plus glitazones with insulin alone, glycemic control was better with insulin-combination therapy (402, 425, 437) than with insulin. In studies comparing insulin plus acarbose with insulin alone, glycemic control has been either better (250) or similar (82) with the combination than with insulin alone. Taken together this analysis shows that insulin combination therapy has not resulted in worse glycemic control in any study hitherto published than treatment with insulin alone. Overall, in approximately half of the studies insulin combination therapy has resulted in better glycemic control than insulin alone.

Only two studies have been conducted to determine which patient characteristics best predict a beneficial response to insulin or combined insulin and glibenclamide therapy. Wolffenbuttel et al. (518) have shown that only duration of diabetes and BMI were independent predictors of response to insulin. In their study poor responders were more obese and had significantly longer known duration of diabetes. In the study of Lewitt et al. (293) patients were previously insulin treated and then
glibenclamide was added to insulin therapy. Those who responded to that therapy had higher C-peptide concentrations (p < 0.001) and shorter duration of insulin therapy (p < 0.01) than those who did not responded.

*Serum free fatty acids, lipids and lipoproteins*

Elevations in FFA are associated with increased EGO and are reduced by small doses of exogenous insulin (465). Acute increase in FFA induces insulin resistance (144). Insulin treatment has shown to significantly decrease FFA levels in type 2 diabetic patients (284,412). One main effect of insulin on lipid metabolism in peripheral circulation is to stimulate lipoprotein lipase at the surface of endothelial cells to hydrolyze triglyceride-rich lipoproteins. Taskinen et al (464) have shown that intensive insulin treatment significantly reduces fasting FFA, total serum triglyceride, VLDL triglyceride, total cholesterol phospholipids, LDL, apolipoprotein B, and HDL$_3$ subfractions, and increases the mass of the HDL$_2$ subfraction, the ratio of HDL$_2$ to HDL$_3$, apolipoprotein AI and adipose tissue lipoprotein lipase activity (change +230 % in fat biopsies). Total HDL remained unchanged. These antiatherogenic lipid changes can only be achieved with very aggressive insulin therapy but are in general consistent with other data (346).

In a double-blind study, where insulin and glibenclamide were compared, for comparative levels of glycemic control, insulin treatment was found to result in higher HDL cholesterol and a higher HDL - total cholesterol ratio than glibenclamide (351). Also in some cross-sectional studies SU treatment alone has been associated with lower HDL cholesterol levels than insulin treatment (56,64,302). In contrast, another study demonstrated that about 50 % of patients taking low-dose (< 40 U/d) insulin experienced improved control of both blood glucose level and most of the lipids except for HDL cholesterol when transferred from insulin to glipizide (421). Yet in other studies the increase of HDL cholesterol was the same with insulin plus placebo and insulin plus SU treatment (187,246,365).

There are only few long-term data on effects of various therapies on serum lipids in type 2 diabetic patients. In the UGDPS, despite better glycemic control in the insulin variable treatment group, the trends in changes in serum cholesterol and triglycerides were not different from those observed in the placebo or insulin-standard
treatment group (4). In an uncontrolled 5-year insulin treatment study with different insulin treatment regimens there was a significant decrease of serum cholesterol and triglycerides (267). In the Veterans Affairs Cooperative Study in Type 2 Diabetes, where the mean follow-up time was 27 months, there was no difference in plasma lipids between the intensive and conventional insulin treatment groups (21).

Also, in one study using bedtime insulin and oral agents serum cholesterol concentration decreased while those of triglycerides and HDL remained unchanged (430).

**Blood pressure**

When type 2 diabetic patients have been treated with an implantable insulin pump (IIP) or with multiple doses of insulin (MDI) blood pressure has been reported to decrease with IIP but not with MDI (432). In an uncontrolled five year follow-up study of insulin treatment in type 2 diabetic patients, 68 % of patients had increased blood pressure values at the start and 67 % at five year after onset of insulin therapy (NS) (267). During the few studies, where treatment with insulin alone were compared to insulin plus sulphonylurea, there was no change in blood pressure and no difference within the groups (414,415). In one study, where insulin combination therapy was compared with insulin glargine and NPH, blood pressure remained unchanged for 1 year compared with baseline in both groups (534).

**Coagulation and fibrinolysis**

Studies concerning effects of insulin treatment on components of coagulation and fibrinolysis are sparse. In three studies PAI-1 activity has slightly but significantly decreased during insulin treatment (233,306,374). In two of these studies insulin and sulphonylurea treatments were compared, with the latter treatment no change in PAI-1 activity was found. In one study t-PA concentrations were higher when the treatment consisted only of insulin (ultralente or soluble) or ultralente and sulphonylurea than when only OAD were used (217). In contrast in one study no change in PAI-1 and t-PA levels was found with insulin treatment (497).

Recently Melidonis et al. have demonstrated that PAI-1 and fibrinogen increase remarkably in type 2 diabetic patients with acute myocardial infarction and that intensive insulin therapy may prevent these increments (336).
**Urinary albumin excretion**

Many studies including the study of Ohkubo et al. (361) and the UKPDS (14,15) show that UAER decreases when glycemic control improves regardless of whether insulin or SU is used to improve control. In the VA Study by Levin et al. (292) 153 male type 2 diabetic patients were randomly assigned to either intensive (HbA1c goal 7.1 %) or standard (HbA1c goal 9.1 %) insulin treatment and found that in intensive insulin treatment group the progression of microalbuminuria was retarded and the retardation was most pronounced in those patients who had microalbuminuria at baseline.

**Serum insulin concentrations**

Serum free insulin concentrations increase significantly with all insulin treatment regimens including those with evening NPH insulin (412,415), twice daily 30/70 regular/NPH insulin (300) with multiple insulin injections therapy (300,432) and with implantable insulin pump therapy (432). Diurnal serum free insulin levels have seldom been compared between two insulin regimens (412).

**Markers of endothelial activation**

Effect of insulin treatment on markers of endothelial dysfunction including circulating adhesion molecules have only been investigated in two studies. In one study 16 patients with type 2 diabetes received 2 weeks of insulin treatment with continuous subcutaneous insulin infusion (23). In this study, both sE-selectin and sVCAM-1 concentrations decreased significantly. In the other study 22 poorly controlled type 2 diabetic patients were treated for 16 weeks with insulin (542). HbA1c decreased from 11.8 % to 8.6 % but there were no significant changes in circulating concentrations of vWF, cellular fibronec tin, trombomodulin, t-PA, sE-selectin and sVCAM-1. Serum C-reactive protein concentrations were, however, found to decrease significantly (542).

**Body weight**

Modest but significant weight gain commonly occurs with insulin treatment alone (58,267,432) or in combination with SU (217,351,412,415,444,519,534) in type 2 diabetic patients. In three studies the weight gain was greater with insulin plus sulphonylurea treatment than with insulin alone (246,284,412). In one study the
weight gain was more pronounced when preprandial doses of regular insulin was used with glibenclamide than when glibenclamide was used with bedtime dose of NPH insulin (284). When Saudek et al. (432) compared IIP-therapy and MDI-therapy in type 2 diabetic patients, there was no weight gain in IIP group while weight continuously increased in the MDI group.

**Hypoglycemia**

It is not possible to prevent all hypoglycemic reactions with insulin treatment if the goal is good glycemic control (71), but in those studies where hypoglycemic episodes have been reported, they have not been frequent and usually well tolerated. In the UKPDS with conventional insulin treatment, the rate of major hypoglycemic episodes per year was 0.7 % and with the intensive insulin treatment 1.8 % (p < 0.0001). In one sulphonylurea and insulin combination treatment study the hypoglycemic episodes were significantly fewer in the evening insulin group than in the morning insulin group (444) and in another study, where bedtime insulin glargine was compared with bedtime insulin NPH during insulin combination therapy, there were less overall and nocturnal hypoglycemia with insulin glargine (534). Regarding severe hypoglycemia (requiring the assistance of another person), it can be estimated that the frequency is 19-fold higher in type 1 diabetes (31 episodes/100 pt years, HbA1c 7.8 %) (8,401,404) than in type 2 diabetes (1.6 episodes/100 pt years, HbA1c 8.0 %) (415,420).

Compared to type 1 diabetes (61 episodes/pt year, HbA1c 7.4 %) (278,401) the frequency of mild hypoglycemia can be calculated to be 14-fold lower with type 2 diabetes (4 episodes/ pt year, HbA1c 8.0 %) (420).

### 2.3.2. Effect of insulin therapy on diabetic long-term complications

**Macrovascular disease**

The UKPDS tested whether intensive glucose control with insulin influences macrovascular complications compared with conventional treatment (14). The incidence of MI was reduced by 16 %, which was marginally significant (p = 0.052) (Fig. 6) The predicted decrease, based on epidemiological data from the same study was 18 % for a 1 % reduction in HbA1c. The Diabetes Insulin-Glucose in
Acute Myocardial Infarction (DIGAMI I) study (316,317) tested whether insulin-glucose infusion initiated as soon as possible after the onset of MI and followed by long-term subcutaneous insulin treatment may have beneficial effect on the prognosis of type 2 diabetic patients. In the insulin treated group there were 306 patients and in the control group 314. The overall mortality after one year was 19 % in the insulin group compared to 26 % among controls (p < 0.05). The treatment effect was most pronounced in patients without prior insulin medication and who were at a low cardiovascular risk. In this stratum the in-hospital mortality was reduced by 58 % (p < 0.05) and the one year mortality by 52 % (p < 0.02). The most frequent cause of death in all patients was congestive heart failure (66 %), but cardiovascular mortality tended to be decreased in insulin-treated patients. However, this difference did not reach the level of statistical significance.

In a recently published study (387) 2 years of intensive glycemic control with insulin treatment did not affect the left ventricular systolic or diastolic function in patients with type 2 diabetes. Insulin therapy has been shown to normalize endothelium-dependent vasodilatation, an early functional alteration in patients with type 2 diabetes (491).

**Microvascular disease**

In a 6 year prospective study Ohkubo et al. (361) showed that intensive glycemic control by multiple insulin injection therapy can delay the onset and the progression of diabetic retinopathy, nephropathy and neuropathy in Japanese patients with type 2 diabetes.

In the UKPDS (14) intensive insulin therapy decreased the risk of microvascular end points in type 2 diabetes patients by 25 % (p < 0.01) (Fig. 8). The reduction in microvascular risk was principally due to fewer cases of retinal photocoagulation (29 % risk reduction, p < 0.005). There were also a decreased risk of deterioration in retinopathy (21 % risk reduction at 12 years follow-up, p < 0.02) and microalbuminuria (33 % risk reduction at 12 years follow-up, p < 0.0001). Importantly, there was no threshold for the development of microvascular complications in the UKPDS.
2.3.3. Causes of weight gain during insulin therapy

Weight gain increases insulin resistance and thereby naturally also increases its consequences e.g. blood pressure (405).

Although most patients with type 2 diabetes are overweight, weight loss generally precedes the diagnosis of type 2 diabetes (262). This weight loss is likely caused by hyperglycemia-induced wasting of energy because glucosuria and excessive consumption are associated with glucose overproduction (60). When glucose control is improved with insulin or sulphonylureas, energy loss in the urine decreases or ceases, weight increases and basal metabolic rate (kJ/min) (157,503) and dietary intake (313) are unchanged. The increase of body weight increases basal metabolic rate, the decrease in glycemia decreases basal metabolic rate by decreasing the energy consumed for glucose overproduction. If dietary intake remains unchanged, as has been observed (313), weight gain is proportional to reduction of glucosuria and its magnitude can be predicted from fasting glucose concentration (313). Because glucosuria appears when the fasting glucose increases to 10 - 12 mmol/l, weight gain is inevitable if insulin therapy is postponed until fasting glucose exceeds this concentration. A 5 mmol/l decrease in fasting glucose or a decrease in HbA1c by 2.5% from a baseline of 15mmol/l is associated with 5 kg of weight gain within 1 year, or 2 kg for a 1% decrease in HbA1c (313). The patient with poor glycemic control before the start of insulin therapy but with a good treatment response is therefore at greatest risk for weight gain. The possibility that metformin counteracts weight gain when used in combination with insulin has not been studied.

2.3.4. Causes of variation in insulin requirements in type 2 diabetes

Theoretically, exogenous insulin requirements could depend on 1) β-cell function, 2) the amount of insulin absorbed, 3) the action of absorbed insulin on glucose metabolism, 4) other factors such as insulin antibodies. Causes of variation in insulin absorption have been sparsely studied in patients with type 2 diabetes. In one study, subcutaneous fat thickness was not found to influence the disappearance rate of iodinated insulin (86). Insulin antibodies have not been found to influence insulin re-
quirements (209). Whether inter-individual variation in insulin sensitivity modulates insulin requirement in type 2 diabetes has not been studied.

One study with type 2 diabetic patients on continuous ambulatory peritoneal dialysis has shown in multivariate analysis that duration of diabetes, HbA1c, and body weight were independent determinants of insulin requirement in these patients (520).
3. AIMS OF THE STUDY

The present studies were conducted to answer the following questions:

1) What is the optimal insulin treatment regimen for type 2 diabetic patients (I, II)?
2) Do different bedtime insulin regimens differ with respect to their effects on weight gain in type 2 diabetic patients (II)?
3) What are the causes of inter-individual variation in insulin requirements in type 2 diabetes (III)?
4) Does long-term improvement of glycemic control by insulin therapy decrease endothelial activation as measured by serum levels of the soluble adhesion molecules sE-selectin and serum vascular cell adhesion molecule sVCAM-1 (IV)?

4. SUBJECTS AND STUDY DESIGNS

Baseline characteristics of the subjects are presented in Table 9. The study designs are described below. All studies were approved by the local ethics committees.
Table 9. Characteristics of the study groups

<table>
<thead>
<tr>
<th></th>
<th>Study I</th>
<th>Study II</th>
<th>Study III</th>
<th>Study IV</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Insulin treatment group</td>
<td>Control group</td>
<td></td>
<td>Type 2 diabetes Patients</td>
</tr>
<tr>
<td>Number of subjects</td>
<td>119</td>
<td>30</td>
<td>88</td>
<td>20</td>
</tr>
<tr>
<td>Men/Women</td>
<td>119</td>
<td>30</td>
<td>88</td>
<td>20</td>
</tr>
<tr>
<td>Age (y)</td>
<td>60±2</td>
<td>59±7</td>
<td>57±8</td>
<td>58±2</td>
</tr>
<tr>
<td>Duration of diabetes (y)</td>
<td>11±1</td>
<td>10±4</td>
<td>10±3</td>
<td>9±1</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>28.2±3.5</td>
<td>28.6±3.7</td>
<td>29±0.7</td>
<td>28.9±0.7</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>12.6±0.9</td>
<td>12.2±0.3</td>
<td>11.8±0.5</td>
<td>11.9±0.3</td>
</tr>
<tr>
<td>HbA₁c (%)</td>
<td>10±2</td>
<td>9.9±2</td>
<td>9.8±0.8</td>
<td>7.6±0.2</td>
</tr>
<tr>
<td>Serum triglycerides (mmol/l)</td>
<td>2.40±0.2</td>
<td>2.2±0.2</td>
<td>2.5±0.3</td>
<td>2.1±0.2</td>
</tr>
<tr>
<td>Serum total cholesterol (mmol/l)</td>
<td>5.8±0.9</td>
<td>5.9±0.5</td>
<td>5.8±0.3</td>
<td>5.1±0.2</td>
</tr>
<tr>
<td>Serum LDL-cholesterol (mmol/l)</td>
<td>3.4±0.2</td>
<td>3.3±0.3</td>
<td>3.7±0.6</td>
<td>3.07±0.2</td>
</tr>
<tr>
<td>Serum HDL-cholesterol (mmol/l)</td>
<td>1.4±0.2</td>
<td>1.4±0.2</td>
<td>1.1±0.5</td>
<td>1.1±0.4</td>
</tr>
</tbody>
</table>

Data are shown as mean±SEM * p < 0.05 †† p < 0.0001 for type 2 diabetic patients versus normal subjects, y = years
4.1 Comparison of insulin regimens in type 2 diabetic patients (FINMIS-study) (I)

*Design.* 153 type 2 diabetic patients who were treated with maximal doses of either glipizide (15 mg per day) or glibenclamide (10.5 mg per day) alone or in combination with metformin (0.5 to 2.0 g per day), a FBG concentration above 8.0 mmol/l and C-peptide concentration above the lower limit of normal (0.33 nmol/l), were randomized to be treated with continued OAD and NPH insulin in the evening (evening NPH group) or continued OAD and NPH insulin in the morning (morning NPH group), a 2 insulin injection regimen NPH insulin and regular insulin, in a ratio of 70 units to 30 units before breakfast and dinner (2 insulin injection group) without OAD, or a multiple insulin injection regimen, NPH insulin in the evening and regular insulin given before breakfast, lunch and dinner (multiple insulin injection group) without OAD, or to continued OAD. Baseline clinical characteristics of the five groups are shown in Table 10.
Table 10. Baseline clinical characteristics of the five groups of type 2 diabetic patients in study I (FINMIS)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Morning-NPH group</th>
<th>Evening-NPH group</th>
<th>Two-injection group</th>
<th>Multiple-injection group</th>
<th>Control group</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of patients</td>
<td>32</td>
<td>28</td>
<td>29</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>Men/women</td>
<td>12/20</td>
<td>15/13</td>
<td>12/17</td>
<td>18/12</td>
<td>11/19</td>
</tr>
<tr>
<td>Age (y)</td>
<td>59±8</td>
<td>60±6</td>
<td>59±7</td>
<td>60±5</td>
<td>59±7</td>
</tr>
<tr>
<td>Body-mass index (kg/m$^2$)</td>
<td>27.6±3.6</td>
<td>27.9±3.2</td>
<td>28.7±4.0</td>
<td>28.8±3.2</td>
<td>28.6±3.7</td>
</tr>
<tr>
<td>Duration of diabetes (y.)</td>
<td>11±4</td>
<td>10±6</td>
<td>10±4</td>
<td>11±5</td>
<td>10±4</td>
</tr>
<tr>
<td>Fasting blood glucose (mmol/l)</td>
<td>12.5±2.4</td>
<td>12.2±2.4</td>
<td>12.4±2.4</td>
<td>12.5±2.8</td>
<td>12.2±2.1</td>
</tr>
<tr>
<td>Fasting serum C peptide (nmol/l);‡</td>
<td>0.69±0.31</td>
<td>0.66±0.31</td>
<td>0.68±0.23</td>
<td>0.64±0.22</td>
<td>0.67±0.31</td>
</tr>
<tr>
<td>Serum creatinine (μmol/l);§</td>
<td>80±9</td>
<td>80±9</td>
<td>80±9</td>
<td>80±9</td>
<td>80±9</td>
</tr>
<tr>
<td>Urinary albumin excretion (mg/24h);¶</td>
<td>65±76</td>
<td>62±85</td>
<td>36±40</td>
<td>51±49</td>
<td>45±37</td>
</tr>
</tbody>
</table>

Oral hypoglycemic agents dose (percent of group)

| Glibenclamide (mg/day)                        | 11±2 (81)         | 11±2 (89)         | 12±2 (81)           | 11±2 (88)                | 11±1 (89)    |
| Glipizide (mg/day)                            | 18±3 (17)         | 19±3 (14)         | 17±3 (18)           | 22±6 (10)                | 18±3 (10)    |
| Metformin (g/day)                             | 1.3±0.5 (59)      | 1.3±0.5 (64)      | 1.2±0.5 (55)        | 1.4±0.5 (60)             | 1.4±0.5 (57) |

Beta-adrenergic-antagonists percent of group

|                | 27                | 25                | 35                  | 36                       | 44           |
| Diuretics      | 28                | 21                | 23                  | 12                       | 33           |
| Other drugs    | 68                | 61                | 54                  | 64                       | 65           |

* Plus-minus values are means ±SD
† Reference range, 0.33 to 0.69 nmol per liter
‡ Reference range < 115 μmol/l
¶ Patients with rates of albumin excretion ≥ 300 mg per 24 hours were excluded.
At the start of the study, the patients were admitted to hospital for 5 days. On day 1, fasting blood glucose, HbA1c, serum lipoproteins, body weight and blood pressure were measured. On day 2 insulin therapy was started and during the following days adjusted to achieve normoglycemia, defined as a FBG < 7 mmol/l and PBG < 10 mmol/l. The patients visited the treatment center at two weeks, one month and two months after start of the treatment period. At these visits, results of the daily fasting and weekly diurnal blood glucose measurements and hypoglycemia episodes were reviewed, body weight and fasting blood glucose were measured and the insulin dose was adjusted. After three months, patients were readmitted to hospital to repeat the tests performed on day 1.

4.2. Comparison of bedtime insulin regimens in type 2 diabetes (FINFAT-study) (II)

Design. 96 type 2 diabetic patients, with a FBG above 8 mmol/l, C-peptide concentration above 0.33 nmol/l who were treated with maximal dose of either glipizide (>15 mg per day) or glibenclamide (>10 mg per day), were recruited. Exclusion criteria were congestive heart failure, myocardial infarction or stroke during the past six months; epilepsy or other severe disease; liver disease; nephropathy as determined by a serum creatinine concentration greater than 120 µmol/l or macroalbuminuria; proliferative retinopathy or severe maculopathy; previous insulin therapy for more than 2 weeks; excessive alcohol consumption; night work. The patients were randomized in four treatment groups: 1) bedtime NPH insulin and glibenclamide (10.5 mg), 2) bedtime insulin and metformin (2 g), 3) bedtime NPH insulin and glibenclamide (10.5 mg) and metformin (2 g), 4) bedtime NPH insulin and morning NPH insulin.

Baseline clinical and biochemical characteristics of the different groups are shown in Table 11.
Table 11. Baseline clinical and biochemical characteristics of type 2 diabetic patients in study II (FINFAT).

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Bedtime insulin plus glibenclamide group (n = 22)</th>
<th>Bedtime insulin plus metformin group (n = 19)</th>
<th>Bedtime insulin glibenclamide and metformin group (n = 23)</th>
<th>Bedtime and morning insulin group (n = 24)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, (y)</td>
<td>61±2</td>
<td>57±2</td>
<td>55±2</td>
<td>58±2</td>
</tr>
<tr>
<td>Men, (%)</td>
<td>59</td>
<td>58</td>
<td>61</td>
<td>67</td>
</tr>
<tr>
<td>Body mass index, (kg/m²)</td>
<td>29.7±1.0</td>
<td>28.9±1.1</td>
<td>29.5±0.9</td>
<td>28.5±1.1</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.93±0.02</td>
<td>0.93±0.02</td>
<td>0.95±0.02</td>
<td>0.94±0.02</td>
</tr>
<tr>
<td>Glycosylated hemoglobin value, (%) †</td>
<td>9.8±0.3</td>
<td>9.8±0.4</td>
<td>9.9±0.3</td>
<td>10.1±0.4</td>
</tr>
<tr>
<td>Fasting blood glucose level, (mmol/l)</td>
<td>11.7±0.5</td>
<td>12.3±0.5</td>
<td>11.5±0.6</td>
<td>12.1±0.5</td>
</tr>
<tr>
<td>Fasting serum C peptide level, (nmol/l) §</td>
<td>1.1±0.1</td>
<td>1.1±0.2</td>
<td>1.0±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Antihypertensive drugs, (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thiazides or ß-blockers</td>
<td>8</td>
<td>7</td>
<td>3</td>
<td>7</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitors</td>
<td>9</td>
<td>10</td>
<td>9</td>
<td>7</td>
</tr>
<tr>
<td>Other drugs, (%)</td>
<td>50</td>
<td>42</td>
<td>40</td>
<td>42</td>
</tr>
<tr>
<td>Serum triglyceride level, (mmol/l)</td>
<td>2.7±0.5</td>
<td>2.4±0.4</td>
<td>2.3±0.2</td>
<td>2.6±0.5</td>
</tr>
<tr>
<td>Serum high-density lipoprotein cholesterol level, (mmol/l)</td>
<td></td>
<td>1.1±0.1</td>
<td>1.2±0.1</td>
<td>1.1±0.1</td>
</tr>
<tr>
<td>Serum total cholesterol level, (mmol/l)</td>
<td>5.7±0.2</td>
<td>5.9±0.3</td>
<td>5.8±0.2</td>
<td>5.8±0.3</td>
</tr>
<tr>
<td>Mean arterial blood pressure, (mmHg)</td>
<td>106±2</td>
<td>105±2</td>
<td>108±2</td>
<td>103±3</td>
</tr>
<tr>
<td>Median urinary albumin excretion rate (25th, 75th percentiles), (µg/min)</td>
<td>26 (8, 37)</td>
<td>10 (9, 22)</td>
<td>10 (8, 31)</td>
<td>11 (7, 31)</td>
</tr>
</tbody>
</table>

* Unless otherwise specified, data are expressed as the mean ±SE. All data except lipid measurements were obtained 6 weeks before the start of therapy; lipid data were obtained at the 0-month visit.
† Reference range, 4.0 % to 6.0 %.
§ Reference range, 0.33 to 0.75 nmol/l (1.0 to 2.0 ng/ml). To convert to ng/ml, divide by 0.333.
Patients were taught to self adjust their insulin doses according to home glucose measurements. The patients were given written instructions as shown in Table 12.

Table 12. Instructions to patients regarding self adjustment of bedtime insulin doses

<table>
<thead>
<tr>
<th>FBG</th>
<th>Insulin dose ↑</th>
<th>Insulin dose ↓</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 8.0 mmol/l on 3 consecutive days</td>
<td>4 IU/day</td>
<td></td>
</tr>
<tr>
<td>&gt; 6.0 mmol/l on 3 consecutive days</td>
<td>2 IU/day</td>
<td></td>
</tr>
<tr>
<td>&lt; 4.0 mmol/l on 3 consecutive days</td>
<td>2 IU/day</td>
<td></td>
</tr>
</tbody>
</table>

↑ = increase insulin dose, ↓ = decrease insulin dose, FBG = fasting blood glucose

Before start of insulin therapy, concentrations of FBG, HbA1c, fasting serum free insulin, C-peptide, triglycerides, cholesterol, and HDL cholesterol, W/H and blood pressure were measured. Following visits took place at 3 and 6 weeks and every 3 months for 1 year. At these visits, body weight, blood pressure, insulin dose, and side effects were recorded and FBG was measured. HbA1c was measured every 3 months. Measurement of serum C-peptide and lipids and W/H were repeated at 12 months. Compliance was monitored through pill counting.

4.3. What are the causes of inter-individual variation in insulin requirements in type 2 diabetic patients (III)

Design. Twenty type 2 diabetic patients with stable glucose control and insulin dose for at least 6 months and on combination therapy with bedtime NPH insulin and metformin for at least 1 year were admitted to the hospital 1 day before the studies. The patients participated in the following studies: 1) measurement of action of intravenous insulin on endogenous glucose production and utilization (Fig. 9), 2) measurement of absorption and action of subcutaneous insulin (Fig. 10) and 3) measurement of liver fat content (proton spectroscopy) (Fig. 11), intra-abdominal fat content (MRI) and subcutaneous fat thickness (ultrasound). In addition, body weight and body composition were determined as described below.
Fig. 9. Study design: measurement of the action of intravenous insulin on endogenous glucose production and utilization, using euglycemic insulin clamp technique combined with \([3-^3\text{H}]\)glucose.

Fig. 10. Study design: measurement of insulin absorption, (increase in free insulin and total insulin over 8 h after a subcutaneous dose (36 IU of regular insulin) and action of subcutaneous insulin (glucose infusion rate required to maintain euglycemia and suppression of FFA).
Fig. 11. Proton magnetic resonance spectra from 2 patients with fat percentages of 6 and 28%. The water peak has a chemical shift of 0 ppm, and the methylene (CH$_2$) signal of fat has the chemical shift of 3.4 ppm relative to the water peak. The height of the signal (y-axis) is in arbitrary units.

4.4. Effect of long-term improvement in glycemic control on serum sE-selectin and sVCAM-1 concentrations (IV)

Design. Subjects: 81 type 2 diabetic patients, who participated on study II (FIN-FAT) and 41 normal subjects were studied. The type 2 diabetic patients were treated with the following regimens: 1) bedtime NPH insulin and sulphonylurea (n = 20), 2) bedtime NPH insulin and metformin (n = 17), 3) bedtime NPH insulin and sulphonylurea and metformin (n = 21), 4) bedtime insulin and morning insulin (n = 23). The study design and measurements were as in study II. Serum sE-selectin and sVCAM-1 concentrations were determined in extra serum samples taken at 0, 3 and 12 months.
5. METHODS

5.1. Action of intravenous insulin (Study III)

At 8:00 p.m. on the evening before the study, an indwelling catheter, equipped with an obturator, was placed in an antecubital vein. To determine total rates of glucose production (Ra) - i.e., the sum of hepatic and renal glucose production (455) and utilization (Ru) - a primed continuous intravenous infusion of [3-³H]glucose was started at 3:00 a.m. and continued for a total of 660 min. The priming dose of [3-³H]glucose was adjusted according to the fasting blood glucose concentration measured at 3:00 a.m., as follows: priming dose = [glucose (mmol/l) at 3:00 a.m./5] x 20µCi. The dose was infused intravenously over 10 min. The continuous rate infusion of [3-³H]glucose was thereafter started at a rate of 0.2 µCi/min. At 7:30 a.m. another catheter was placed retrogradely in a heated (65 °C chamber) hand vein to obtain arterialized venous blood (331). Baseline blood samples were taken for measurements of insulin antibodies, total and HDL cholesterol, triglyceride and C-peptide concentrations. At 8:00 a.m., after a 300-min equilibrium period a primed continuous (0.3 mU · kg⁻¹ · min⁻¹) infusion of insulin was started, as previously described (123,395). Plasma glucose was allowed to decrease to 8 mmol/l and was then maintained at this level for 360 min using a variable rate infusion of 20 % glucose based on plasma glucose measurements, which were made from arterialized venous blood every 5 min. Blood samples for measurements of glucose specific activity (SA) were taken at -30, -20, -10 and 0 min and at 15 to 30 min intervals between 120 and 360 min. Serum free insulin concentrations were measured every 60 min and serum FFA at 0, 10, 15, 20, 25 and 30 min and then at 30 to 60 min intervals during the insulin infusion.

[³-³H]glucose SA and calculation of glucose kinetics. Plasma was deproteinized with Ba(OH)₂ and ZnSO₄ and evaporated as previously described (538). The dried glucose residue was resuspended in water and counted in a double-channel liquid scintillation counter (Reckbeta 1215; Wallac, Turku, Finland) after adding 10 ml Aquasol liquid scintillation fluid (NEN-DuPont, Boston, MA) and corrected for quenching. The [³-³H]glucose SA (in disintegrations per minute per micromole)
was calculated by dividing the disintegrations per minute in 0.3 ml plasma by the plasma glucose concentration (in micromoles per milliliter). The infusate was diluted 1:100 and 1:1000, and duplicates were counted to determine the infusate [3-$^3$H]glucose concentration. Glucose $R_a$ and $R_d$ were calculated using the Steele equation, assuming a pool fraction of 0.65 for glucose and distribution volume of 200ml/kg for glucose (395). Endogenous glucose $R_a$ was calculated by subtracting the exogenous glucose infusion rate required to maintain euglycemia during hyperinsulinemia (0-360 min) from the rate of total glucose $R_a$. The percent suppression of basal endogenous glucose $R_a$ during the final hour (300-360 min) by insulin was used as an index of the sensitivity of endogenous glucose production to insulin (percent suppression of endogenous $R_a$).

5.2. Absorption and action of subcutaneous insulin (Study III)

The patients did not take their bedtime NPH insulin injection before the insulin absorption study, which was performed after 10 to 12 h overnight fast starting at 7:30 - 8:00 a.m. Two indwelling catheters were inserted, one retrogradely in a heated hand vein for withdrawal of arterialized venous blood and another for infusion of 20 % glucose. A fixed dose of 36 U regular insulin was injected subcutaneously by the same investigator with a 29-gauge needle 4 cm left of the umbilicus. The temperature in the room was recorded in each study and was between 24 and 25 °C. We chose a large insulin dose for two reasons. First, we wished to quantitate insulin absorption from the increment in circulating free insulin concentrations, which can only be done reliably if a relatively large dose is used. Second, we also wished to quantitate the action of the subcutaneous insulin from the glucose infusion rate needed to maintain glucose at its fasting concentration. We chose a fixed dose of insulin and related the increment in circulating insulin concentrations to the estimated distribution space of insulin. The latter is roughly equal to the extracellular space and therefore closely correlated with fat-free mass (FFM) (537). A fixed rather than a variable dose of insulin adjusted to body size was chosen, since the absolute insulin dose injected influences the profile of absorbed insulin (285). Serum free and total insulin concentrations were measured before and for 480 min after the insulin injection, at 30 min intervals until 270 min and at 330, 420, and 480 min. Insulin absorption was calculated from the increase in free and total insu-
lin concentrations above basal during the 480 min period and expressed as area above basal divided by FFM (insulin absorption [mU/l] * min/kg FFM).

The action of subcutaneous insulin on glucose metabolism was assessed by determining the glucose infusion rate needed to maintain euglycemia during the 480 min period. The glucose infusion rate was adjusted based on plasma glucose measurements, which were performed from arterialized venous blood every 5 min for the entire 480 min. The action of subcutaneous insulin on FFA metabolism was assessed by measuring how serum FFA were suppressed by the insulin injection. Serum FFA were measured basally, at 10 min intervals between 0 and 60 min, at 30 min intervals between 60 and 270 min, and at 330, 420, and 480 min.

5.3. Measures of body composition (Study III)

Liver fat content measured by proton spectroscopy

Single-voxel (2x2x2 cm) proton spectra from the liver were acquired using 32 excitations, a loop surface coil, and an 1.5 T magnetic resonance device (Magnetom Vision; Siemens, Erlangen, Germany). Spatial location was achieved using a stimulated echo acquisition mode applied with a repetition time (TR) of 3000 ms and an echo time (TE) of 20 ms. A long TR and short TE were chosen to minimize effects of T1 and T2 relaxation, respectively, on signal intensities. Chemical shifts were measured relative to water signal intensity (Swater) at 4.8 ppm. Methylene signal intensity (Sfat), which represents intracellular triglyceride in the liver (459) was measured at 1.4 ppm. Signal intensities were obtained by time domain fitting routine VARPRO-MRUI (carbon.uab.es/mrui). This measurement of percent hepatic fat by proton spectroscopy has been validated against the lipid content of liver biopsies in humans (469) and animals (459). It has also been validated against liver density measurements performed by computed tomography (305). The latter validation was repeated by quantifying liver density in 8 of the patients in the present study with the HiQ computed tomography device (Siemens, Erlangen, Germany). The entire liver was scanned with a 10-mm collimator. By using a standard region-of-interest method, the density of normal liver parenchyma was calculated in Hounsfield units. The percent liver fat measured by proton spectroscopy correlated inversely (r = -0.85, p < 0.01) with liver density measured with computed tomogra-
phy, so the measurements correlated closely with respect to the fatty liver. Hepatic fat percentage was calculated by dividing \((100 \times S_{\text{fat}})\) by the sum of \(S_{\text{fat}}\) and \(S_{\text{water}}\). (Fig. 11)

**Abdominal fat distribution (MRI)**

A series of T1-weighted transaxial scans for the determination of visceral and subcutaneous fat were acquired from a region extending from 4 cm above to 4 cm below the fourth and fifth lumbar interspace (6 slices, field of view 375 x 500 mm², slice thickness 10 mm, breath-hold TR/TE 138.9 ms/4.1 ms). Visceral and subcutaneous fat areas were measured using image analysis program Alice (Alice version 3.0 by Hayden Image Processing Group, Waltham, MA). A histogram of pixel intensity in the intra-abdominal region was displayed, and the intensity corresponding to the nadir between the lean and fat peaks was used as a cut point. Visceral adipose tissue was defined as the areas of pixels in the intra-abdominal region above this cut point. For correlation of subcutaneous adipose tissue area, a region of interest was first manually drawn at the demarcation of subcutaneous adipose tissue and visceral tissue. The measurement could not be performed in 3 subjects because of their large body size.

**Subcutaneous fat thickness (ultrasound)**

The thickness, in millimeters, of the subcutaneous fat layer at the injection site was determined by high-frequency (10 MHz) ultrasound using an ImagePoint Multispacial Ultrasound System (Hewlett Packard, Andover, MA)

**Body weight and fat content**

Body weight was recorded and fat free mass and percent body fat were determined using a single frequency bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model #BIA-101A; RJA Systems, Detroit, MI) (156).

5.4. Cardiovascular risk predictors and markers (Studies I, II,III, IV)

**Glycosylated hemoglobin (I, II, III, IV)**

HbA1c was measured centrally in the same laboratory in all studies by high-
performance liquid chromatography (453) using the fully automated Glycosylated Hemoglobin Analyzer System (Bio-Rad, Richmond, CA).

Serum lipids and lipoproteins (I, II)
Total cholesterol, HDL cholesterol, triglycerides and lipoproteins were measured as previously described (171,464).

Adhesion molecules (IV)
Serum sE-selectin (Parameter catalog no. BBE 2B; R&D Systems, Minneapolis, MN) and sVCAM-1 (Parameter catalog no. BBE 3; R&D Systems) were measured by solid-phase enzyme-linked immunoassays.

Serum free and total insulin concentrations (I, II, III)
Serum free insulin concentrations were determined after precipitation with polyethylene glycol (124) using the Phadesph insulin radioimmunoassay (RIA) kit (Pharmacia, Uppsala, Sweden). Serum total insulin (IV) concentrations were measured with the same RIA kit without the polyethylene glycol precipitation.

5.5. Other measurements (Study III)

Insulin antibodies
Insulin antibodies were determined after incubation of serum with $^{125}$I-insulin at room temperature overnight by measuring total radioactivity and that remaining in the supernatant after precipitation with polyethylene glycol (124,168).

5.6. Statistical analyses

(I) The distribution of data was analyzed and changes between the groups compared with BMPD programs for detailed data description (program 2D) and analysis of variance (program 7D), followed by the Bonferroni test. Changes within a group were calculated by analysis of variance for repeated measures (program 2V). Symptomatic hypoglycemias were analyzed by multiway tables (program 4F), followed by Pearson’s chi-square statistic for comparison between groups. Linear re-
gression analysis were performed by simple and multiple linear regression analysis (program 8D and 1R).

(II) Analysis of variance for repeated measures was used for comparisons of normal distributed variables between the groups. Post hoc pairwise comparisons between the four groups were performed by using a Bonferroni correction if analysis of variance for repeated measures had significant results. Kruskal-Wallis test was used for comparison of means when the variance was not normally distributed. The GraphPad Prism program (GraphPad Software, San Diego, CA) was used to fit data relating indices of glycemia to frequency of biochemical hypoglycemias by searching for the best fit among linear and various nonlinear regression models. Goodness of fit was evaluated by the runs test and the $ F $ test (GraphPad Software). Frequencies of hypoglycemia among the groups were compared by using the chi-square test.

(III) Spearman's nonparametric rank correlation coefficient was used for all correlation analyses. Areas above basal were calculated using the trapezoid rule. Goodness of fit of individual nonlinear equations to mean data in the insulin absorption test was analyzed using the runs test and $ R^2 $. Goodness of fit of different equations was compared by calculating the significance of $ F $ ratio: whether, when using a complicated versus simple model, the relative increase in sum of squares was greater than the relative increase in degree of freedom. GraphPad Prism was used for all calculations.

(IV) Normal distribution was tested using Kolmogorov-Smirnov nonparametric test, and parameters non-normally distributed were log-transformed for statistical analysis. Comparison between patients and controls were performed using the unpaired Student's $ t $ test. Data between treatment groups were compared using analysis of variance for repeated measures, followed by post hoc pairwise comparison using the Fisher's least significant differences test. All correlation analyses were calculated using Spearman's rank correlation coefficient. GrapPad Prism (GraphPad Software, San Diego, CA or Systat statistical package, SYSTAT, Evenston, IL) were used for calculations.
6. RESULTS


Baseline clinical characteristics of the five groups of type 2 diabetic patients are shown in Table 10.

Insulin dose
The mean (± SE) doses of NPH insulin in the two groups receiving an OAD and NPH insulin were similar at three months (morning NPH group, 19 ± 1 U per day; evening NPH group, 20 ± 2 U per day). The total doses of insulin were also comparable in the two-injection group (total 43 ± 2 U per day; 27 ± 2 U per day before breakfast, and 16 ± 1 U per day before dinner) and the multiple injection group (total, 45 ± 3 U per day, regular insulin, 9.5 ± 0.7; 8.9 ± 0.7, and 9.4 ± 0.4 U per day before breakfast, lunch, and dinner, respectively; and 17 ± 1 U of NPH insulin per day at 9 p.m.)
Serum free insulin concentrations (Fig. 12)

Fig. 12. Changes in the mean diurnal serum free insulin concentrations after 3 months in each insulin-treatment groups as compared with the control group, as measured in the hospital. The control group (+), the morning NPH group (○), the evening NPH group (●), the two injection group (△), and the multiple injection group (■). * p < 0.05 and † p < 0.01 for insulin treatment group compared to with the control group. B = breakfast, L = lunch, D = dinner.

At baseline, mean diurnal free insulin concentrations were comparable among the groups and averaged 21 ± 1 mU/l. After 3 months of treatment, the mean diurnal free insulin concentration had increased by 29 % in the morning NPH insulin group, 14 % in the evening NPH insulin group, 39 % in the 2 injection group and 36 % in the multiple insulin group. When the insulin-treatment groups were compared with each other, the increment in the evening NPH insulin group was significantly less than in the other three groups (p < 0.05).
Glycemic control during the 6 week run-in period and during the treatment period are shown in Fig. 13, 14 and 15 and Table 13.

Fig. 13. Glycemic control during the 6 week run in period and during treatment in type 2 diabetic patients. The curves for mean diurnal blood glucose concentrations represent 70 % of the requested number of measurements in the five groups: the control group (+), the morning NPH group (o), the evening NPH group (●) the two injection group (■), and the multiple injection group (■). During the run-in period, the mean value for glycosylated hemoglobin decreased by 0.30 ± 0.09 percent (p < 0.001), the mean diurnal blood glucose concentration by 0.89 ± 0.24 mmol/l (p < 0.001), and the mean FBG concentration by 1.7 ± 0.2 mmol/l (p < 0.001). Each diurnal profile included blood glucose measurements before and 1.5 hours after breakfast, lunch and dinner and at 10 p.m. and 4 a.m.. The mean diurnal blood glucose concentration was significantly lower during treatment in each insulin-treated group than in the control group p < 0.01 to p < 0.001. p < 0.01 for the difference in values for glycosylated hemoglobin between the insulin -treatment groups and the control group at 12 weeks.
Fig. 14. Changes in mean diurnal blood glucose concentrations after 3 months in each insulin-treatment group as compared with the control group, as determined by home glucose monitoring. * p < 0.05 and † p < 0.01. B = breakfast, L = lunch, D = dinner. The control group (+). To convert values for glucose millimoles per liter, multiply by 0.05551

Fig. 15. Changes in mean diurnal blood glucose concentrations after 3 months in each insulin-treatment group as compared with the control group, as measured in the hospital. * p < 0.05 and † p < 0.01. B = breakfast, L = lunch, D = dinner. The control group (+). To convert values for glucose millimoles per liter, multiply by 0.05551
<table>
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<tr>
<th>Measure</th>
<th>Morning NPH group</th>
<th>Evening NPH group</th>
<th>Two-injection group</th>
<th>Multiple-injection group</th>
<th>Control group</th>
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<tbody>
<tr>
<td>Body weight (kg)</td>
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<td>Change</td>
<td>Baseline</td>
<td>Change</td>
<td>Baseline</td>
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<tr>
<td></td>
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<td></td>
<td>††§</td>
<td></td>
<td>††§</td>
<td>-0.5±0.2§</td>
</tr>
<tr>
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<td>20±2</td>
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<td>23±3</td>
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<tr>
<td></td>
<td></td>
<td>§**</td>
<td></td>
<td></td>
<td>23±3</td>
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<tr>
<td>Blood pressure (mmHg)</td>
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<td>Systolic</td>
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<td>138±2</td>
<td>5±4</td>
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<td>1±2</td>
<td>84±2</td>
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<td>Serum triglycerides (mmol/l)</td>
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<tr>
<td>Total</td>
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<tr>
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<td></td>
<td>††§</td>
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<td>VLDL</td>
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<td>1.4±0.1</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>-0.1±0.1</td>
</tr>
<tr>
<td>Serum cholesterol (mmol/l)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
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<td>6.1±0.2</td>
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<td>5.9±0.2</td>
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<tr>
<td>LDL</td>
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<td>3.6±0.2</td>
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<td>3.3±0.1</td>
</tr>
<tr>
<td>HDL</td>
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<td>-0.1±0.1</td>
<td>1.4±0.1</td>
<td>-0.1±0.1</td>
<td>1.4±0.1</td>
</tr>
</tbody>
</table>

* To convert values for insulin picomoles per liter, multiply by 7.2
§P < 0.05 for the change from base line
‡P < 0.05 for the comparison with the changes in the control group
†P < 0.001 for the change from base line
∥P < 0.001 for the comparison with the changes in the control group
††P < 0.01 for the change from base line
∥∥P < 0.01 for the comparison with the changes in the control group
†††P < 0.001 for the changes between the evening-NPH group and the multiple-injection group

Table 13. Effect of insulin therapy on body weight, glycemic control, blood pressure, and serum lipid concentrations before and after three months of treatment in type 2 diabetic patient. (FINMIS)
The mean diurnal blood glucose concentration was significantly lower in each insulin-treated group than in the control group (p < 0.01 to p < 0.001). HbA1c values decreased significantly and to similar extent in all the insulin-treated groups but changed little in the control group. (Fig. 13).

The glycemic control improved equally well with all insulin-therapy regimens though not to ideal level.

*Body weight (Table 13)*

The patients in all insulin-treatment groups gained weight during the three-month period. The smallest increment in body weight occurred in the evening NPH group (mean 1.2 ± 0.5 kg) and the largest in the multiple-injection group (2.9 ± 0.5 kg), p < 0.05 for comparison with the evening NPH group. The change in body weight was inversely correlated with the change in glycosylated hemoglobin (r = -0.20, p < 0.001) and positively correlated with the change in the mean diurnal serum free insulin concentration (r = 0.23, p < 0.001), suggesting that weight gain occurred concomitantly with improved glycemic control and hyperinsulinemia.

*Hypoglycemia*

The frequency of blood glucose concentrations below 4 mmol/l was 2 %, with no significant differences between the insulin treatment groups.

*Blood pressure (Table 13)*

Blood pressure remained unchanged in all groups.

*Lipids (Table 13)*

The concentration of serum VLDL triglycerides decreased by 13 to 28 % in the insulin treatment groups and by 7 % in the control group. The concentrations of total, LDL, and HDL cholesterol remained unchanged.
6.2. Comparison of bedtime insulin regimens in type 2 diabetes (FINFAT-study)

Baseline clinical and biochemical characteristics of the different treatment groups are shown in Table 11.

**Insulin dose**

After 12 months, the dosages of bedtime insulin were 24 ± 3 IU/d in patients receiving bedtime insulin and glibenclamide, 36 ± 9 IU/d in patients receiving bedtime insulin and metformin (p < 0.01 compared with all other groups), 20 ± 3 IU/d in patients receiving bedtime insulin and both oral drugs, and 24 ± 3 IU/d in patients receiving bedtime insulin and morning insulin. The dose of bedtime insulin was significantly higher in patients receiving bedtime insulin and metformin than in other patients at all time points starting 3 weeks after therapy (Fig. 16). In patients receiving bedtime insulin and morning insulin the morning insulin dose averaged 16 ± 1 IU at 3 weeks and 29 ± 3 IU at 12 months.
Fig. 16. Changes in body weight (top left), bedtime insulin doses (bottom left), glycosylated hemoglobin value (top right) and changes in the glycosylated hemoglobin value (bottom right) during 12 months of insulin treatment in patients receiving bedtime insulin and metformin (●), those receiving bedtime insulin and glibenclamide ( ), those receiving bedtime insulin and both oral agents (○), and those receiving bedtime insulin and morning insulin (△). In the top left and bottom left panels x = p < 0.05, xx = p < 0.01, and xxx = p < 0.001 for bedtime insulin and metformin compared with all other treatments, in top right and bottom right panels x = p < 0.05 and xx = p < 0.01 for bedtime insulin and metformin compared with bedtime and morning insulin and bedtime insulin and glibenclamide, in the top right and bottom right panels, * = p < 0.05 and ** = p < 0.01 for bedtime insulin and glibenclamide and metformin compared with bedtime and morning insulin.
Serum free insulin concentrations

Serum fasting free insulin concentrations were similar among the groups at baseline and averaged 12 ± 1 mU/L. At 12 months, the mean fasting serum free insulin levels were significantly increased (p < 0.05 for 12 months compared with 0 month), they averaged 17 ± 2 mU/L in patients receiving bedtime insulin and glibenclamide, 22 ± 4 mU/L in patients receiving bedtime insulin and metformin, 16 ± 2 mU/L in patients receiving bedtime insulin and both oral drugs, and 19 ± 3 mU/L in patients receiving bedtime and morning NPH insulin. Differences among groups were not statistically significant.

Glycemic control

HbA₁c concentrations were similar among groups before start of the treatment period (Fig. 16). At 3 and 6 months after therapy, patients receiving bedtime insulin and glibenclamide and metformin had a significantly lower HbA₁c value than those receiving bedtime insulin and glibenclamide and those receiving bedtime and morning insulin (Fig. 16). This difference disappeared after 6 months (Fig. 16). Unlike the other patients, patients receiving bedtime insulin and metformin showed a progressive decrease in HbA₁c values over time. At 12 months, HbA₁c values in this group averaged 7.2 % ± 0.2 %; this change and the absolute change (-2.5 ± 0.4 percentage points) differed significantly from that in the other groups (Fig. 16). Diurnal glucose profiles showed that the greatest decreases occurred at 4 a.m. and before breakfast, whereas the smallest decreases occurred after dinner and at 10 p.m. Glycemic control was significantly better in patients receiving bedtime insulin and metformin than in the other groups during the last 3 months of the study (Fig. 16).

Body weight

At 12 months, the patients receiving bedtime insulin and metformin had not gained weight compared with 0 month, (mean change 0.9 ± 1.2 kg, Fig. 16). This was significantly less than in the other groups, in which weight gain during 12 months therapy averaged 3.9 ± 0.7 kg in patients receiving bedtime insulin and glibenclamide, 3.6 ± 0.8 kg in patients receiving bedtime insulin and both two oral drugs, and 4.6 ± 1.0 kg in patients receiving bedtime and morning insulin.
Hypoglycemia

During 12 months therapy, the mean number of symptomatic hypoglycemic episodes per patient was 3.4 ± 1.0 in the bedtime insulin and glibenclamide group, 1.8 ± 0.4 in the bedtime insulin and metformin group, 3.3 ± 1.6 in the bedtime insulin and both oral drugs group, and 3.9 ± 1.6 in the bedtime and morning insulin group. The frequency of hypoglycemic episodes in patients receiving bedtime insulin and metformin was significantly lower (p < 0.05) than that seen in patients receiving bedtime and morning insulin. The mean frequency of biochemical (glucose level < 3.5 mmol/l) hypoglycemic episodes in fasting blood glucose measurements was significantly lower in the bedtime insulin and metformin group (1.1 %) and bedtime and morning insulin group (1.2 %) than in the bedtime insulin and glibenclamide group (2.2 %; p < 0.01 for comparison with both groups) and in the bedtime insulin and glyburide and metformin group (1.8 %; p < 0.05). The frequency of biochemical hypoglycemic episodes was correlated with the mean blood glucose level in a nonlinear fashion (Fig. 17).
Fig. 17. Relation between the mean annual fasting blood glucose concentration and the mean annual glycosylated hemoglobin value (top) and the frequency of biochemical hypoglycemias (bottom) (fasting glucose < 3.5 mmol/l). The regression equation relating fasting glucose and glycosylated hemoglobin was as follows: glycosylated hemoglobin = 4.4 [95% CI, 3.3 to 5.5] + 0.50[CI, 0.35 to 0.66] x fasting plasma glucose level [measured in mmol/l]. The gray area in the top panel indicates the normal range of glycosylated hemoglobin value. The dotted lines indicate glycosylated hemoglobin values that correspond to fasting glucose concentrations of 3 and 6 mmol/l.

The mean frequency of biochemical hypoglycemic episodes in the diurnal profiles was 1 % during 12 months of insulin therapy. The frequency was significantly lower among patients receiving bedtime insulin and metformin (0.6 %) than in those receiving bedtime insulin plus glyburide (1.0 %; p < 0.05), those receiving
bedtime insulin and both oral drugs (1.5%; p < 0.001), and those receiving bedtime and morning insulin (1.0%; p < 0.05). The highest frequency of biochemical hypoglycemic episodes was at 4 a.m. in all groups (Fig. 18).

**Fig. 18.** Frequency (percentage of all measurements at a given time point) of biochemical hypoglycemias (blood glucose concentration < 3.5 mmol/l) during home glucose monitoring and diurnal glucose profiles. These frequencies differed significantly among the groups before lunch (2.7% of 648 measurements before lunch in patients receiving bedtime insulin and glibenclamide and metformin compared with 0% patients receiving bedtime insulin and metformin [*** p < 0.001] and 0.1% in patients receiving bedtime insulin and glibenclamide [*** p < 0.001]) and at 4 a.m. (4.7% of 603 measurements at 4 a.m. in patients receiving bedtime insulin and glibenclamide compared with 2.2% in patients receiving bedtime and morning insulin; * p < 0.05). The frequency of hypoglycemia was also higher before lunch in the bedtime insulin and morning insulin groups than in the bedtime insulin and glibenclamide or metformin groups (** p < 0.01). B = breakfast, L = lunch and D = dinner.

**Blood pressure**

Blood pressure remained unchanged in all groups during the treatment period (II).
Lipids
Serum triglyceride concentrations were similar in all groups at baseline (Table 11). During therapy, these concentrations decreased by 0.8 ± 0.3 mmol/l in the bedtime insulin and glibenclamide group, 0.7 ± 0.3 mmol/l in the bedtime insulin and metformin group, 0.4 ± 0.2 mmol/l in the bedtime insulin and both oral drug groups, and 0.9 ± 0.3 mmol/l in the bedtime and morning insulin group. P < 0.001 for change compared with 0 months in all groups. Differences between the groups were not significant. Serum total cholesterol and HDL cholesterol remained unchanged in all groups during insulin therapy (II).

6.3. Causes of inter-individual variation in insulin requirements in type 2 diabetes (III)

Variation in insulin absorption and action as causes of variation in insulin requirements

Absorption of subcutaneous insulin
The area above basal under the insulin absorption curve, measured as free or total insulin, during the 480-min period was calculated for each patient to obtain an index of the amount of insulin absorbed. This index for free insulin, expressed as milliunits per liter times minutes, was closely correlated to that expressed as milliunits per liter times minutes per kilogram of body weight (r = 0.93, p < 0.0001) or milliunits per liter times minutes per kilogram of FFM (r = 0.92, p < 0.0001). The correlation between insulin absorbed, measured as the area under the total serum insulin curve (milliunits per liter times minutes) and that expressed as milliunits per liter times minutes per kilogram body weight, was r = 0.95, p < 0.0001. The correlation between the amount of insulin absorbed and that expressed as milliunits per liter times minutes per kilogram of FFM was r = 0.95, p < 0.0001. The relationships of parameters thought to influence insulin absorption are shown in Table 14. The best correlates for both total and free insulin were visceral fat volume, subcutaneous fat volume, and BMI. The thickness of subcutaneous fat, varied between 1.6 and 3.8 cm, but was not significantly correlated with insulin absorption (Table 14).
Table 14. Relationships (Spearman’s) between measures of overall adiposity and body fat distribution and the amount of absorbed insulin during the 480-min period in patients with type 2 diabetes.

<table>
<thead>
<tr>
<th>Measures of overall adiposity</th>
<th>Insulin area above basal (mU·l⁻¹·min⁻¹)</th>
<th>Insulin area above basal (mU·l⁻¹·min⁻¹·kg⁻¹FFM)</th>
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<tr>
<td></td>
<td>Free</td>
<td>Total</td>
</tr>
<tr>
<td></td>
<td>r</td>
<td>P</td>
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<td>Visceral/subcutaneous fat ratio</td>
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</table>
**Action of the subcutaneous insulin**

**Fig. 19** depicts the mean ± SE for serum free and total insulin, plasma glucose, and FFA concentrations and glucose infusion rate during 480-min period after subcutaneous insulin injection. The amount of free insulin absorbed, calculated as the area above basal, area above basal divided by FFM and area above basal divided by kilogram of body weight, varied 9.2, 10.6 and 10.9-fold.

![Graph showing absorption and action of subcutaneous insulin](image)

**Fig. 19.** Absorption and action of subcutaneous insulin. Plasma glucose concentrations (A), serum insulin concentrations (B), glucose infusion rate (C), and serum FFA concentrations (D) during 480-min period after subcutaneous injection of fixed dose of regular insulin. Free insulin •, total insulin €, mean values, dotted line patient with an exceptionally high bedtime insulin dose (176 U).

The action of absorbed insulin was assessed by 2 parameters, suppression of serum FFA (area under the FFA curve) and the amount of glucose infused to maintain euglycemia between 0 and 480 min (M-value). The M-value varied 11.5-fold. The ability of subcutaneous insulin to suppress FFA varied 7.5-fold. The amount of free insulin absorbed (area above basal over the 480 min period) was significantly correlated with both the area under the FFA curve during the 480-min period ($r = -0.63$, $p < 0.005$) and $M$ value ($r = 0.74$, $p < 0.001$) (**Fig. 20**). The corresponding correlations for total insulin were $r = -0.48$, $p < 0.05$, and $r = 0.62$, $p < 0.001$ (**Fig. 20**).
Fig. 20. The relationship between the amount of free (upper panels) and total (lower panels) insulin absorbed after subcutaneous injection (area above basal) and the action of subcutaneous insulin on FFA (A) and \(M\)-values (B).

**Action of intravenous insulin**

The time course for plasma glucose, serum free insulin, and FFA concentrations and the glucose infusion rate for the intravenous insulin action study are shown in Fig. 21.
Plasma glucose concentrations were similar in all subjects between 240 and 360 min and averaged 8.2 ± 0.2 mmol/l. The M-value (0-360min) varied 10.8-fold. During the final 2 h, glucose Rd averaged 2.75 ± 0.20 mg·Kg⁻¹·min⁻¹ and endogenous Ra 1.17 ± 0.23 mg·kg⁻¹·min⁻¹, which represented a 67 ± 7 % suppression below basal. FFA averaged 759 ± 53µmol/l basally and decreased to 352 ± 38 µmol/l between 240 and 360 min. Action of intravenous insulin to suppress FFA (area under the FFA concentration curve between 0 and 360 min) varied by 4.6-fold.

Relationships between the sensitivity of endogenous Ra to insulin during the final hour of intravenous insulin infusion and measures of overall adiposity and fat distribution in type 2 diabetic patients are shown in Table 15. The % hepatic fat was the best correlate of the % suppression of EGO by insulin (Fig. 22).

**Fig. 21.** Plasma glucose (A), serum free insulin (B), glucose infusion rate (C), and FFA (D) during the euglycemic insulin clamp study. Data are means ± SE.
**Table 15.** Relationship (Spearman’s) between measures of overall adiposity and body fat distribution and the sensitivity of endogenous $R_1$ to insulin in patients with type 2 diabetes

<table>
<thead>
<tr>
<th>Measures of overall adiposity</th>
<th>Percent suppression 300-360 min</th>
<th>Range</th>
<th>Fold variation</th>
<th>r</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver fat (%)</td>
<td>2 – 28</td>
<td>14.0</td>
<td>0.72</td>
<td>0.0013</td>
<td></td>
</tr>
<tr>
<td>Visceral fat volume (ml)</td>
<td>179 – 2053</td>
<td>11.5</td>
<td>0.56</td>
<td>0.0297</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat volume (ml)</td>
<td>526 – 1765</td>
<td>3.4</td>
<td>0.67</td>
<td>0.0065</td>
<td></td>
</tr>
<tr>
<td>Subcutaneous fat (cm)</td>
<td>1.6 – 3.8</td>
<td>2.3</td>
<td>0.32</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.3 – 36.4</td>
<td>1.6</td>
<td>0.54</td>
<td>0.026</td>
<td></td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>15 – 39</td>
<td>2.6</td>
<td>0.60</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>22 – 39</td>
<td>1.8</td>
<td>0.51</td>
<td>0.037</td>
<td></td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>67 – 127</td>
<td>1.9</td>
<td>0.44</td>
<td>NS</td>
<td></td>
</tr>
<tr>
<td>Measur es of fat distribution</td>
<td>WHR</td>
<td>0.92</td>
<td>1.3</td>
<td>0.58</td>
<td>0.01</td>
</tr>
<tr>
<td>Visceral/subcutaneous fat ratio</td>
<td>0.34 – 1.37</td>
<td>4.0</td>
<td>-0.02</td>
<td>NS</td>
<td></td>
</tr>
</tbody>
</table>
**Insulin antibodies**

The titer of insulin antibodies averaged 9.8 ± 1.6 % (range 3.6 - 27.3 %). The correlation between the titer of insulin antibodies and the daily insulin dose did not reach statistical significance (r = 0.37, p = 0.11)

**Relationship between insulin absorption and action, insulin antibodies, and the daily insulin dose**

The amount of insulin absorbed, measured either as free (r = 0.38, NS) or total insulin (r = 0.27, NS) during any 30-min time period between 0 and 480 min did not correlate significantly with the insulin dose, whereas the ability of subcutaneous insulin to suppress FFA (r = 0.70, p < 0.001) and M-value (r = -0.61, p < 0.005) were significantly correlated with the daily insulin dose (Fig. 23).
The relationship between the action of intravenous insulin to suppress serum FFA ($r = 0.46$, $p < 0.05$, FFA area under curve at 0 - 360 min) and $M$-value ($r = -0.46$, $p < 0.05$, 0 - 360 min) and the daily insulin dose were significant, albeit weaker than the relationship between the action of subcutaneous insulin and insulin dose (Fig. 23).

**Fig. 23.** The relationship between actions of intravenous (i.v.) (A and C) and subcutaneous (s.c.) (B and D) insulin on glucose infusion rate (A and B) and antilipolysis (C and D) and insulin dose. GINF = glucose infusion rate.

To examine the combined effects of insulin absorption, action and antibodies on daily insulin requirements, multiple linear regression analysis was used. The greatest $F$ ratio (13.4, $p < 0.001$ for model) and $R^2$ (61.3%) were found when the daily insulin dose (units per day) was the dependent variable and the action of subcutaneous insulin to suppress FFA ($p < 0.001$) and insulin antibodies ($p = 0.05$) were the independent variables. The $F$ ratio was 10.2 ($p < 0.002$ for model), and $R^2$ was 54.7% when the actions of intravenous insulin to suppress FFA ($p < 0.002$) and insulin antibodies ($p = 0.05$) were included as the dependent variables. Inclusion of the amount of absorbed insulin, measured as either free or total insulin, did not im-
prove the model. Use of $M$-value or intravenous insulin action on $R_d$ or endogenous $R_a$ instead of insulin suppression of FFA as a measure of insulin action did not improve the model.

**Hepatic fat content and its relationship to measures of total adiposity and fat distribution**

The % fat in the liver varied 14-fold, from 2 to 28%. Relationships with the % liver fat and measures of total adiposity and fat distribution were calculated with Spearman’s rank correlation coefficients. In univariate analyses, several measures of adiposity (BMI, $r = 0.63$, $p = 0.006$; % body fat $r = 0.60$, $p = 0.011$; subcutaneous fat volume, $r = 0.62$, $p = 0.016$) but especially total fat mass were significantly correlated with % of liver fat (Fig. 22), but neither W/H ($r = 0.40$, NS) nor the ratio between visceral and subcutaneous fat volume ($r = 0.02$, NS) was correlated with the % of liver fat.

**Hepatic fat role in insulin requirement in type 2 diabetes**

Table 16 and Fig. 22 show the relationships (Spearman’s $r$) between measurements of overall adiposity and fat distribution and the daily insulin dose. The % hepatic fat was the parameter best correlated with the insulin dose.

**Table 16.** Relationship (Spearman's) between measures of overall adiposity and body fat distribution and the daily insulin dose expressed as units per day and units per kilogram per day in patients with type 2 diabetes

<table>
<thead>
<tr>
<th></th>
<th>Insulin dose (U/day)</th>
<th>Insulin dose (U·kg$^{-1}$·day$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$r$</td>
<td>$P$</td>
</tr>
<tr>
<td>Liver fat (%)</td>
<td>0.76</td>
<td>0.0004</td>
</tr>
<tr>
<td>Measures of overall adiposity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Visceral fat volume (ml)</td>
<td>0.46</td>
<td>0.0048</td>
</tr>
<tr>
<td>Subcutaneous fat volume (ml)</td>
<td>0.47</td>
<td>0.0041</td>
</tr>
<tr>
<td>Subcutaneous fat (cm)</td>
<td>0.04</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>0.68</td>
<td>0.0009</td>
</tr>
<tr>
<td>Fat mass (kg)</td>
<td>0.60</td>
<td>0.005</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>0.31</td>
<td>NS</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>0.63</td>
<td>0.003</td>
</tr>
<tr>
<td>Measures of fat distribution</td>
<td></td>
<td></td>
</tr>
<tr>
<td>WHR</td>
<td>0.69</td>
<td>0.0008</td>
</tr>
<tr>
<td>Visceral/subcutaneous fat ratio</td>
<td>0.01</td>
<td>NS</td>
</tr>
</tbody>
</table>
6.4. Does improvement of glycemic control by long term insulin therapy affect endothelial activation measured by sE-selectin and sVCAM-1(IV)?

The characteristics of the type 2 diabetic patients and control subjects are shown in Table 17, and the metabolic parameters in patients with type 2 diabetes grouped according to the treatment regimens in Table 18.

### Table 17. Characteristics of the study groups. Study IV

<table>
<thead>
<tr>
<th></th>
<th>Patients with type 2 diabetes (n = 81)</th>
<th>Normal subjects (n = 41)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>58 ± 1</td>
<td>51 ± 1 **</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>52 / 29</td>
<td>28 / 13</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>82.4 ± 1.7</td>
<td>83.8 ± 1.7 §#</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>56.5 ± 1.3</td>
<td>58.4 ± 1.7 #</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94 ± 0.01</td>
<td>0.94 ± 0.01</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>9.7 ± 0.2</td>
<td>9.1 ± 0.1 §##</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>11.9 ± 0.3</td>
<td>6.8 ± 0.3 §</td>
</tr>
<tr>
<td>Serum triglyceride level (mmol/l)</td>
<td>2.5 ± 0.1</td>
<td>1.6 ± 0.1 §</td>
</tr>
<tr>
<td>Serum HDL cholesterol level (mmol/l)</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.04 †</td>
</tr>
<tr>
<td>Serum LDL cholesterol level (mmol/l)</td>
<td>3.5 ± 0.1</td>
<td>3.4 ± 0.1</td>
</tr>
</tbody>
</table>

Data are n or means ±SEM. * Reference range 4.0 - 6.0%. †P < 0.05, ‡P < 0.001 for 3 or 12 vs. 0 months; §P < 0.05, ¶P < 0.01, #P < 0.001 for 12 vs. 3 months, **P < 0.05, ‡P < 0.001 for patients with type 2 diabetes versus normal subjects, †† p < 0.0001.

### Table 18. Metabolic parameters in the patients with type 2 diabetes grouped according to the treatment regimen

<table>
<thead>
<tr>
<th></th>
<th>BI+SU</th>
<th>BI+MET</th>
<th>BI+SU+MET</th>
<th>BI+MI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>7/13</td>
<td>8/9</td>
<td>8/13</td>
<td>6/17</td>
</tr>
<tr>
<td>BMI (kg/m²), 0 months</td>
<td>28.7 ± 0.8</td>
<td>27.8 ± 0.9</td>
<td>28.6 ± 0.7</td>
<td>28.0 ± 1.1</td>
</tr>
<tr>
<td>Change in weight (kg), 12-0 months</td>
<td>3.6 ± 0.7 *</td>
<td>2.1 ± 1.0 *§</td>
<td>3.7 ± 0.9 *</td>
<td>4.6 ± 1.1 *</td>
</tr>
<tr>
<td>HbA1c (%) , 0 months</td>
<td>9.4 ± 0.3</td>
<td>9.9 ± 0.4</td>
<td>9.8 ± 0.4</td>
<td>9.8 ± 0.4</td>
</tr>
<tr>
<td>Change in HbA1c (%) , 12-0 months</td>
<td>-1.8 ± 0.3</td>
<td>-2.6 ± 0.4</td>
<td>-2.3 ± 0.3</td>
<td>-1.9 ± 0.4</td>
</tr>
<tr>
<td>Serum triglyceride level (mmol/l), 0 months</td>
<td>2.7 ± 0.6</td>
<td>2.3 ± 0.4</td>
<td>2.4 ± 0.2</td>
<td>2.7 ± 0.5</td>
</tr>
<tr>
<td>Change in serum triglyceride level (mmol/l), 12-0 months</td>
<td>0.3 ± 0.1</td>
<td>0.5 ± 0.5</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.3</td>
</tr>
<tr>
<td>Serum sE-selectin level (ng/ml), 0 months</td>
<td>72 ± 7</td>
<td>94 ± 11</td>
<td>66 ± 7</td>
<td>80 ± 7</td>
</tr>
<tr>
<td>Serum sE-selectin level (ng/ml), 3 months</td>
<td>65 ± 7 †</td>
<td>77 ± 11 *</td>
<td>60 ± 6 *</td>
<td>68 ± 5 ‡</td>
</tr>
<tr>
<td>Serum sE-selectin level (ng/ml), 12 months</td>
<td>67 ± 8 *</td>
<td>78 ± 10 †</td>
<td>62 ± 6</td>
<td>75 ± 7 *</td>
</tr>
<tr>
<td>Serum sVCAM-1 level (ng/ml), 0 months</td>
<td>363 (318-55)</td>
<td>362 (272-508)</td>
<td>356 (244-401)</td>
<td>448 (280-596)</td>
</tr>
<tr>
<td>Serum sVCAM-1 level (ng/ml), 3 months</td>
<td>334 (259-399) *</td>
<td>335 (236-429) †</td>
<td>312 (227-355) *</td>
<td>447 (288-593)</td>
</tr>
<tr>
<td>Serum sVCAM-1 level (ng/ml), 12 months</td>
<td>353 (309-439)</td>
<td>347 (308-509)</td>
<td>315 (227-487)</td>
<td>453 (317-593)</td>
</tr>
</tbody>
</table>

Data are n, means ±SEM, and median (range). *P < 0.05, †P < 0.01, §P < 0.001 for 3 or 12 vs. 0 months; §P < 0.05 versus other groups. BI=bedtime insulin, MI=morning insulin

Serum sE-selectin was 71% higher in the type 2 diabetic patients than in the normal subjects at baseline. This difference remained essentially unchanged after adjustment for serum triglyceride and HDL cholesterol concentrations, blood pressure, BMI, W/H, and age and also for CHD. Sixteen type 2 diabetic patients had CHD. In the type 2 diabetic patients there was no gender difference in sE-selectin concentrations, whereas the sE-selectin concentration was 64% higher in normal
men than women. Serum sVCAM-1 concentrations were comparable between the type 2 diabetic patients and the normal subjects (Table 18).

**Effect of insulin therapy in glucose control, body weight and serum lipids**

During 1 year of insulin therapy, HbA1c decreased from 9.7 ± 0.2 to 7.6 ± 0.1 % (p < 0.001) and fasting blood glucose from 11.9 ± 0.3 to 6.3 ± 0.1 mmol/l (p < 0.001). Body weight increased by 3.6 ± 0.5 kg (p < 0.001). Serum triglycerides decreased by 24 % from 2.5 ± 0.1 to 1.9 ± 0.2 mmol/l (p < 0.001). Serum LDL cholesterol concentrations remained unchanged and were comparable to those of the normal subjects (Table 18).

**Effect of insulin therapy in serum sE-selectin and sVCAM-1 concentrations**

After 3 and 12 months insulin therapy serum sE-selectin concentrations had decreased significantly compared to 0 months (Fig. 24), but the concentration at 12 months was still 55 % higher than in the normal subjects (Fig. 24). Serum sVCAM-1 decreased transiently during the first 3 months and then increased back to baseline (Table 18) by 12 months.
Correlations between clinical and biochemical characteristics and serum sE-selectin and sVCAM-1 concentrations

At baseline, HbA1c correlated with sE-selectin concentrations in diabetic men ($r = 0.29$, $p < 0.05$), but not in diabetic women ($r = -0.07$, NS). None of the other clinical or biochemical parameters correlated with sE-selectin at baseline. Within the normal subjects, the % of body fat correlated with sE-selectin both in men ($r = 0.48$, $p < 0.01$) and women ($r = 0.63$, $p < 0.05$). Serum triglycerides were also positively correlated with sE-selectin concentrations both in women ($r = 0.65$, $p < 0.05$) and in men ($r = 0.38$, $p < 0.05$).
The change in HbA\textsubscript{1c} by 1 year of insulin therapy both in diabetic men and women, was significantly correlated with the change in sE-selectin concentrations (Fig. 25). Changes in other parameters did not correlate with the change in serum sE-selectin concentrations.

sVCAM-1 concentrations correlated with HbA\textsubscript{1c} at baseline both in diabetic women (r = 0.47, p < 0.001) and men (r = 0.28, p < 0.05). In the control subjects, the % fat (r = 0.56, p < 0.005) in women and systolic blood pressure in men (r = 0.38, p < 0.05) were significantly correlated with sVCAM-1 concentrations.

**Fig. 25.** Relationship between change in glycemic control versus serum sE-selectin concentrations within the patients with type 2 diabetes. • BI+SU, ◆ BI + MET, ▼ BI + SU + MET, ▲ BI + ML. BI=bedtime insulin; ML=morning insulin
7. DISCUSSION

7.1 Comparison of insulin treatment regimens in patients with type 2 diabetes

Type 2 diabetes is regarded as a progressive disorder (13, 14, 114), where use of insulin is frequently necessary at some stage of the natural course of this disease. When these studies were begun more than 10 years ago, data comparing effects of various insulin regimens on glycemic control and other cardiovascular risk markers were sparse (191, 238, 382, 394, 398). Insulin treatment was frequently started in a hospital following the principles used to treat patients with type 1 diabetes. These principles often included prescription of a diet which included snacks in addition to main meals and use of multiple insulin injections. We decided to question this approach given that type 1 and 2 diabetes are fundamentally different diseases. From the point of view of insulin therapy, it is important to remember that 80% of patients with type 2 diabetes are obese (169) when insulin therapy is started and that weight gain is a frequent and undesirable side effect. Because of obesity and possibly other factors, type 2 diabetic patients are also markedly insulin resistant even when normoglycemic, in contrast to type 1 diabetic patients, who are normally sensitive to insulin if normoglycemic (535, 536). On the other hand, there are type 2 diabetic patients who are lean and not necessarily insulin resistant. These considerations suggest that patients with type 2 diabetes need greater insulin doses than patients with type 1 diabetes and also that variation in insulin requirements is much greater than in patients with type 1 diabetes. Another factor which could have implications with respect to insulin therapy is the presence of residual endogenous insulin secretion in type 2 diabetic patients. It might be possible to use simple insulin regimens perhaps together with one or two oral agents, which allow satisfactory glycemic control to be achieved without the use of multiple insulin injections and frequent blood glucose monitoring. Possibly use of agents such as metformin might counteract weight gain or use of insulin at bedtime rather than in the morning. The timing of the insulin injection might simply influence eating patterns and possibly be beneficial in counteracting weight gain.
Glycemic control, weight gain and insulin requirements during various insulin treatment regimens

To test the validity of the above hypotheses, 2 major studies comparing insulin treatment regimens were performed. In the first study called FINMIS, 153 poorly controlled type 2 diabetic patients were treated with four different insulin regimens or with continued maximal doses of two oral agents. In this study (I), we found, for the first time, that use of evening NPH insulin combined with oral drugs improved glycemic control as much as other insulin regimens including the regimen where multiple insulin injections was used but was associated with less weight gain than the other regimens. This beneficial effect on body weight has subsequently been confirmed in many studies (85, 412, 415, 430). Clearly, the beneficial effect on body weight of the evening NPH and sulfonylurea and metformin combination regimen was not due to metformin, since the group using identical oral drugs but NPH in the morning gained more weight than the group injecting insulin in the evening. However, the study did raise the question of whether a similar result could have been obtained by using only one agent and if so whether this agent then should be a sulfonylurea or metformin. The FINFAT study (II) was designed to answer this question. In this study 4 different bedtime insulin regimens were compared for 1 year in 96 patients with type 2 diabetes. The patients were previously treated with sulfonylurea only to maximize the likelihood of observing differences in weight gain which could be attributable to use of metformin. Results of this study were striking in the sense that the group using bedtime NPH and metformin differed from the other groups in several respects. This group experienced the least weight gain, had the best glycemic control and also the lowest number of hypoglycemia. The latter also explained why this group was able to increase their insulin dose more than the group using bedtime NPH and sulfonylurea. From data available in the literature, one could calculate that on the average use of a maximal dose of either a sulfonylurea or metformin reduces insulin requirements by 32 % and use of two agents by 62 % (531). In the FINFAT study, the insulin dose in the 2-injection group was 53 IU/day, in the bedtime NPH and glibenclamide group 24 IU/day, in the bedtime NPH and metformin group 36 IU/day, in the bedtime NPH plus glibenclamide and metformin 20 IU/day. Thus, the insulin dose was 32 % lower in the bedtime insulin and metformin and 62 % lower in the group using bedtime NPH and 2 oral agents than in the 2-injection group. The unexpected find-
ing was that the group using NPH and glibenclamide used only 24 IU/day i.e. 55 % instead of the expected 30 % less than the 2-injection group. This finding was, however, explained by greater frequency of hypoglycemia in this as compared to the other groups. Of note, these results may not be generally applicable to all intermediate or long acting insulins and not necessarily to all sulfonylureas. For example, insulin glargine, which has a peakless time-action profile compared to NPH (290) may not induce as much hypoglycemia with a sulfonylurea than NPH (534).

In our experience the combination of metformin and bedtime insulin can be used both in obese and lean type 2 diabetic patients. The obese patients may have even greater benefit than lean, because of the ability of metformin to counteract weight gain. In another study (539), we have shown that after an initial good response, glycemic control deteriorates more in obese than in non-obese patients with insulin alone or with insulin and sulphfonylurea plus metformin. In that study, after one year of treatment, HbA1c was not significantly lower than that at the baseline, but in the FINFAT study - after one year of treatment with metformin and bedtime insulin, glycemic control was still good.

**Lipids and blood pressure during insulin therapy**

In both studies I and II, serum lipids and lipoproteins changed rather similarly regardless of the insulin treatment regimen. Serum triglycerides decreased by 12 to 25 % in 3 months with the insulin regimens in study I and by 17 to 35 % in 12 months with the regimens in study II. Serum total, HDL and LDL cholesterol remained unchanged in all studies. The lack of change in HDL cholesterol can be expected as insulin therapy increases LPL activity and the mass of HDL2 particles but decreases that of HDL3 (464). These data indicate that from the point of view of lipids and lipoproteins, use of a simple combination therapy regimen is not disadvantageous compared to more complicated regimens.

In study I systolic and diastolic blood pressures decreased significantly in the control group but remained unchanged in all insulin-treatment groups. In study II blood pressure also remained unchanged in all treatment groups. It is important to note that neither study was powered to detect changes in blood pressure. The decrease in blood pressure in the control group but not in the insulin treatment groups in study I
could be due to changes in body weight i.e. body weight decreased in the control
group and increased in all insulin treatment groups. In a follow-up of the FINMIS
patients, a highly significant increase in systolic blood pressure was observed (539).
In the latter analysis, the increase in blood pressure was significantly correlated
with changes in body weight and an increase in LDL cholesterol.

**Serum insulin concentrations**

In study I during the 3 months of treatment, the mean diurnal free insulin concen-
tration increased by 29 % in the morning-NPH group, 14 % in the evening-NPH
group, 39 % in the two injection group and 36 % in the multiple-injection group as
compared with the values at baseline. The increment in the bedtime NPH and sul-
fonylurea and metformin group was significantly smaller than in the other groups.
This suggests, in the face of similar improvement in glycemic control that either
nocturnal insulinization is more effective in lowering glucose concentrations than
daytime increments in insulin concentrations, or that endogenous insulin can act
better during the day to suppress EGO during meals during bedtime NPH combina-
tion than during morning NPH or multiple insulin injection therapy.

**Body weight**

Modest but significant weight gain commonly occurs with insulin treatment alone
(58, 267) or combination with sulphonylureas (217, 351, 412, 415, 444, 519, 534)
in type 2 diabetic patients. In study I improvement in glycemic control was signifi-
cantly correlated with weight gain in the insulin treatment groups, whereas the op-
posite was true in the control group. As discussed above, the weight gain in the in-
sulin treated patients is likely to be caused by inhibition of hyperglycemia- induced
wasting of energy because of glucosuria and excessive energy consumption associated
with glucose overproduction (60). If dietary intake remains unchanged, as we have
previously observed during treatment with insulin alone as well as with insulin plus metformin (313), weight gain is proportional to reduction of glucosuria and can even be predicted based on how much the fasting glucose concentration decreases (313).

Factors other than correction of glycemia also influence weight gain. In study I, the
patients in the evening-NPH group gained less weight than the patients treated with
other insulin regimens. The cause of this phenomenon remains speculative. One possibility is that insulin itself promotes weight gain. Since serum free insulin increased less in the evening-NPH than in the other groups, peripheral hyperinsulinemia may perhaps have been responsible for weight gain via stimulation of lipogenesis in adipose tissue and stimulation of appetite (62).

The data from study II demonstrate that metformin prevents weight gain during insulin therapy. This beneficial effect of metformin is consistent with previous data from studies comparing oral agents such as the Multicenter Metformin Study (118) and more recently the UKPDS (16). The mechanisms underlying the beneficial effect of metformin on body weight is unclear. Recent data of causes of weight gain in patients using insulin alone and those using insulin and metformin suggests, however, that the weight gain-sparing effect of metformin is due to reduced energy intake (313).

Hypoglycemia
The frequency of biochemical hypoglycemia in study I (blood glucose < 4 mmol/l) was 2% of all measurements, with no significant differences between the insulin treatment groups and there were no serious hypoglycemia episodes. When compared to e.g. the DCCT (12) study, the frequency of biochemical hypoglycemia in the present study was close to that of severe hypoglycemia in the DCCT implying that hypoglycemia is much less common in type 2 than in type 1 diabetes. In study II the frequency of symptomatic and biochemical (blood glucose < 3.5 mmol/l) hypoglycemic episodes was approximately 2-fold lower in patients receiving bedtime insulin plus metformin than in the other groups (Fig.18). These data are consistent with those of the UKPDS (11), in which the frequency of any hypoglycemia in patients using metformin was 4-fold lower than in patients with equally well-controlled type 2 diabetes who received glibenclamide. Data from studies I and II suggest that metformin combined with glibenclamide does not reduce hypoglycemic episodes i.e. that use of glibenclamide masks any beneficial effect of metformin on hypoglycemia.

As in patients with type I diabetes (9), the frequency of hypoglycemic episodes was closely correlated with mean fasting glycemia. Although no severe hypoglycemic
episodes occurred, the frequency of biochemical hypoglycemic episodes increased markedly when the mean annual fasting glucose level was less than 6 mmol/l (Fig. 17). The latter glucose level corresponded to a mean annual HbA1c value of 7.4% (Fig. 17). These data imply that normoglycemia is not necessarily a safe target during insulin therapy and that it is not realistic to achieve normoglycemia by using bedtime insulin combined with metformin, glibenclamide or a second injection of insulin.

**Self adjustment of insulin dose – a key to successful insulin therapy**

In the FINMIS and FINFAT studies the range of the insulin requirements varied at least 20-fold *i.e.* from 8 to 168 IU/day (I-II). The causes of this large variation are unknown but were explored in study III. Even after the knowledge accumulated in study III, it is, however, impossible to exactly predict insulin requirements in an individual patient, which means that the insulin dose has to be titrated individually. If a patient needs 168 IU of bedtime insulin, and treatment is started with a low dose such as 10 IU/day, and the dose is adjusted only at outpatient visit every 3 months by 4 units, it would take 119 months or approximately 10 years before the treatment goal would be achieved. In the FINFAT study, the 168 IU dose was achieved in 6 months because the patient was taught self-adjustment of the insulin dose. This extreme example illustrates how important it is to develop flexible and simple algorithms which can be used by the patient, to achieve glycemic targets in a reasonable time frame. It is also noteworthy that in the FINFAT study, insulin therapy was not, as in study I started in the hospital but on an outpatient basis.

**7.2. Causes of variation in the insulin dose (III)**

Study III was undertaken to determine causes of variation in the insulin dose of patients with type 2 diabetes, who were in relatively good glycemic control with a stable insulin dose. Theoretically, insulin requirements could depend on the amount of insulin absorbed, the action of absorbed insulin and possibly other factors such as insulin antibodies. Previous insulin absorption studies have been performed almost exclusively in patients with type I diabetes using iodinated insulin ([125I]-labeled regular insulin) to trace insulin absorption (109, 161, 209, 264, 285, 496). Those studies have shown that insulin absorption, at constant temperature under
resting conditions, is slowed by increases in subcutaneous fat thickness (208, 441),
total dose injected (285), decreases in subcutaneous blood flow (207, 495, 496) and
injection site (208). Insulin antibodies have not been found to influence insulin re-
quirements (209), nor has the amount of insulin absorbed been shown to correlate
with insulin action in normal subjects (544). Causes of variation in insulin absor-
ption have been sparsely studied in patients with type 2 diabetes. In one study, sub-
cutaneous fat thickness was not found to influence the disappearance rate of iod-
nated insulin (86). Whether interindividual variation in insulin sensitivity modu-
lates insulin requirements in type 2 diabetes has not been studied. For those rea-
sons, we wished in study III to quantify the extent to which insulin absorption and
action determine insulin requirements in patients with type 2 diabetes. On separate
days, using [3-3H]glucose and the euglycemic insulin clamp technique, we deter-
mined the amount of absorbed insulin from the increment in free and total insulin
concentrations after a subcutaneous injection of regular insulin and the action of
intravenous insulin on suppression of FFA and glucose production and utilization.
The action of subcutaneous insulin was also quantitated by measuring the ability of
subcutaneous insulin to suppress FFA. To search for causes of variation in insulin
absorption and action, several parameters characterizing body size, fat content, and
fat distribution were quantitated. These parameters included measurements of he-
patic fat content by proton spectroscopy, visceral fat by MRI, body fat content by
bioimpedance plethysmography, and subcutaneous fat thickness by ultrasound.

**Insulin absorption**

The mean insulin dose of the patients using bedtime NPH insulin and metformin
was 42 IU/day (36 IU/day excluding the patient using 176 IU/day). To mimic the
mean dose, to enable ranking of patients with respect to their insulin sensitivity, and
to make the absorption study feasible to perform, 36 IU of regular insulin was in-
jected subcutaneously, and the increase in free and total insulin concentrations and
glucose requirements for the ensuing 8-h period were followed. This period was not
long enough to allow the entire absorption and action profile to be determined (Fig.
19). The amount of absorbed insulin therefore only provides an index of insulin ab-
sorption. Even in normal subjects, absorption of regular insulin is markedly slow.
In the study by Ziel et al. (544), the maximal concentration of regular insulin in
non-diabetic non-obese subjects after a 10 IU subcutaneous injection was observed
at 112 min, and the glucose infusion rate was maximal at 256 min. Even after subcutaneous injection of a short-acting insulin analog (21 IU to non-obese non-diabetic subjects), the glucose infusion rate remains increased until 8 h (204). Consistent with these observations, the possibly slower insulin absorption in obese type 2 diabetic patients than in type 1 diabetic patients (86) and the decrease in the rate of insulin absorption at increasing insulin doses, the glucose infusion rate did not reach maximum until ~360 min after the subcutaneous injection in the present study (Fig. 19). Although the insulin concentrations, the action of subcutaneous insulin on serum FFA concentrations, and the glucose infusion rate did not return to the baseline within the 8-h period, the absorption study nevertheless provided some useful information about absorption and action of the subcutaneous insulin. First, the significant correlation between the amount of absorbed insulin and its action on both FFA and glucose metabolism (Fig. 20) suggests that variation in insulin absorption had biologically significant consequences. Second, the significant correlation between the action of absorbed insulin and the insulin dose on one hand (Fig. 23) and the better correlation between subcutaneous than intravenous insulin action and the insulin dose on the other, support the idea that measurement of insulin absorption provided physiologically meaningful information, and that the amount of absorbed insulin does influence insulin requirements because it influences insulin action.

**Insulin action**

In searching for factors associated with insulin requirements, the correlation between the ability of insulin to suppress serum FFA and the insulin dose was better than that between the \( M \)-value and the insulin dose (Fig. 23). Also the correlation between actions of subcutaneous and intravenous insulin was clearly better for action on FFA than on glucose metabolism. The superiority of FFA suppression versus the \( M \)-values as a measure of insulin action is likely to be technical because the glucose concentrations were variable during the insulin absorption study. Such variations in the glucose concentrations will induce some variation in \( M \)-values because of the mass action effect of hyperglycemia (540). Because antilipolysis is not regulated by glucose in humans (532), use of the area under the FFA curve during insulin infusion or injection is subject to less variability than \( M \)-value under conditions of varying glycemia. Insulin action seemed a more important determinant of
variation in insulin requirements than insulin absorption since there was no correlation between the amount of insulin absorbed and insulin dose, but there was highly significant correlation between subcutaneous insulin action and the insulin dose.

**Measures of obesity**

Regarding causes of variation in insulin absorption several measures of body fat (visceral and subcutaneous fat volume, BMI, weight, and fat mass) rather than subcutaneous thickness at the injection site were found to be associated with the amount of insulin absorbed, regardless of whether the area under the absorption curve or the area divided by FFM was used as the measure of the amount of insulin absorbed. Previous data in type 2 diabetic patients are sparse. In the study of Clauson et al. (86) in type 2 diabetic patients, insulin absorption was followed using of 5 U regular $^{125}$I-insulin. In that study, which included obese and non-obese patients, no correlation was observed between depth of the fat layer and residual radioactivity at the 3 injection sites examined (86). On the other hand, consistent with the present data, studies performed in type 1 diabetic patients have demonstrated significantly slower insulin absorption, measured as residual radioactivity at the injection site, in obese than in non-obese patients (441, 495). As in the present study, however, subcutaneous fat thickness did not explain variation in insulin requirements in these studies (441, 495).

Of the simple parameters measured, BMI was one that correlated with the amount of absorbed insulin (Table 14), the percent fat in the liver, the sensitivity of EGO (Table 15), and insulin dose (Table 16). Thus insulin is more slowly absorbed and acts less efficiently to suppress EGO and FFA concentrations in obese than in non-obese patients. These factors increase not only the absolute insulin dose but also the dose needed per kilogram of body weight.

**Hepatic fat content**

Because inhibition of EGO represents the major target for insulin therapy (338, 465), it was particularly interesting to search for parameters that might explain inter-individual variation in hepatic insulin sensitivity. Percent liver fat was found to be most closely correlated with suppression of EGO by insulin (Table 15). It was also correlated with the insulin dose. The results suggest that 60% of the variation
in daily insulin dose (Fig. 22) was attributable to variation in hepatic fat content, possibly via effects of hepatic adiposity on the sensitivity of EGO to insulin (Fig. 22). This relationship may seem surprisingly strong considering that daily insulin requirement should also be influenced by inter-individual differences in diet composition and exercise habits. When body weight is stable and physical activity habits are constant, however, insulin requirements also should stabilize. Because physical activity and body weight are key determinants of insulin sensitivity (529), and inhibition of EGO is the primary target of insulin therapy (528), the correlations between liver fat content, insulin sensitivity, and insulin requirements are physiologically feasible and expected.

The relationship between liver fat percentage and hepatic insulin sensitivity supports recent evidence that has led to the classification of nonalcoholic steatohepatitis (NASH) as a disease of affluence and part of the insulin resistance syndrome (234). In the Third National Health and Nutrition Examination Survey, 2.6% of the U.S. population had raised values of serum alanine aminotransferase (ALT) for which no potential cause of chronic liver disease could be found (234). The raised ALT concentration was significantly and independently associated with indexes of insulin resistance and with HbA1c concentrations. The risk of steatosis increases exponentially with each addition of a component of the insulin resistance syndrome, such as IGT, BMI, hypertension, and dyslipidemia (322). Patients in this study had normal transaminases, but percent liver fat varied 14-fold, from 2 to 28%, suggesting that noninvasive measurement of liver fat content is a more sensitive index of steatosis than elevation of ALT, although the diagnosis of NASH still rests on histopathological features (234). The mechanisms linking insulin resistance and fatty liver are unclear. It has been suggested, but not confirmed, because of the inaccessibility of the portal vein for blood sampling in humans, that fatty liver is a consequence of fatty acid mobilization from visceral fat depots to the liver (31). In the present study, visceral fat volume, WHR, and the visceral/subcutaneous fat ratio were not significantly correlated with percent liver fat, whereas measures of overweight (total and subcutaneous fat mass, percent body fat, and BMI) were. No causal conclusions can be made based on these correlation analyses. They do not exclude the possibility that hyperinsulinemia, which could result from obesity-
induced primary insulin resistance in skeletal muscles (296) and from exogenous insulin injections, itself might increase hepatic fat content (318).

7.3. Effect of insulin therapy on markers of endothelial activation

CVD is the major complication of type 2 diabetes (273). Endothelial activation and dysfunction are amongst the first steps in its evolution (393). In study IV sE-selectin and sVCAM-1 concentrations were determined in 81 patients with type 2 diabetes before and after 3 and 12 months of treatment with four different bedtime insulin regimens. Improvement in glycemic control was associated with a significant decrease in serum sE-selectin concentrations. The decrease was highly significantly correlated with improvement in glycemic control and was not dependent on the pharmacological agent used to improve glycemia. In contrast to sE-selectin, sVCAM-1 exhibited a transient decrease at 3 months and was not significantly different from concentrations in normal subjects at any time point.

In previous cross-sectional studies, sE-selectin levels have been an average 43 % higher in patients with type 2 diabetes than in normal subjects (23, 42, 97, 125, 128, 271), also in type 2 diabetic patients without CHD (42, 128, 271, 367). In study IV the increase in the type 2 diabetic patients before insulin therapy averaged 71 % and there was no gender difference in type 2 diabetic patients as there is in normal subjects (42). In contrast to sE-selectin, sVCAM-1 concentrations did not differ between patients with type 2 diabetes and normal subjects in this study. This negative finding is in keeping with lack of a difference in sVCAM-1 concentrations between type 2 diabetic patients and normal subjects in half of the previous studies (23, 24, 108, 125, 242, 297, 451), and a small mean 12 % difference in sVCAM-1 concentrations between type 2 diabetic patients and normal subjects, when all studies are analyzed together (23, 24, 42, 95, 97, 108, 125, 128, 242, 297, 451).

Although hyperglycemia in vitro stimulates the production of both E-selectin, VCAM-1 and ICAM-1 (341), this study demonstrating a sustained decrease in sE-selectin but not in sVCAM-1 supports the idea that in vivo sE-selectin is a sensitive marker of hyperglycemia induced endothelial activation, and these data support the
potential usefulness of sE-selectin as compared with sVCAM-1 to monitor reversal of early vascular dysfunction by antihyperglycemic therapies.

Regarding the mechanisms linking hyperglycemia and sE-selectin concentrations, it seems possible that glucose-induced increased oxidative stress is involved (325, 341). Glycosylated hemoglobin concentrations are associated with reduced plasma antioxidant trapping capacity in patients with type 2 diabetes (312), a finding consistent with the concept that clinically relevant hyperglycemia increases oxidative stress. However antioxidant treatment (N-acetyl-L-cysteine) has decreased sVCAM-1 levels in one study (108), although it has not been possible to decrease sE-selectin or sVCAM-1 levels by tomato juice (478), α-tocopherol (478) or gliclazide (125). Because treatment of hyperglycemia has reduced sE-selectin levels in three studies (23, 94, 95) and in the present study, it could be considered a better tool than antioxidants to lower sE-selectin concentrations in type 2 diabetes. Such lowering might be important because sE-selectin concentrations have been shown to predict restenosis in patients with intermittent claudication undergoing percutaneous transluminal angioplasty (51) and levels of soluble forms correlate with endothelial expression (384). On the other hand, no data presently exist regarding the predictive value of sE-selectin concentrations for future vascular events in patients with type 2 diabetes, and no data exist to demonstrate that lowering of sE-selectin reflects a beneficial change in vascular function.

7.4. Choice of the insulin treatment regimen

In type 2 diabetes there are abnormalities in insulin secretion and insulin action, so it is reasonable to treat them both. If the hypothesis of progressively deteriorating β-cell function in type 2 diabetes (286) is correct, insulin therapy will ultimately be required by all of type 2 diabetic patients if the patient lives long enough.

We have shown that similar metabolic control can be achieved with different simple combination treatment regimens as with multiple insulin injections (FINMIS and FINFAT). In addition the FINFAT study showed that treatment with bedtime insulin and metformin gives best glycemic control and is associated with less hypoglycemia and weight gain than other bedtime insulin regimens.
Many factors influence the choice of insulin treatment regimen, such as the patients’ psychosocial background, lifestyle, propensity for hypoglycemia, age, obesity and ability of the patient to self-administer insulin. Thus, there is no universally applicable regimen. It is therefore important to carefully examine these factors in each individual patient before recommending an insulin treatment regimen, also including the patients motivation.
SUMMARY AND CONCLUSIONS

I In poorly controlled type 2 diabetic patients receiving oral hypoglycemic drug therapy, the addition of NPH insulin in the evening improves glycemic control in a similar manner as a two-insulin-injection therapy regimen and multiple-insulin-therapy regimen, but induces less weight gain and hyperinsulinemia.

II Combination therapy with bedtime insulin plus metformin prevents weight gain and seems superior to other bedtime insulin regimens with respect to improvement in glycemic control and frequency of hypoglycemia.

III S.c. insulin absorption is a determinant of the action of s.c. insulin but not of the insulin dose. Actions of s.c. and i.v. insulin to suppress FFA are significantly correlated. The actions of i.v. insulin and s.c. insulin are both correlated with the insulin dose. Of various measurements of adiposity, the % hepatic fat is the parameter best correlated with hepatic sensitivity to insulin and insulin dose.

These data demonstrate that the major reason for inter-individual variation in insulin requirements in type 2 diabetes is the variation in insulin action. Variation in hepatic fat content may influence insulin requirements via an effect on the sensitivity of EGO to insulin.

IV Improvement in glycemic control by administration of insulin alone or insulin combined with either glibenclamide, metformin, or both agents induces a sustained decrease in sE-selectin, the magnitude of which seems to be dependent on the degree of improvement in glycemia. sE-selectin might provide a marker of effects of treatment of chronic hyperglycemia on endothelial activation.
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