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# **INSULIN THERAPY IN TYPE 2 DIABETES**

## **INITIATION, IMPACT ON LIVER FAT, ROLE OF COMBINATION THERAPY WITH GLITAZONES OR GLINIDES**

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ACADEMIC DISSERTATION

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# ABSTRACT

## AIMS

The overall aims of the present studies were to understand the role of the liver in regulating the response to anti-hyperglycemic treatment, and to find ways to optimize basal insulin therapy in patients with type 2 diabetes. Specifically, we determined whether i) insulin therapy changes liver fat content (LFAT) or hepatic insulin sensitivity (I), ii) a peroxisome proliferator-activated receptor gamma (PPAR $\gamma$ ) agonist decreases insulin requirements, hepatic insulin resistance and LFAT in patients using high doses of insulin (II), iii) adding nateglinide decreases postprandial glucose excursions (PPGEs) in patients treated with the combination of basal insulin and metformin (MET) (III), iv) insulin can be started in groups as well as individually (IV).

## SUBJECT AND METHODS

In study I, 14 patients with type 2 diabetes treated with MET alone received additional basal insulin for 7 months. Liver fat content (by proton magnetic resonance spectroscopy,  $^1\text{H-MRS}$ ), fat distribution (magnetic resonance imaging, MRI), fat-free mass and fat mass, whole body and hepatic insulin sensitivity (6-h euglycemic hyperinsulinemic clamp combined with infusion of [ $3\text{-}^3\text{H}$ ]-glucose), and substrate oxidation rates (indirect calorimetry) were measured. In study II, we determined the effect of the PPAR $\gamma$  agonist rosiglitazone (8 mg/d) for 8 months on liver fat and directly measured hepatic insulin sensitivity in 14 patients with type 2 diabetes, who were poorly controlled, i.e. HbA $_{1c}$  ( $8.9 \pm 0.4\%$ ) despite using high doses of insulin ( $1.98 \pm 0.2$  IU/kg) in combination with MET. In study I and II, liver fat content ( $^1\text{H-MRS}$ ), hepatic insulin sensitivity [6 h hyperinsulinemic euglycemic clamp (insulin 0.3 mU/kg-min) combined with infusion of [ $3\text{-}^3\text{H}$ ]-glucose], body composition (MRI), substrate oxidation rates (indirect calorimetry), clinical parameters, and liver enzymes were measured before and after additional basal insulin (I) and rosiglitazone treatment (II). Study III was an investigator-initiated, double-blind, randomized, parallel-group study in the five centers. Patients with type 2 diabetes ( $n = 88$ , age  $56.0 \pm 0.9$  years, duration of diabetes  $9.4 \pm 0.5$  years, HbA $_{1c}$   $7.8 \pm 0.1\%$ ) treated with basal insulin and MET entered a 24-week period, during which basal insulin was titrated to optimize glucose control. Thereafter, the patients were randomized to receive either nateglinide (120 mg three times daily) or placebo for 24 weeks HbA $_{1c}$ , the episodes of hypoglycemia, diurnal glucose profiles and postprandial glucose excursions were recorded. Study IV was a randomized (1:1), multicenter, two-arm, parallel design study with 3 to 14-week run-in phase and

a 24-week treatment period. 121 insulin-naive type 2 diabetic patients with an HbA<sub>1c</sub> of 7.0–12.0 % were randomized to initiate bedtime insulin glargine either in groups of 4–8 or individually, using the same personnel and education program. The patients visited the treatment center before and at the time of insulin initiation and at 6, 12, and 24 weeks. Patients self-adjusted the insulin dose to achieve a fasting plasma glucose target of 4.0–5.5 mmol/l. Glycemic control, the episodes of hypoglycemia, treatment satisfaction, and time spent on patient education were measured.

## RESULTS

Insulin therapy decreased significantly LFAT (-20 %), and enhanced hepatic insulin sensitivity. Insulin requirements correlated with LFAT. The change in LFAT was significantly correlated with that in hepatic insulin sensitivity. Rosiglitazone significantly improved glycemic control, decreased liver fat (by 46 %) and insulin requirements (by 41 %), and enhanced hepatic insulin sensitivity. The change in liver fat correlated with the decrease in HbA<sub>1c</sub>, and improvement in hepatic insulin sensitivity. Nateglinide added to basal insulin and MET slightly but significantly decreased HbA<sub>1c</sub> due to a decrease in postprandial glucose as compared to placebo. In the study comparing methods to initiate basal insulin, HbA<sub>1c</sub> decreased from  $8.7 \pm 0.2$  to  $6.9 \pm 0.1$  % in those starting insulin therapy individually and from  $8.8 \pm 0.2$  to  $6.8 \pm 0.1$  % in those starting insulin in groups (not significant [NS]). Insulin doses averaged  $62 \pm 5$  IU and  $56 \pm 5$  IU at 24 weeks (NS), respectively. The frequency of hypoglycemia was similar in both groups. The total time (visits and phone calls) spent in initiating insulin in groups was 48 % less than in those treated individually. Diabetes treatment satisfaction improved similarly in both groups.

## CONCLUSIONS

In conclusion, insulin therapy has favorable effects on the liver as it improves hepatic insulin sensitivity and significantly reduces liver fat content. Rosiglitazone decreases liver fat content and enhances hepatic insulin sensitivity. Addition of a short-acting insulin secretagogue slightly but significantly improves postprandial hyperglycemia during combination therapy with basal insulin and MET. Similar glycemic control and treatment satisfaction can be achieved by initiating insulin in groups and individually but initiation in groups takes half as much time as individual initiation.

# CONTENTS

<b>ABSTRACT</b>	3
<b>LIST OF ORIGINAL PUBLICATIONS</b>	7
<b>ABBREVIATIONS</b>	8
<b>1. INTRODUCTION</b>	10
<b>2. REVIEW OF THE LITERATURE</b>	12
2.1. PATHOGENESIS OF TYPE 2 DIABETES	12
2.1.1. Insulin resistance, the MetS and NAFLD	12
Definitions	12
Methods to measure insulin sensitivity	12
Insulin resistance in the liver	13
Regulation of liver fat in humans	14
Insulin resistance in adipose tissue	15
Insulin resistance in skeletal muscle	16
Defects of insulin action in other tissues	16
2.1.2. Defects in insulin secretion	17
2.2. TREATMENT OF HYPERGLYCEMIA IN TYPE 2 DIABETES	17
Treatment targets and effects of insulin therapy on micro- and macrovascular disease	17
2.2.1. Weight loss and exercise	17
2.2.2. Oral agents and GLP-1-analogues	18
Metformin	18
Sulfonylureas	18
Glinides	19
Glitazones	19
DPP-4- inhibitors	20
GLP-1 - analogues	20
SGLT-inhibitors	21
2.2.3. Insulin	22
Choice of the insulin regimen	22
Practical aspects of initiation of insulin therapy	24
<b>3. AIMS OF THE STUDY</b>	25

<b>4. SUBJECTS AND STUDY DESIGN</b>	26
4.1 Subjects	26
4.2 Study designs	27
<b>5. METHODS</b>	<b>31</b>
5.1. Hepatic insulin sensitivity (I, II)	31
5.2. Liver fat content (I, II)	32
5.3 Measures of body composition (I, II)	33
5.4 Measurements of substrate oxidation rates (I, II)	33
5.5 Transthoracic echocardiography (II)	34
5.6 Optimization of insulin therapy (III, IV)	34
5.7. Analytical procedures	35
5.8. Statistical analyses	36
<b>6. RESULTS</b>	<b>38</b>
6.1. Effect of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes (I)	38
6.2. Liver fat content and hepatic insulin sensitivity after combination of rosiglitazone to metformin and insulin therapy (II)	43
6.3. Postprandial hyperglycemia after combination of nateglinide to metformin and insulin therapy (III)	44
6.4. Initiation of insulin therapy individually or in groups (IV)	48
<b>7. DISCUSSION</b>	51
<b>8. SUMMARY AND CONCLUSIONS</b>	56
<b>9. ACKNOWLEDGEMENTS</b>	57
<b>10. REFERENCES</b>	59
<b>ORIGINAL PUBLICATIONS</b>	74

# LIST OF ORIGINAL PUBLICATIONS

THIS THESIS IS BASED ON THE FOLLOWING PUBLICATIONS, WHICH ARE REFERRED TO THE TEXT BY THEIR ROMAN NUMERALS.

- I *Juurinen Leena, Tiikkainen Mirja, Häkkinen Anna-Maija, Hakkarainen Antti, Yki-Järvinen Hannele*  
**Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes.**  
*American Journal of Physiology, Endocrinology and Metabolism* 292: E829–E835, 2007.
- II *Juurinen Leena, Kotronen Anna, Granér Marit, Yki-Järvinen Hannele*  
**Rosiglitazone reduces liver fat and insulin requirements and improves hepatic insulin sensitivity and glycemic control in patients with type 2 diabetes requiring high insulin doses.** *Journal of Clinical Endocrinology and Metabolism* 93: 118–124, 2008.
- III *Juurinen Leena, Tiikkainen Mirja, Saltevo Juha, Nikkilä Katriina, Lanki, Helena, Leppävuori Eeva, Kock Tuula, Teikari-Myyrä Tarja, Kauppinen-Mäkelin Ritva, Kotronen Anna, Yki-Järvinen Hannele*  
**Nateglinide combination therapy with basal insulin and metformin in patients with type 2 diabetes.** *Diabetic Medicine* 26: 409–415, 2009.
- IV *Yki-Järvinen Hannele, Juurinen Leena, Alvarsson Michael, Bystedt Tord, Caldwell Ian, Davies Melanie, Lahdenperä Sanni, Nijpels Gil, Vähätalo Markku*  
**Initiate Insulin by Aggressive Titration and Education (INITIATE): A randomized study to compare initiation of insulin combination therapy in type 2 diabetic patients individually and in groups.**  
*Diabetes Care* 30: 1364–1369, 2007.

Publication II has also been included in the doctoral thesis of *Anna Kotronen* entitled *Liver fat in the metabolic syndrome and type 2 diabetes*, University of Helsinki, 2008.

## ABBREVIATIONS

ALP	alkaline phosphatase
ALT	alanine aminotransferase
AST	aspartate aminotransferase
ANOVA	analysis of variance
BMI	body mass index
CoA	coenzyme A
DAG	diacylglycerol
DNL	de novo lipogenesis
DPP-4	Dipeptidyl peptidase-4
DQTS	diabetes treatment satisfaction questionnaire
EGP	endogenous glucose production
FDG	fluorodeoxyglucose
FFA	free fatty acids
fm	fat mass
ffm	fat free mass
FPI	fasting plasma insulin
fP	fasting plasma
FPG	fasting plasma glucose
fS	fasting serum
GAD	glutamic acid decarboxylase
GLP-1	glucagon like peptide-1
GLUT4	glucose transporter 4
GCP	good clinical practice
HDL	high density lipoprotein
HbA1c	glycosylated hemoglobin A1c
HGP	hepatic glucose production
<sup>1</sup> H-MRS	proton magnetic resonance spectroscopy
HOMA-IR	homeostasis model assessment of insulin resistance
IA	intra-abdominal
IFG	impaired fasting glucose
IGT	impaired glucose tolerance
IMCL	intramyocellular lipid
IR	insulin resistance
LDL	low density lipoprotein
LFAT	liver fat
LPL	lipoprotein lipase
MET	metformin

Mets	metabolic syndrome
MRI	magnetic resonance imaging
MRS	magnetic resonance spectroscopy
NAFLD	non-alcoholic fatty liver disease
NASH	non-alcoholic steatohepatitis
NEFA	non-esterified fatty acid
NPH	neutral protamine Hagedorn
NS	not significant
OAD	oral antidiabetic drug(s)
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor 1
PET	positron emission tomography
PKC	protein kinase C
PPGE	postprandial glucose excursion
ppm	parts per million
PPAR $\gamma$	peroxisome proliferator-activated receptor gamma
QUICKI	quantitative insulin sensitivity check index
Ra	rate of appearance
Rd	rate of disappearance
S	serum
SA	specific activity
SAT	subcutaneous adipose tissue
SC	subcutaneous
SEM	standard error of mean
SU	sulphonylurea
TZD	thiazolidinedione(s)
TAG	triacylglycerol(s)
TG	triglycerides
VAT	visceral adipose tissue
VLDL	very low density lipoprotein
WHR	waist to hip ratio
wks	weeks

# 1. INTRODUCTION

Increased fat accumulation in the liver due to non-alcoholic fatty liver disease (NAFLD) is a key feature of the insulin resistance/metabolic syndrome and the most common cause of elevated liver enzymes (1). The liver is the source of glucose and triglycerides (TG), two of the key components of the metabolic syndrome (MetS). The liver, once fatty, becomes resistant to the normal actions of insulin to inhibit the production of glucose and VLDL (2), (3). This leads to hyperglycemia and stimulation of insulin secretion as well as hypertriglyceridemia (2), (4).

Achievement of good glycemic control is a major challenge in the clinic in patients with type 2 diabetes. In Finland it has been estimated that less than 25 % of type 2 diabetic patients achieve the average HbA1c target of 7.0 % often regarded as the goal in treating hyperglycemia (5), (6). This is also true even if insulin therapy is used to control glycemia. One possible reason for this failure is fat accumulation in the liver due to NAFLD. Consistent with the knowledge that the fatty liver is insulin resistant in type 2 diabetes, the amount of insulin needed depends on the amount of fat in the liver (7), (8). However, insulin also directly stimulates lipogenesis in the liver (9) raising the possibility that insulin therapy actually worsens hepatic insulin resistance by increasing liver fat content. Therefore we determined the effect of insulin therapy on hepatic insulin sensitivity and liver fat content.

PPAR $\gamma$ -agonists such as rosiglitazone and pioglitazone increase hepatic insulin sensitivity and lower liver fat content when given as monotherapy to patients with type 2 diabetes (10), (11). In the present study, we examined these drugs are helpful in improving glycemic control particularly in patients who have a high liver fat content and high insulin requirements.

Basal insulin combination therapy is recommended as the regimen of choice in initiating insulin therapy in patients with type 2 diabetes poorly controlled on oral agents alone (6). Although the treatment target of 7.0% can on the average be achieved with basal insulin (12), (13) some patients have residual postprandial hyperglycemia. A possible option is to treat postprandial hyperglycemia with short-acting insulin regimens, dipeptidyl peptidase-4 (DPP-4)-inhibitors, glucagon like peptide-1 (GLP-1)-analogues, or glinides. Nateglinide is a derivate of meglitinide, which stimulates insulin secretion (14). In the present study, we examined whether this drug improves postprandial hyperglycemia also when added to basal insulin therapy.

Although basal insulin therapy is a simple and effective way in initiating insulin therapy on an out-patient basis (15), it nevertheless requires a nurse to teach basic skills such as measurement of fasting glucose concentrations and injection of insulin. This is time-consuming and perhaps limits initiation of insulin therapy. There is thus a need to develop new ways to ease the transition from oral agents to insulin.

The present series of studies were undertaken to improve treatment of type 2 diabetic patients. We examined the effect of insulin and rosiglitazone on hepatic insulin sensitivity and liver fat content and the effect of adding nateglinide on postprandial glycemia in patients treated with basal insulin combined with metformin. We also studied whether the patient education how to start basal insulin therapy can be carried out in patient groups as well as individually.

## 2. REVIEW OF THE LITERATURE

### 2.1. PATHOGENESIS OF TYPE 2 DIABETES

#### 2.1.1. INSULIN RESISTANCE, THE METS AND NAFLD

##### DEFINITIONS

Insulin resistance is defined as the inability of insulin to produce its biological actions at circulating concentrations that are effective in normal subjects (16). Insulin resistance may involve any of the metabolic actions of insulin, a cluster of metabolic and cardiovascular risk factors. The most recent criteria for diagnosis of the MetS are as follows: the presence of any three of the following five risk factors: waist circumference  $\geq 80$  cm in women and  $\geq 94$  cm in men, fasting glucose  $\geq 5.6$  mmol/l or drug treatment for hyperglycemia, fS-TG  $\geq 1.7$  mmol/l or drug treatment for hypertriglyceridemia, HDL-cholesterol  $< 1.3$  mmol/l, women and  $< 1.0$  mmol/l, men or drug treatment for low HDL-cholesterol, systolic blood pressure  $\geq 130$  mmHg and/or diastolic blood pressure  $\geq 85$  mmHg or antihypertensive drug treatment (17).

NAFLD is defined as excess fat in the liver in the absence of excess alcohol abuse ( $< 20$ g/day) or other causes of liver disease (16). It covers conditions from simple steatosis to steatohepatitis (NASH) (18). In NAFLD, liver fat exceeds 10 % as determined by histology (19), and over 5.6 % measured by spectroscopy (20). Liver fat content have been shown to correlate significantly with all features of MetS independent of BMI (1), (21).

##### METHODS TO MEASURE INSULIN SENSITIVITY

The golden standard for measurement of insulin sensitivity is the euglycemic hyperinsulinemic clamp technique (22). With this technique, whole body insulin sensitivity is measured as the amount of glucose required to maintain normoglycemia during intravenously induced hyperinsulinemia (22). Without isotopes, the glucose infusion rate corrected for changes in glucose concentrations, provides a measure of whole body insulin sensitivity but does not allow distinction between hepatic and peripheral insulin resistance (23), (24), (25). To determine the tissue localization of insulin resistance (liver vs. periphery), a glucose isotopes needs to be used (25). For the calculation of glucose kinetics the Steele equation is used (26).

Insulin sensitivity in adipose tissue (insulin sensitivity of lipolysis) can be quantified by infusing a radioactive or stable glycerol or free FFA tracer (27), (28). In addition,

PET with  $^{18}\text{F}$ FDG (fluorodeoxyglucose) can be used to determine glucose uptake in adipose tissue (29), in skeletal muscle (30), (31), and the liver (32).

Other methods to measure insulin sensitivity and glucose metabolism include the intravenous insulin tolerance test (33), the insulin suppression test (34), (35), and the frequently sampled intravenous glucose tolerance test (FSIVGT) with minimal model assessment (36). Methods based on measurement of fasting insulin concentrations, such as fP-insulin, homeostatic model assessment [ $\text{HOMA}_{\text{IR}} = (\text{fasting insulin} \times \text{fasting glucose})/22.5$ ], qualitative insulin check index, [QUICKI =  $1/(\log \text{fasting insulin} + \log \text{fasting glucose})$ ] cannot be used to reliably measure insulin sensitivity in type 2 diabetes since it is unclear how well these tests function in subjects with decreased  $\beta$ -cell secretion (37). Fasting insulin also increases because of impaired insulin clearance in subjects with a fatty liver (38). Thus, e.g. HOMA overestimates hepatic insulin resistance compared to insulin resistance in skeletal muscle. The HOMA method is, however used to measure insulin sensitivity in large epidemiologic studies (39).

## **INSULIN RESISTANCE IN THE LIVER**

The liver is mainly responsible for glucose production in the postabsorptive phase (40), (41), (42). Approximately 50–64 % of total hepatic glucose production is due to gluconeogenesis. Insulin inhibits hepatic glucose production (HGP) (23). This occurs both via inhibition of glycogenolysis (43) and gluconeogenesis (44). Insulin also suppresses HGP via a decrease in glucagon secretion (45). In addition, insulin inhibits the production of VLDL from the liver (46).

### ***Hepatic insulin resistance***

Hepatic insulin resistance of glucose production is characterized by a defect in the ability of insulin to suppress HGP (47), (2). A high LFAT content impairs insulin suppression of HGP in non-diabetic subjects (2) and in type 2 diabetic patients (7). This leads to an increase in basal HGP in the fasting state (2). Postprandial hyperglycemia is also characterized by persistent HPG after a meal (48). Patients with type 2 diabetes have more LFAT than non-diabetic subjects for the same BMI (49). LFAT also correlates with insulin requirements in patients with type 2 diabetes (7).

Insulin resistance affects not only glucose but also lipid metabolism in the liver. The increase in LFAT has been shown to lead to overproduction of VLDL (4) due to a defect in insulin suppression of VLDL production (3). In addition, due to the defect in the antilipolytic effect of insulin, the suppression of FFA by insulin is blunted (2) contributing to increased hepatic uptake of FFA (50).

## REGULATION OF LIVER FAT IN HUMANS

### *Overfeeding and weight loss*

Excessive energy intake (43 % fat, 45 % calories from carbohydrate, and 12 % from protein) increases liver fat content significantly within four weeks in healthy, normal weight subjects (51). We recently showed that liver fat increased by 27 % in three months by carbohydrate overfeeding when body weight increased only by 2 % (52). Several studies have shown that weight loss markedly decreases LFAT in non-diabetic subjects (53), (54), (55) and in patients with type 2 diabetes (56), (57).

### *Glitazones*

TZDs are PPAR $\gamma$  (peroxisome-proliferator-activated receptor-gamma) agonists. TZDs bind to the transcription factor PPAR $\gamma$ , which regulates gene expression in response to ligand binding. PPAR $\gamma$  is mainly expressed in adipose tissue and slightly also in the liver, in skeletal muscle, in heart, and in endothelial cells and macrophages (58). PPAR $\gamma$  regulates the transcription of numerous genes involving carbohydrate and lipid metabolism (59) and inflammation (58). TZDs increase hepatic and peripheral insulin sensitivity (60), (61), (10) and have been shown to decrease liver fat content in several studies (60), (61), (62), (10).

The improvement in insulin sensitivity and decrease in LFAT by TZDs are accompanied by a marked increase in serum adiponectin concentrations (63), (10). In mice, adiponectin, which is produced exclusively in adipose tissue, decreases liver fat content and hepatic inflammation (64). Adiponectin is thus one possible mediator of the beneficial effects of TZDs on the liver. TZDs have reduced steatosis, inflammation, and ballooning necrosis in randomized controlled clinical trials addressing treatment of NASH (61), (65), (66), (67).

During insulin combination therapy in patients with type 2 diabetes, insulin doses vary at least 20-fold from 10 to 200 IU/d (8), (15), (68). In 2000, LFAT was shown to correlate with insulin requirements in type 2 diabetes (7). This suggests that especially patients with high insulin requirements could benefit from therapies such as TZDs, which lower LFAT. However, because of fear of hepatotoxicity after withdrawal of troglitazone due to liver reactions, patients with elevated liver enzymes were carefully excluded from insulin-TZD studies (69), (70), (71), (72), (73), (74). Use of rosiglitazone or pioglitazone has not been accompanied by hepatotoxicity but rather beneficial effects on LFAT, hepatic insulin sensitivity and liver histology (58).

### *Metformin*

We previously compared effects of rosiglitazone and MET on LFAT in patients with type 2 diabetes (10). In a previous study, MET had no effect on LFAT while rosiglitazone decreased LFAT by 51 % (10). The lack of an effect of MET on LFAT as measured by computed tomography in type 2 diabetes was confirmed by Gupta et al (75) and by

Teranishi et al who used <sup>1</sup>H-MRS to determine LFAT before and 6 months after MET in 20 Japanese type 2 diabetic patients (76).

### ***Insulin***

In patients with NAFLD, lipolysis and de novo lipogenesis are the main sources of excessive intrahepatocellular triglycerides (77). Insulin, even at low concentrations effectively lowers S-FFA and may therefore lower liver fat content (2). On the other hand, insulin stimulates fatty acid and VLDL synthesis in the liver, which could increase LFAT (78). Body weight also increases during insulin therapy in proportion to improved glycemic control (79), while weight loss reduces LFAT (53). There are no studies addressing effects of insulin therapy on liver fat content.

### ***Genetic factors***

Genetic factors have been shown to associate with NAFLD (80), (81), (82). The genetic variation in the gene patatin-like phospholipase domain-containing protein 3 (PLPNA3, adiponutrin gene) was found to associate with LFAT (83). The meta-analysis also showed that subjects with NAFLD and the I148M variant (rs738409 C>G) in PNPLA3 gene not only were in risk for 73 % higher LFAT but also were predisposed to have 3.2-fold risk for NASH and the fibrosis in the liver (84).

## **INSULIN RESISTANCE IN ADIPOSE TISSUE**

The main function of adipose tissue is to store excess energy in the form of triacylglycerols (TAG). On the other hand, under fasting conditions lipolysis of TAG-stores in adipose tissue releases glycerol and FFA into the circulation for use in other tissues (85). The main regulator of both processes is insulin. Insulin inhibits lipolysis via inhibition of hormone-sensitive lipase (HSL). It also inhibits intravascular lipolysis, i.e. hydrolysis of VLDL and chylomicrons via inhibition of lipoprotein lipase (LPL). Adipose tissue also acts as an endocrine organ. It secretes adipokines, peptide hormones, and cytokines such as adiponectin, leptin, tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ) and interleukin 6 (IL-6) (86), (87). Adiponectin is produced exclusively by adipose tissue and appears to act mainly in the liver, where it enhances hepatic insulin sensitivity and decreases inflammation (88), (89).

Adipose tissue insulin resistance is characterized by enhanced lipolysis (90). The increase in circulating FFA decreases insulin-stimulated glucose uptake and the ability of insulin to suppress HGP (91). Adipose tissue lipolysis has been shown to be insulin resistant in obese subjects (92) and patients with type 2 diabetes (49). Adipose tissue insulin resistance correlates with LFAT in obese subjects (93) and in type 2 diabetes (49).

Adipose tissue in obese insulin resistant subjects is inflamed (89). This inflammation is characterized by an increase in the number of macrophages surrounding dead adipocytes (94). Plasma concentrations of adiponectin are low in obesity and in type 2 diabetes (95), in non-diabetic subjects with family history of type 2 diabetes (96),

and in NAFLD (97). The severity of whole body insulin resistance is inversely related to low serum adiponectin concentrations (95).

## **INSULIN RESISTANCE IN SKELETAL MUSCLE**

After an overnight fast, when serum insulin concentration is low, only ~10 % (25), (98) of glucose utilization occurs in skeletal muscle. Most of glucose is used in the brain and other insulin-independent tissues (25). In healthy subjects insulin stimulates glucose uptake by skeletal muscle (23), (99).

The ability of insulin to increase glucose uptake is impaired in patients with type 2 diabetes (100), (101), (102). Both insulin-stimulated glucose oxidation and non-oxidative glucose metabolism are deteriorated in type 2 diabetes (103), (104). The defect in insulin stimulated glucose disposal is observed in type 2 diabetes when studied under normoglycemic conditions. However, glucose itself is a potent stimulator of glucose uptake (105). When glucose uptake is measured under hyperglycemic conditions mimicking those prevailing under everyday conditions, glucose uptake is similar in type 2 diabetic patients and matched normal subjects (105), (106). In the basal state, when glucose uptake is largely non-insulin-dependent, rates of absolute whole body and muscle glucose utilization are increased in patients with type 2 diabetes compared to normal subjects, in proportion to hyperglycemia (98).

Hyperglycemia (107), (108), or high S-FFA (91), (92), (30), (109) induce insulin resistance in human skeletal muscle. The rate of insulin-stimulated glucose uptake in type 2 diabetic patients is inversely proportional to the degree of chronic hyperglycemia (105). Increases in S-FFA within the physiological range have also been shown to decrease insulin-stimulated glucose uptake and impair insulin signaling (110).

## **DEFECTS OF INSULIN ACTION IN OTHER TISSUES**

Insulin is known to cause vasodilatation and decrease the stiffness of large arteries (111). Normally insulin inhibits platelet aggregation (112). Insulin stimulates sympathetic nervous system (113) and regulates heart rate (114). Several defects of insulin action in cardiovascular system are found in insulin resistant state, such as the inability of insulin to decrease central aortic pressure in large arteries (111), (115), the defects in endothelium-dependent vasodilatation (116), (117), and in myocardial insulin resistance as measured by PET (118). Insulin action on platelets has shown to be blunted in insulin resistant subjects (112). The action of insulin in central nervous system has been studied using mouse models. Insulin action in brain is supposed to regulate glucose and fat metabolism, and central insulin resistance may lead to insulin resistance in other tissues (119).

## 2.1.2. DEFECTS IN INSULIN SECRETION

The insulin response to intravenous glucose is biphasic. An early first-phase burst is followed by a second, sustained phase. Glucose tolerance deterioration from normal to impaired means a progressive decrease in the acute insulin response to glucose (120), and an increase in the response of total insulin to oral glucose load (121). Despite this, most patients with type 2 diabetes are hyperinsulinemic (122).

The increase in total insulin concentrations is an attempt of  $\beta$ -cells to maintain normal glucose tolerance. Once the 2-h plasma glucose in an oral glucose tolerance test (OGTT) exceeds 11.0 mmol/l, insulin secretion starts to decrease relative to insulin resistance and hyperglycemia (122). This means the onset of type 2 diabetes. There is thus absolute hyperinsulinemia but relative deficiency of insulin in type 2 diabetic patients.

The defects in insulin secretion may be familial, inherited or acquired. Polymorphisms in genes involved in insulin secretion seem to be more common in patients with type 2 diabetes than in non-diabetic subjects. Identification of these genetic markers has not, however, helped in identification of subjects at risk of developing type 2 diabetes (123). Acquired factors such as gluco- and lipotoxicity, and amyloid deposition may also contribute to  $\beta$ -cell exhaustion (124), (125), (126).

## 2.2. TREATMENT OF HYPERGLYCEMIA IN TYPE 2 DIABETES

### *Treatment targets and effects of therapy on micro-and macrovascular disease*

Lowering of HbA<sub>1c</sub> below 7.0 % has been shown to decrease the risk of microvascular disease (6). This goal therefore has been recommended as an average HbA<sub>1c</sub> target for patients with type 2 diabetes. On the other hand, the treatment target should be defined individually after considering hypoglycemia, age, and the presence of complications. Long-term follow-up of patients in the UKPDS, where sulphonylureas, metformin and insulin were used as antihyperglycemic agents suggested that intensive glucose control may be beneficial also for the prevention of macrovascular disease (6), (127).

### 2.2.1 WEIGHT LOSS AND EXERCISE

Obesity and sedentary lifestyle together with genetic susceptibility are main reasons for deterioration of glucose metabolism. Lifestyle intervention such as weight reduction, healthy diet and increased physical activity have shown to decrease the risk of diabetes (128). In addition, the changes in lifestyle can reduce the risk of type 2 diabetes in subjects with impaired glucose tolerance (129). Several studies have shown that dietary changes resulting in weight loss decrease HbA<sub>1c</sub> (130), (131), (132), (133). Bariatric

surgery reverses type 2 diabetes in 36 % when assessed 10 years after gastric bypass surgery (134).

Daily physical activity associates closely to insulin sensitivity in non-diabetic subjects (135). Whole body insulin sensitivity correlates closely with maximal aerobic power ( $VO_2$ max). Aerobic exercise, already within one week, improves significantly peripheral insulin sensitivity in subjects with type 2 diabetes (136). Both diet and exercise have shown to decrease  $HbA_{1c}$  similarly in patients with type 2 diabetes (137). Aerobic training together with high-intensity resistance training have decreased  $HbA_{1c}$  by approximately 1.0–0.7% (138), (139), (140) in type 2 diabetes.

## 2.2.2 ORAL AGENTS AND GLP-1 -ANALOGUES

### ***Metformin***

MET, a biguanide, the first-line therapy in type 2 diabetes (6) has been used over 40 years. MET decreases the rate of HGP (141), (142), (10). This is mainly due to the decrease in gluconeogenesis (143). MET promotes weight loss (141) which may improve whole body insulin sensitivity. MET has been reported to decrease serum lipids, especially TG concentrations (144), (143). The risk of lactic acidosis, a serious adverse effect of MET, is very low (145). MET has shown to decrease  $HbA_{1c}$  approximately 1.5% (144) without increasing the risk of hypoglycemia (146), (145).

MET has been shown to prevent type 2 diabetes (147) and to reduce the risk of cardiovascular diseases (146) (127). Furthermore, MET may decrease the risk of cancer in patients with type 2 diabetes (148), (149).

### ***Sulfonylureas***

Sulfonylureas (SUs) stimulate insulin secretion by closing ATP dependent  $K^+$ -channels in beta cells (150). SUs bind to the  $\beta$ -cell sulphonylurea receptor -1, which forms a transmembrane complex together with Kir 6.2 potassium channels. Reduced potassium efflux leads to cascade of the membrane depolarisation, opening of  $Ca^{2+}$  channels, and the activation of calcium-dependent proteins controlling insulin release (151).

SUs have been shown to decrease  $HbA_{1c}$  by ~1–2% (144). The efficacy depends on the adequacy of the beta-cell function (152). Within five years, however, the glycemic control has shown to deteriorate in patients treated with SU (153). Weight gain and hypoglycemia (153), concerning especially elderly individuals and patients with renal insufficiency (154) are the main adverse effects of SU. Since SU are bound to plasma proteins, interaction with other drugs, such as salicylates, warfarin, and sulphonamides, may lead to the increase in the risk of hypoglycemia (155).

## Glinides

Derivates of meglitinide, such as repaglinide (carbamoymethyl benzoic acid derivate) and nateglide (D-phenylalanine derivate), stimulate the release of insulin from  $\beta$ -cells, and nateglide is eliminated primarily by kidney (156) and repaglinide via the biliary tract. The absorption and the elimination are rapid. Both of these short-acting insulin secretagogues have been shown to decrease postprandial hyperglycemia (157), (158). The meal-related dosing of repaglinide has been shown to decrease HbA<sub>1c</sub> by ~1 % (159). The effect of nateglinide vs. placebo on HbA<sub>1c</sub> in various studies in type 2 diabetic patients has been summarized in **Table 1**. The geometric mean change by nateglinide vs. placebo in the studies in Table 1 has averaged 0.5 %.

Glinides increase the risk of hypoglycemia but less than long-acting sulphonylureas (14). The percentage of patients reporting hypoglycemia was 3 % for nateglinide compared to 14 % for glyburide (14). In a 24-week study performed in 675 type 2 diabetic patients confirmed hypoglycemia occurred in 5.3 % of the patients treated with nateglinide (160). Whether glinides are helpful in improving glycemic control in patients treated with basal insulin – MET combination therapy has not been examined. In study IV, we hypothesized that addition of nateglinide to basal insulin combined with MET might help in controlling postprandial hyperglycemia in type 2 diabetes.

**Table 1.** The effect of nateglinide as compared to placebo on glucose control in drug-naïve patients with type 2 diabetes.

<i>Author Reference</i>	n	Study Duration (wks)	Basal HbA1c (%)	Nateglinide HbA1c $\Delta$ (%)	Placebo HbA1c $\Delta$ (%)	p-value
<i>Horton ES 161</i>	701	24	8.3	-0.5	0.5	<0.001
<i>Hanefeld M 162</i>	289	12	8.3	-0.55	0.07	<0.05
<i>Rosenstock J 163</i>	585	16	8.4	-0.6	0.5	<0.001
<i>Saloranta C 160</i>	334	24	6.6	-0.39	0.16	<0.001
<i>Mari A 164</i>	108	24	6.6	-0.31	-0.1	NS
<i>Schwarz SL 165</i>	52	12	7.6	-0.7	-0.2	0.004

## Glitazones

TZDs are insulin-sensitizers enhancing whole body insulin sensitivity (166), (167), (142) hepatic insulin sensitivity (10) and adipose tissue insulin sensitivity (60). TZDs increase the suppression of HGP by insulin (166) and decrease HbA<sub>1c</sub> approximately 1 to 1.5%.

From three TZDs only pioglitazone is in clinical use. Troglitazone was withdrawn due to hepatotoxicity (168) and rosiglitazone due to increase in cardiovascular events, especially heart failure (169). TZDs decrease liver fat content approximately 50% (10), (11), (60). In the previous studies the combination therapy of insulin and TZDs decreased insulin requirements slightly. Glycemic control improved in spite of the increase in body weight. LFAT was not measured in these studies (69), (70), (71), (72), (73).

TZDs increase the incidence of congestive heart failure (170), (58), (70). In addition, TZDs are associated with weight gain, a small decrease in hemoglobin (171), (167), and fluid retention (58). The reason for fluid retention and peripheral edema may be due to reduction of renal extraction of sodium (172). Pioglitazone and placebo was shown to have similar congestive heart failure related mortality (170) but with rosiglitazone the increase in mortality due to heart failure was shown to be doubled compared to placebo (169). Use of TZDs also increases the risk of fractures (173).

### ***DPP-4 inhibitors***

Glucagon-like peptide 1 (GLP-1) is secreted from L-cells in response to a meal and stimulates insulin secretion as well as lowers glucagon secretion (174). Dipeptidyl peptidase-4 (DPP-4) is an aminopeptidase, which postprandially rapidly degrades the incretin hormones, glucose-dependent insulinotropic polypeptide (GIP) and GLP-1. DPP-4 is expressed in many tissues, and on the surface of lymphocytes, macrophages, and endothelial cells (175). Inhibition of DPP-4 by DPP-4 inhibitors such as sitagliptin, vildagliptin, saxagliptin and linagliptin, increases the concentration of endogenous active plasma GLP-1 (176), (177).

DPP-4 inhibitors have been shown to decrease HbA<sub>1c</sub> by ~0.5% from the baseline HbA<sub>1c</sub> of 7.4% (178), (179), (180), (181). The elimination of DPP-4-inhibitors occurs mainly by the kidney with the exception of linagliptin, which is metabolized mainly by the liver (182). Vildagliptin had no effect on fasting TG or total cholesterol concentrations (183). DPP-4 inhibitors are well-tolerated, weight neutral and do not cause hypoglycemia (182).

DPP-4 inhibitor linagliptin when added to basal insulin therapy improved glycemic control and HbA<sub>1c</sub> decreased approximately 0.6% (184). The frequency of hypoglycemia was low. Similar results are reported with other DPP-4 inhibitors combined to basal insulin therapy (185), (186).

### ***GLP-1 analogues***

Oral glucose induces a higher insulin response than intravenous glucose load has been attributed to incretin hormones, GLP-1 and GIP (187). Over 50% of the glucose-stimulated insulin response is suggested to be due to incretin effect, which is blunted in patients with type 2 diabetes (174). The response of insulin to glucose is decreased due to a resistance of GIP-action and a deficiency of GLP-1 in patients with type 2

diabetes (119). Thus, the lower GLP-1 levels after mixed meal contribute to increase in glucagon secretion leading hyperglycemia (174).

GIP is synthesised in K-cells of the proximal small intestine and GLP-1 in L-cells in the distal ileum and colon (174). They increase insulin secretion in a glucose-dependent manner. Incretins act by decreasing basal and postprandial glucagon secretion, delaying gastric emptying, and increasing satiety. Furthermore, incretins may have beneficial effects on beta cell function (188).

Endogenous GLP-1 has a short half-life due to quick enzymatic deactivation in the circulation by DPP-4. GLP-1 analogues such as exenatide, liraglutide and lixisenatide, are resistant to enzymatic deactivation by DPP-4 (189).

When used as monotherapy GLP-1-analogues decrease HbA<sub>1c</sub> by ~0.8% (190), (191), (178). Despite improved glycemic control, body weight decreases significantly by 2-4 kg (191). Gastrointestinal side-effects and nausea are common. A low risk of hypoglycemia, weight loss and the efficacy in the combination therapy with other OAD (192) and with basal insulin (193) are the advantages of these drugs for patients with type 2 diabetes.

### ***SGLT-inhibitors***

Sodium-glucose cotransporter (SGLT) 2 inhibitors have been recently introduced as antihyperglycemic agents for patients with type 2 diabetes. SGLT2 is expressed at the luminal membrane of proximal renal tubules. SGLT2 is the major transporter responsible for glucose reabsorption. SGLT1 reabsorbs the remaining 10% of the filtered glucose. The average of the daily amount of glucose reabsorbed by the kidney in healthy subjects is around 160 grams of glucose. No glucose normally appears in the urine (194).

Dapagliflozin is an SGLT2 inhibitor. It thereby inhibits reabsorption of glucose and increases urinary excretion, which lowers plasma glucose concentrations. The glucose-lowering effect in patients with type 2 results in approximately a 0.7 % decrease in HbA<sub>1c</sub> without increasing hypoglycemia when compared to placebo (195). Weight loss by dapagliflozin was approximately 3 kg in 24-week placebo-controlled study. The adverse events, such as signs and symptoms of urinary infections and genital infections, were more common in group treated with dapagliflozin as compared to placebo (195).

## 2.2.3 INSULIN

### *Choice of the insulin regimen*

Studies comparing basal insulin with OAD to mixed insulin with OAD are shown in Table 2 and studies comparing basal insulin with OAD to mixed insulin without OAD in Table 3. The comparison of studies using basal insulin with OAD and multiple injections with OAD is shown in **Table 4**.

In the various comparisons there was no difference in glycemic control except in studies where the insulin doses differed significantly (**Table 2-4**). Weight gain was significantly less with basal insulin and OAD than with mixed insulin with OAD (Table 2) or multiple injections with OAD (Table 4) in 5 out of 8 comparisons and not significantly different in the remaining 3 comparisons. Hypoglycemia was also significantly less in 5 out of 8 comparisons with basal insulin and OAD than with mixed or multiple insulin injections combined with OAD. These data support initiation of insulin therapy with the addition of basal insulin to OAD (6).

**Table 2.** Studies comparing basal insulin with OAD to mixed insulin twice daily with similar OAD in insulin-naïve patients with type 2 diabetes. Studies after year 1990 with duration  $\geq 3$  months are included.

<i>Author (Reference)</i>	<b>Comparator with OAD</b>	<b>ENDHbA<sub>1c</sub> %</b>	<b><math>\Delta</math>HbA<sub>1c</sub> %</b>	<b>ID IU/kg, IU/day</b>	<b><math>\Delta</math>Weight kg</b>	<b>Hypoglycemia</b>
<i>Kilo et al (196)</i> NPH+MET, n=91 Bas. <sub>1c</sub> 9.5 %	MIX30+MET	8.3 / 8.2 (NS)	1.2 / 1.3 (NS)	28 / 26 (ND)	0.1 / 0.7(NS)	0.55 / 1.04 13 % / 24 % (ND)
<i>Malone et al (197)</i> GLARGIN+MET, n=105 Bas.HbA <sub>1c</sub> 8.7 %	MIX25BID+MET	7.8 / 7.4**	0.9 / 1.3**	0.57 / 0.62***	1.6 / 2.3**	4.75 / 8.27*
<i>Raskin et al (198)</i> GLARGIN+OAD, n=209 Bas.HbA <sub>1c</sub> 9.8 %	MIX30BID+OAD	7.41 / 6.91**	2.36 / 2.79**	51 / 79*	3.5 / 5.4**	0.7 / 3.4**
<i>Jacober et al (199)</i> GLARGIN+OAD, n=60 Bas.HbA <sub>1c</sub> 9.2 %	MIX BID+OAD	7.34 / 7.08**	0.75 / 1.0**	0.28 / 0.35** 28 / 37**	1.52 / 1.98 (NS)	10.7 / 2.8*
<i>Holman et al (200)</i> DETEMIR+OAD, n=469 Bas.HbA <sub>1c</sub> 8.5 %	MIX30BID+ OAD	7.6 / 7.3***	0.8 / 1.3***	0.53 / 0.49 42 / 48 (NS)	1.9 / 4.7**	0 / 3.9**

ID=Insulin dose. Bas HbA<sub>1c</sub>, Baseline HbA<sub>1c</sub> (mean of both study groups). \*p<0.05; \*\*p<0.01; \*\*\* p<0.001; NS, not significant; ND, not documented. OD, once daily; BID, twice daily; TID, three times a day.

† Cross-over study. Hypoglycemia: Events/patient-year (% of patients). OAD, oral antidiabetic drug.

**Table 3.** Studies comparing basal insulin with OAD (oral antidiabetic drug) to mixed insulin without OAD in insulin-naïve patients with type 2 diabetes. Studies after year 1990 with duration  $\geq 3$  months are included.

<i>Author (Reference)</i>	<b>Comparator</b>	<b>ENDHbA<sub>1c</sub></b>	<b><math>\Delta</math>HbA<sub>1c</sub></b>	<b>ID</b>	<b><math>\Delta</math>Weight</b>	<b>Hypoglycemia</b>
<i>Yki-Jarvinen et al (68)</i> NPH evening+OAD, n=57 Bas. HbA <sub>1c</sub> 9.8 %	MIX 30 BID	8.0 / 7.9 (NS)	1.9 / 1.8 (NS)	20 / 43 (ND)	1.2 / 1.8*	NS
<i>Yki-Jarvinen et al (68)</i> NPH morning+OAD, n=61 Bas. HbA <sub>1c</sub> 9.6 %	MIX 30 BID	7.8 / 7.9 (NS)	1.7 / 1.8 (NS)	19 / 43 (ND)	2.2 / 1.8 (NS)	NS
<i>Wolffenbuttel et al (201)</i> NPH evening +SU, n=62 Bas.HbA <sub>1c</sub> 10.9 %	MIX 30 BID	8.1 / 8.2 (NS)	2.4 / 3.0 (NS)	24 / 39*	4.4 / 4.0 (NS)	ND
<i>Wolffenbuttel et al (201)</i> NPH morning+SU, n=67 Bas.HbA <sub>1c</sub> 11.2 %	MIX 30 BID	8.5 / 8.2 (NS)	2.6 / 3.0 (NS)	26 / 39*	3.4 / 4.0 (NS)	ND
<i>Janka et al (202)</i> GLARGIN evening+OAD, n=364 Bas.HbA <sub>1c</sub> 8.8 %	MIX 30 BID	7.15 / 7.49***	1.64 / 1.31***	28.2 / 64.5 (ND)	1.4 / 2.1 (NS)	2.6 / 5.7***

ID, Insulin dose; Bas HbA<sub>1c</sub>, Baseline HbA<sub>1c</sub> (mean of both study groups); \* p<0.05; \*\*p<0.01; \*\*\* p<0.001. NS, not significant. ND, not documented. OD, once daily; BID, twice daily; TID, three times a day. Hypoglycemia: Events/patient-year (% of patients).

**Table 4.** Studies comparing basal insulin and OAD to multiple preprandial injections with similar OAD in insulin-naive patients with type 2 diabetes.

<i>Author (Reference)</i>	<i>Comparator</i>	<i>ENDHbA<sub>1c</sub> (%)</i>	<i>ΔHbA<sub>1c</sub> (%)</i>	<i>IU (IU/kg, IU/day)</i>	<i>ΔWeight (kg)</i>	<i>Hypo-glycemia</i>
<i>Landstedt-Hallin et al (203)</i> NPH+SU, n=80 Bas. HbA <sub>1c</sub> 9.1 %	PREPRANDIAL TID+SU	7.5/7.1 (NS)	1.7/2.1 (NS)	26/29 (NS)	1.9/3.4 **	ND
<i>Bastyr et al (204)</i> NPH+SU, n=91 Bas. HbA <sub>1c</sub> 10.2 %	PREPRANDIAL TID+SU	8.5/7.7 **	1.89/2.33 (NS)	0.29/0.42 (ND)	2.3/3.4 (NS)	7.3/13.3 (NS)
<i>Holman et al (200)</i> DETEMIR+SU+MET, n=475 Bas. HbA <sub>1c</sub> 8.5 %	PREPRANDIAL TID+SU+MET	7.6/7.2 ***	0.9/1.3 ***	0.49/0.61 **	1.9/5.7 ***	grade 2 ***
<i>Bretzel et al (205)</i> GLARGIN+OAD, n=418 Bas. HbA <sub>1c</sub> 8.7 %	PREPRANDIAL TID+OAD	7.0/6.8 (NS)	1.7/1.8 (NS)	42/45 (ND)	3.0/3.5 (NS)	4.2/13.5 ***

\*\*p<0.01; \*\*\* p<0.001; NS, not significant; ND, not documented. OD, once daily; BID, twice daily; TID, three times a day. OAD, oral antidiabetic drug. BasHbA<sub>1c</sub>; Baseline Hba<sub>1c</sub> (mean of both groups). Hypoglycemia: events/patient-year.

### ***Practical aspects of initiation of insulin therapy***

Although insulin therapy has many beneficial effects, reluctance to initiate insulin is still common. This is in part because of lack of time and resources. There is thus a need to develop innovative strategies to facilitate the transition to insulin therapy. As discussed above, simple addition of basal insulin to existing oral agents is an attractive way to start insulin therapy as it involves only one injection of insulin, the dose of which can be adjusted based on self-monitoring of fasting plasma glucose (8), (12), (15), (68). Historically, insulin therapy has been started individually in patients with type 2 diabetes. Considering limited resources, the large numbers of patients, it would seem worthwhile to establish whether insulin can be started in groups. There are, however, no studies comparing initiation of insulin individually as compared to groups.

### **3. AIMS OF THE STUDY**

- I. To determine the effect of insulin therapy on LFAT content and hepatic insulin sensitivity in patients with type 2 diabetes.
- II. To determine the effects of addition of rosiglitazone to insulin on hepatic sensitivity to insulin, insulin requirements and glycemic control in patients with type 2 diabetes treated with high insulin doses.
- III. To compare the effect of adding nateglinide or placebo on postprandial glucose excursions in patients treated with basal insulin and metformin.
- IV. To determine whether insulin can be started equally well individually and in groups.

## 4. SUBJECTS AND STUDY DESIGNS

### 4.1. SUBJECTS

Baseline characteristics of the study subjects are shown in Table 6. For studies I and IV, insulin-naive patients with type 2 diabetes who were poorly controlled with oral antidiabetic drugs, MET alone (I) and MET and/or SU (IV) were recruited. For study II, patients with type 2 diabetes who had been treated with stable doses of MET, and were poorly controlled despite of high insulin dose were recruited. For study III patients with type 2 diabetes treated with basal insulin therapy and MET were studied.

Subjects were included based on the following criteria: 1) age over 18 years, 2) HbA<sub>1c</sub> between 7.0 and 12 % (IV) or  $\geq 6.5$  % (III), 3) body mass index between 20–50 kg/m<sup>2</sup> (III), less than 45 kg/m<sup>2</sup> (IV) and stable body weight and glycemic control for at least 6 months prior to participation (I, II). Further inclusion criteria were willingness to perform self-monitoring of blood glucose (III, IV) and to share some health information with other members of the group (IV).

In study III the inclusion criterion for entering the treatment period was an HbA<sub>1c</sub> between 6.5 and 9.0 %. Moreover, subjects with non-compliance as determined from failure to perform any diurnal glucose profiles during the optimization period were excluded from this study.

Exclusion criteria were clinical evidence of symptomatic cardiovascular or liver or other severe disease as determined by medical history, physical examination and standard laboratory tests (blood counts, electrolytes, creatinine, urine sample, electrocardiogram). Further exclusion criteria were treatment with drugs likely to interfere with glucose tolerance, macroalbuminuria, proliferative retinopathy, excessive alcohol consumption (>20 g/day), drug abuse and pregnancy (I-IV).

In addition, a history of ketoacidosis or positive GAD-antibodies or liver enzymes (S-ALT, S-AST; S-ALP) higher than 3 times the upper limit of normal or night shift work were exclusion criteria in studies III and IV.

For the study II subjects underwent transthoracic echocardiography and left ventricular ejection fraction equal or less than 50 % or any other clinically significant abnormality was an exclusion criteria.

Written informed consent was obtained from all subjects. The protocols were approved by Ethics Committee of Helsinki University Central Hospital (I-IV). Study III was also approved by local ethics committees of each participating center outside Helsinki.

## 4.2. STUDY DESIGN

### ***Effect of insulin therapy on LFAT and hepatic insulin sensitivity in patients with type 2 diabetes (I)***

Fourteen patients with type 2 diabetes treated with MET (2 g/day) alone were recruited and metabolic studies were performed before and 7 months after the initiation of basal insulin. Metabolic studies included measurements of LFAT, whole body and hepatic insulin sensitivity (**Fig. 3**), intra-abdominal (IA) and subcutaneous (SC) fat volumes, the percentage of body fat and glucose and lipid oxidation rates. Fasting blood samples were obtained for measurements of glucose, insulin, ALT, FFA, adiponectin, and blood lipid concentrations.

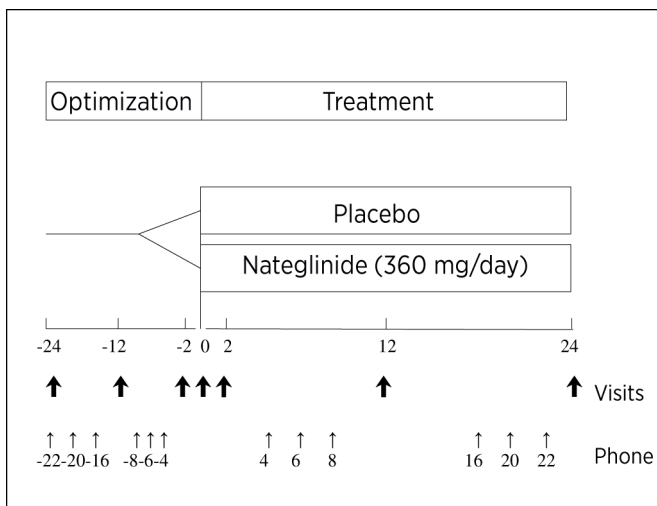
### ***Effect of addition of rosiglitazone to insulin on LFAT and hepatic insulin sensitivity (II)***

Fourteen patients with type 2 diabetes (9 men and 5 women) were enrolled by contacting specialists in Southern Finland. These patients had had a stable and high insulin dose in addition to 2 g of MET for at least 2 years. Despite of high-dose insulin therapy (range 115-400 IU/day) their glycemic control was unsatisfactory. LFAT, fat distribution, fat free and fat mass, whole body and hepatic insulin sensitivity (**Fig. 3**) and rates of substrate oxidation were determined during insulin and MET treatment and 8 months after addition of rosiglitazone (8 mg/day).

### ***Effect of adding nateglinide or placebo on postprandial glucose excursions in type 2 diabetic patients treated with basal insulin and MET (III)***

Study III was an investigator-initiated multicenter, double-blind, randomized, parallel-group, fixed dose trial. The efficacy, safety and tolerability of nateglinide were compared to placebo in patients with type 2 diabetes treated with the combination of basal insulin and MET (2 g/day). Basal insulin was first titrated to optimize glucose control during a 24-week period (optimization period). The goal was to achieve a mean FPG of 4.0-5.5 mmol/l. Thereafter either nateglinide or placebo was added before main meals for 24 weeks (treatment period). Study was performed at five sites in Finland. During first 24-week period basal insulin (NPH or glargine) was titrated to optimize glucose control. At the beginning of the treatment phase eligible patients (n=81) were randomly assigned to treatment with nateglinide or placebo using minimization of differences between the treatment groups (206). The following variables (relative weight of each variable is given in parentheses) were considered: age (1x); gender (0.5x); body mass index (1.5x); HbA<sub>1c</sub> (1.5x), duration of diabetes (0.5x); duration of insulin therapy (1.0x); type of basal insulin (NPH or glargine) (1.5x) and use of diuretics or  $\beta$ -blocking agents (0.25x) (206). Randomization was carried out at the Helsinki University Central Hospital.

Patients measured FPG every morning and performed a diurnal profile with plasma glucose measurements once during weeks -24, -12, -8, -4, -2 and 0, 2, 4, 8, 12, 16, 20, and 24. The visits and phone calls during the study weeks are shown in the **Fig. 1**. At weeks -12, 0, 12 and 24 the measurements of HbA<sub>1c</sub>, blood count, electrolytes, and serum lipid concentrations were performed. Safety laboratory tests included measurement of S-ALT, creatinine and bilirubin at weeks -2, 0, 2, 24.



**Figure 1.** Design (III). Effect of adding nateglinide or placebo on postprandial glucose excursions in type 2 diabetic patients treated with basal insulin and MET. Visits at weeks -24, -12, -2, 0, 2, 12 and 24. Phone calls at weeks -22, -20, -16, -8, -6, -4, 4, 6, 8, 16, 20 and 22.

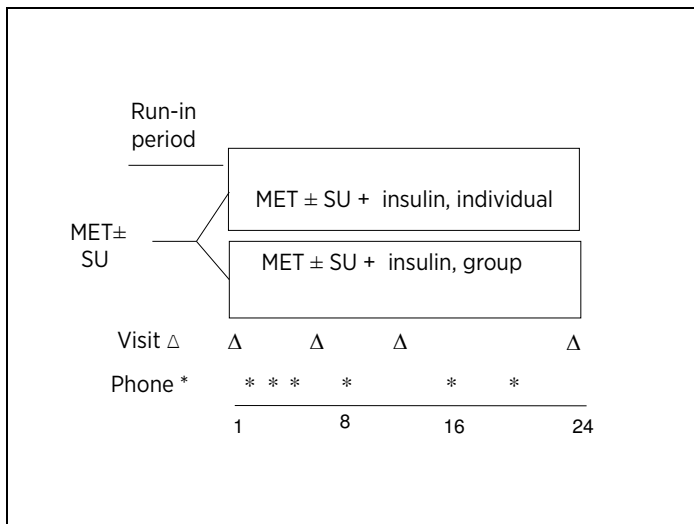
#### ***Initiation of insulin therapy individually or in groups (IV)***

This study was an investigator-initiated, open, randomized, and parallel-group multi-center study. We compared the initiation of insulin in groups vs. individually in insulin-naïve patients with type 2 diabetes. They were poorly controlled on OAD. The study consisted of a 3 to 14-week run-in phase and a 24-week treatment phase. Study groups were in Finland, Sweden, and U.K.

The participants (n= 121) were treated with combination of MET and SU or either drug alone for at least 6 months before the initiation of insulin therapy. The study was performed as the intent-to-treat basis. Week 0 visit the participants were taught how to inject insulin, use the insulin pen, and self-adjust insulin dose. Symptoms and signs of hypoglycemia were discussed. Treatment satisfaction was assessed by asking the patients to fill a Diabetes Treatment Satisfaction Questionnaire DTSQ form (207). This questionnaire was filled in the beginning and the end of the study.

The randomization to either group or individual education was performed centrally, using the method of minimization of differences (206). The group size was 4–8 subjects

(mean 5.3 persons). Patients performed self-monitoring of FPG every morning and sent FPGs using a modem or by mail before calling the study center. During the call, the study nurse reviewed glucose measurements received at a website ([www.prowellness.com](http://www.prowellness.com)), encouraged self-adjustment of the insulin dose, and asked for possible adverse events, incidents of hypoglycemia, and possible changes in medication. The educational content is shown in **Table 5**. The study design is shown in the Fig. 2. HbA<sub>1c</sub>, FPG and body weight were measured at every visit. Time spent by a nurse on education, physician's time, and number and duration of phone calls were recorded. The insulin dose, symptoms and signs of hypoglycemia were recorded at every contact during the study, and treatment satisfaction at week 0 and 24. Measurements of blood count, electrolytes, creatinine, and S-ALT were performed at screening visit -14 to -3, 24 weeks. Serum lipid concentrations were measured at weeks 0, 24.



**Figure 2.** Design (IV). The initiation of insulin therapy individually or in groups. Visits at 0, 6, 12 and 24 weeks, phone calls at 1, 2, 4, 8, 16 and 20 weeks.

**Table 5.** The educational content (IV).

<b>Visit (wks)</b>	<b>Content</b>
-2	Home-glucose monitoring. Pathogenesis of type 2 diabetes Treatment of type 2 diabetes
0	Why and when is insulin therapy needed Injection of insulin Self-adjustment of the insulin dose Hypoglycemia (causes, symptoms, treatment)
+6 +12	Weight gain and insulin therapy Targets of the glycemic control Type 2 diabetes and complications Why do insulin vary between patients
+24	Special situations and insulin therapy (missed injection, acute illness, travel) Encourage to self-adjustment of the insulin dose

## 5. METHODS

### 5.1. HEPATIC INSULIN SENSITIVITY (I, II)

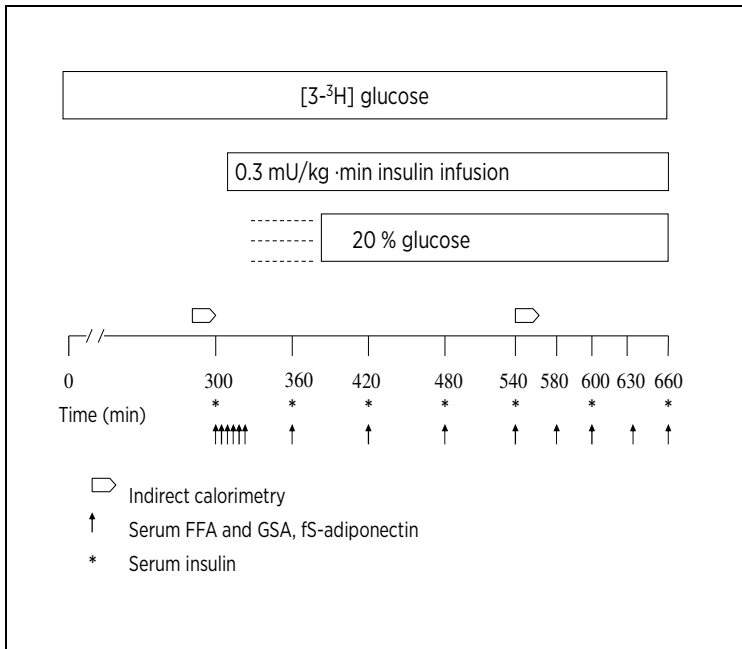
#### *Euglycemic, hyperinsulinemic clamp technique*

Patients were admitted to the hospital on the evening before the study. At 6 PM, an indwelling 18-gauge catheter (Venflon, Viggo-Spectramed, Helsingborg, Sweden) equipped with an obturator was inserted in an antecubital vein. On this evening before the study patients did not take their bedtime insulin injection. To determine total rates of glucose appearance ( $R_a$ ) and disappearance ( $R_d$ ), a primed continuous intravenous infusion of [ $3\text{-}^3\text{H}$ ]-glucose was started at 4 AM and continued for a total of 660 min. The priming dose of [ $3\text{-}^3\text{H}$ ]-glucose was adjusted according to the fasting blood glucose concentration and calculated as follows: priming dose = [glucose (mmol/l) at 4 AM/5] x 20  $\mu\text{Ci}/\text{min}$ . This dose was infused intravenously over 10 min and was followed by a continuous-rate infusion of [ $3\text{-}^3\text{H}$ ]-glucose at a rate of 0.2  $\mu\text{Ci}/\text{min}$  (7).

Before the start of the insulin infusion, a catheter was inserted in a retrograde position in a heated dorsal hand vein for withdrawal of arterialized venous blood. Baseline blood samples were taken for measurement of FPG, glucose specific activity (SA),  $\text{HbA}_{1c}$ , triglycerides, total, HDL, and LDL cholesterol, adiponectin, FFA, and fasting insulin concentrations.

After a 300-min equilibrium period, a primed continuous (0.3 mU/kg·min) infusion of insulin was started (22), (7). Because hepatic glucose production is sensitive to suppression by insulin (45), (24) we used a low insulin infusion rate to be able to accurately assess hepatic insulin sensitivity. Plasma glucose was adjusted to and maintained -8 mmol/l (144 mg/dl) for 360 min. A variable-rate infusion of 20 % glucose based on plasma glucose measurements was used to keep plasma glucose stable. Plasma glucose measurements were made from arterialized venous blood every 5–10 min. Glucose specific activity (SA), serum free insulin concentrations and S-FFA were taken basally and during hyperinsulinemia as shown **Figure 3**. Blood samples for measurement of glucose SA were taken basally at -30, -20, 0 min before the beginning of insulin infusion. Rates of glucose  $R_a$  and  $R_d$  were calculated using the Steele equation (26), assuming a pool fraction of 0.65 for glucose and distribution volume of 200 ml/kg for glucose.

Hepatic glucose  $R_a$  was calculated by subtracting the exogenous glucose infusion rate required to maintain euglycemia during hyperinsulinemia (300–660 min in Study I and 0–360 min in Study II) from the rate of total glucose  $R_a$ . The percent suppression of basal endogenous glucose  $R_a$  during the last 2 hours (540–660 min in Study I and 240–360 min in Study II) by insulin was used as a measure of HGP, *i.e.* the sensitivity of endogenous glucose production to insulin (percent suppression of endogenous  $R_a$ ).



**Figure 3.** Measurement of hepatic and peripheral insulin sensitivity in studies I and II. GSA; glucose specific activity. Blood samples for measurement of GSA were taken during insulin infusion at 420, 480, 540, 580, 600, 630 and 660 min. S-FFA was measured at 310 min and then every 5 min between 310 and 330 min and every 60 min between 360 and 660 min.

## 5.2. LIVER FAT CONTENT (I, II)

### *Proton magnetic resonance spectroscopy (1H-MRS)*

LFAT was measured using <sup>1</sup>H-MRS. Localized single-voxel (2 x 2 x 2 cm<sup>3</sup>) proton spectra were recorded using a 1.5-T whole body system (Magnetom Vision, Siemens, Erlangen, Germany) that consisted of the combination of whole-body and loop surface coils for radiofrequency transmitting and signal receiving (7). T<sub>1</sub>-weighted high-resolution MRI scans were used for localization of the voxel of interest within the right lobe of the liver. Magnetic resonance spectroscopy measurements of LFAT were performed in the middle of the right lobe of the liver at a location that was individually determined for each subject. Vascular structures and subcutaneous fat tissue were avoided when selecting the voxel. Subjects were required to lie on their stomach on the surface coil embedded in a mattress, in order to minimize movement artefact due to breathing. The single voxel spectra were recorded by using the stimulated-echo acquisition mode sequence with an echo time of 20 ms, a repetition time of 3000 ms, a mixing time of 30 ms, 1024 data points over 1000 kHz spectral width with 32 averages. Water-suppressed spectra with 128 averages were recorded to detect weak lipid signals. A short echo time and the long repetition time were chosen to ensure a fully relaxed water

signal, which was used as an internal standard. Chemical shifts were measured relative to water at 4.80 ppm. The methylene signal, which represents intracellular triglyceride, was measured at 1.4 ppm. Signal intensities were quantified by using the analysis program, VARO-MRUI (<http://www.mrui.uab.es/mrui>). Spectroscopic intrahepatocytic triglyceride content was expressed as a ratio of the area under the methylene peak to that under the methylene and water peaks ( $\times 100 =$  liver fat %). All spectra were analyzed by a physicist who was unaware of other research data.

### **5.3. MEASURES OF BODY COMPOSITION (I, II)**

#### ***Intra-abdominal and abdominal subcutaneous fat volumes***

Volumes of IA and abdominal SC fat were measured by analyzing 16 T<sub>1</sub>-weighted two-dimensional trans-axial MRI scans from a region extending from 8 cm above to 8 cm below the 4<sup>th</sup> and 5<sup>th</sup> lumbar interspace (16 slices, field of view 375 × 500 mm<sup>2</sup>, slice thickness 10 mm, breath-hold repetition time 138.9 ms, echo time 4.1 ms). Volumes of adipose tissue deposits were calculated using an image analysis program (Alice 3.0, Parexel, Waltham, MA). A histogram of pixel intensity of each scan was displayed, and the intensity corresponding to the nadir between the lean and fat peaks was used as a cut-off point. Intra-abdominal adipose tissue was defined as the area of pixels in the intra-abdominal region above this cut point. For calculation of subcutaneous adipose tissue area, a region of interest was first manually drawn at the demarcation of subcutaneous adipose tissue and intra-abdominal adipose tissue (7) (**Fig. 4**).

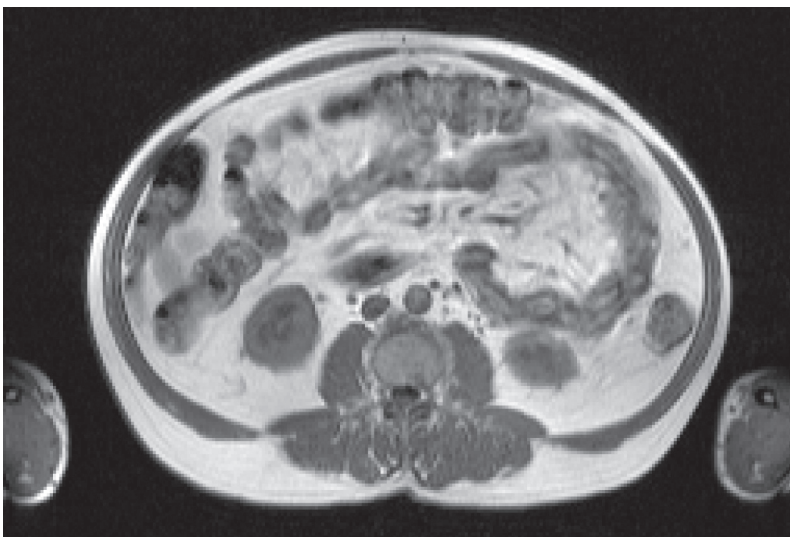
#### ***Whole-body fat % and waist-to-hip -ratio (I, II)***

Body fat-free mass and fat mass were determined by bioelectrical impedance analysis (BioElectrical Impedance Analyzer System model no. BIA-101A, RJL Systems, Detroit, MI) (207). Waist circumference was measured midway between the lower rib margin and spina iliaca superior, and hip circumference at the level of the great trochanters (209).

### **5.4. MEASUREMENTS OF SUBSTRATE OXIDATION RATES (I, II)**

Glucose and lipid oxidation rates were measured by indirect calorimetry using the Deltatrac Metabolic Monitor (Datex, Helsinki, Finland) (210), (211). The measurements were performed for 40 min during the basal period and the last 2 hours of insulin clamp. Samples of inspired and expired air, which were suctioned at 40 l/min, were analyzed for O<sub>2</sub> and CO<sub>2</sub> concentration differences using paramagnetic O<sub>2</sub> and CO<sub>2</sub> analyzers, respectively. Urine was collected and the protein oxidation rate was estimated from urea nitrogen excretion (1 g nitrogen = 6.25 g protein). The constants used for the calculation of glucose and lipid oxidation rates from gas exchange data were as follows:

oxidation of 1 g of protein requires 966 ml of  $O_2$  and produces 782 ml of  $CO_2$ , of 1 g of glucose requires 746 ml of  $O_2$  and produces 746 ml of  $CO_2$ , and of 1 g of lipid requires 2.029 ml of  $O_2$  and produces 1.430 ml of  $CO_2$ . The rate of nonoxidative glucose disposal was calculated by subtracting the rate of glucose oxidation from the rate of total glucose disposal. Energy production rates ( $J/kg\cdot f\cdot m\cdot min$ ) were calculated assuming oxidation of 1 mg carbohydrate produces 15.65 J of energy; 1 mg lipid produces 39.75 J of energy; and 1 mg protein produces 17.15 J of energy (210).



**Figure 4.** One of the 16 abdominal scans of the patient in Study II. Fat is shown in white.

## 5.5. TRANSTHORACIC ECHOCARDIOGRAPHY (II)

Transthoracic echocardiography was performed by using a Vivid 7 digital ultrasonography system (GE Vingmed Ultrasound, Horton, Norway). The left ventricular ejection fraction was calculated by M-mode echocardiography from the parasternal long-axis view. Measurements were made while the subject was lying in the left lateral recumbent position from three consecutive beats. The average of three beats was used for analysis. Subjects with left ventricular ejection fraction less than 50 % were excluded from the study II. Transthoracic echocardiography was performed as a safety concern.

## 5.6. OPTIMIZATION OF INSULIN THERAPY (III, IV)

Patients had been doing home-glucose monitoring before (II, III) and the participants were taught how to inject insulin and to perform glucose self-monitoring (I, IV). The education was led by the same nurse in group and individual sessions (IV). Participants

were asked to measure FPG every morning (I–IV) and to perform a diurnal profile with plasma glucose measurements before and 2.0 hrs after breakfast, lunch and dinner, at 10 p.m. and at 4 a.m (III). The patients were asked to increase the dose of insulin by 2–4 IU when FPG exceeded 5.5mmol/l for 3 consecutive days. The recommended increase was 4 IU/day if FPG exceeded 10mmol/l on 3 consecutive mornings. The goal was to achieve a mean FPG of 4.0–5.5 mmol/l (III, IV). Adjustment of the insulin dose and symptoms of hypoglycemia was monitored at visits and phone calls. Biochemical hypoglycemia was defined as a plasma glucose <4.0 mmol/L. Symptomatic confirmed hypoglycemia was defined as symptoms of hypoglycemia combined with a measured plasma glucose concentration of <4.0 mmol/l. Severe hypoglycemia was defined as an event with symptoms consistent with hypoglycemia during which the subject required the assistance of another person and which was associated with either a plasma glucose level below 3.1 mmol/l or with prompt recovery after oral carbohydrate, intravenous glucose or glucagon administration (8). The patients were instructed to measure plasma glucose whenever hypoglycemia occurred and to record the value and symptoms. If FPGs were <4.0 mmol/l and symptomatic hypoglycemia occurred without an identifiable reason, the patients were asked to decrease the insulin dose by 2 IU/day. Treatment satisfaction was assessed by asking the patients to fill DTSQ - form (207) (IV).

## 5.7. ANALYTICAL PROCEDURES

Plasma glucose concentrations were measured in duplicate with the glucose oxidase method using a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA) (I, II) (211). In order to determine [ $^3\text{-}^3\text{H}$ ]-glucose SA plasma was deproteinized with barium hydroxide,  $\text{Ba}(\text{OH})_2$  and zink sulphate,  $\text{ZnSO}_4$  and evaporated (213) (I, II). Serum free insulin concentrations were measured with the Auto-DELFA kit (Wallac, Turku, Finland) (I, II) and C-peptide concentrations by RIA (214).  $\text{HbA}_{1c}$  was measured by high-pressure liquid chromatography using the Glycosylated Hemoglobin Analyzer System (Bio-Rad, Richmond, CA) (I–IV). Serum total cholesterol, HDL cholesterol, and triglyceride concentrations were measured with the enzymatic kits from Roche Diagnostics using an autoanalyzer (Roche Diagnostics Hitachi 917, Hitachi, Tokyo, Japan) (I, II). The concentrations of LDL cholesterol were calculated using the Friedewald formula (215) (I, II). Serum alanine aminotransferase (ALT) activity was determined as recommended by the European Committee for Clinical Laboratory Standards (I, II). Lipids, ALT, and other measurements were performed using methods in local laboratories (III, IV). Serum adiponectin concentrations were measured by an ELISA kit from B-Bridge International (San Jose, CA) (I), and S-FFA concentrations were measured by fluorometric assay (216) (I, II).

## 5.8. STATISTICAL ANALYSES

In all studies a p-value of less than 0.05 was considered statistically significant. Calculations were made using GraphPad Prism version 3.0 (Study I) or 4.0 (Studies II, III) for Windows (GraphPad, San Diego, CA), SysStat Statistical Package (Systat version 10; SysStat, Evanston, IL) (I, III) and SPSS 14.0 (II) for Windows (SPSS, Chigaco, IL). Data are shown as mean  $\pm$ SEM.

The paired *t*-test was used to compare changes before and after insulin (I) or additional rosiglitazone (II) treatment for normally distributed parameters. Logarithmic transformation was performed if necessary. Correlation analyses were performed using the Spearman`s nonparametric correlation coefficient for the comparison of two continuous variables.

In study III, all statistical analyses were performed on an intent-to-treat basis. The intention-to-treat population was defined as all patients who were randomized to enter the 24-week treatment phase. The mean postprandial glucose excursion (PPGE) was defined as:  $\sum_n (B\text{-gluc}_{2h\text{ pp}} - B\text{-gluc}_{\text{premeal}}) / n$ , where 2-h pp is the postprandial glucose 2 hours after the meal (breakfast, lunch or dinner) and n the number of glucose measurements. To detect a 2 mmol/l difference in postprandial glucose, assuming 90 % power and a significance level of  $p=0.05$ , 50 patients per treatment group were required to observe a clinically significant decrease in postprandial glucose between the groups, assuming a 2.3 mmol/l standard deviation for 2-h glucose (162).

In study III, the change in  $HbA_{1c}$  during 24 weeks between nateglinide and placebo groups was analyzed using two-way ANOVA for repeated measured followed by pairwise comparisons. Changes in body weight, insulin doses, S-ALT, fS-triglycerides, fS-LDL and fS-HDL cholesterol were analyzed similarly. For analysis of changes in diurnal glucose profiles, the mean diurnal profiles of -2 and 0 weeks, and of 20 and 24 weeks were calculated. The 8-point curves of changes in glucose concentrations and insulin doses were compared between the nateglinide and placebo groups using two-way ANOVA for repeated measurements. Pairwise comparisons between nateglinide and placebo groups at all time points were done using paired *t*-test corrected for the number of tests performed. For comparison of symptomatic hypoglycemic events/patient when the variance was not normally distributed, the Mann-Whitney test was used. The frequencies of hypoglycemia between the groups were analyzed using Fisher`s exact test. Data are given as mean $\pm$ SEM for normally distributed, and as median followed by the interquartile range (25<sup>th</sup> and 75<sup>th</sup> percentiles) for non-normally distributed data.

In study IV, all statistical analyses were performed on an intent-to-treat basis, defined as randomized patients who received at least one injection of insulin and using a two-sided significance level of  $\alpha=0.05$ . The primary endpoint,  $HbA_{1c}$  change from baseline

to the end of the study was evaluated using ANCOVA model as a response variable. The comparison of the two educational methods was evaluated using ANCOVA model. These secondary objectives were as follows: time spent by a nurse on education, physician's time, number and duration of phone calls, change in the concentrations of S-HDL and S-LDL cholesterol and S-triglycerides, change in body weight, change in FPG, insulin dose at study end, and change in subject's treatment satisfaction, and incidence of hypoglycemic episodes during the study.

The method of education and center were included as fixed effects, with the baseline value of HbA<sub>1c</sub> as a covariate in the ANCOVA model. Non-normally distributed data were used after logarithmic transformation. The change in the treatment satisfaction was compared between the groups using Mann-Whitney U test. The educational programs were defined as equally successful if HbA<sub>1c</sub> at the end of the study differed by < 0.5 % (8)

## 6. RESULTS

Baseline characteristics of the study subjects are shown in **Table 6**.

**Table 6.** Baseline characteristics of the study subjects in studies I-IV

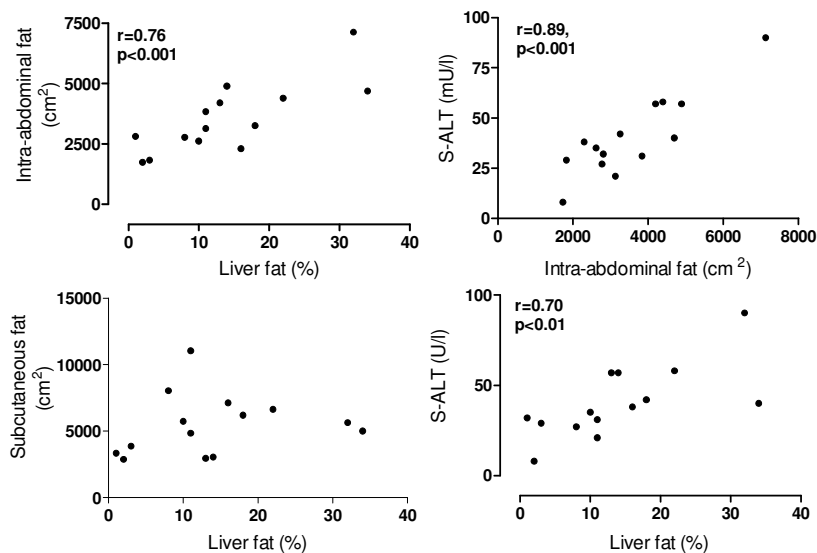
Variable	Study I	Study II	Study III* Nateglinide	Study III* Placebo	Study IV Individual	Study IV Group
Number	14	14	40	41	63	58
Age (y)	51±2	51±3	55±1	57±1	58±0.1	58±0.1
Gender (M/F, %)	79/21	64/36	60/40	51/49	65/35	59/41
Weight (kg)	101±6	111±4	97± 2	93± 3	94±3	90±3
BMI (kg/m <sup>2</sup> )	33±1.4	36.7±1.1	33.6± 0.7	32.1±0.7	31.5±0.7	31.2±0.7
Duration of DM (yrs)	7	12	9	9	8	7
HbA <sub>1c</sub> (%)	8.9 ±0.3	8.9±0.4	7.5±0.1	7.4±0.1	8.7±0.2	8.8±0.2
FPG (mmol/l)	10.7±0.7	9.1±0.6	6.2±0.3	6.0±0.3	9.0±0.1	8.7±0.1
S-ALT (U/l)	51±10	108±14	38±3	32±3	38±3	41±4
Insulin dose (IU/kg)		1.98±0.2	0.83±0.1	0.72±0.1		
OAD	MET	MET	MET	MET	MET±SU	MET±SU

\* at the beginning of treatment period (week 0)

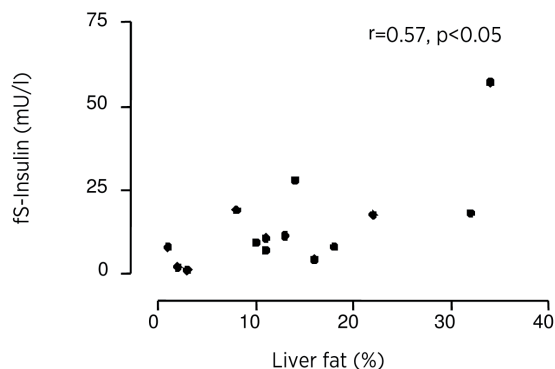
FPG, fasting plasma glucose; S-ALT, serum alanine aminotransferase; OAD, oral antidiabetic drug

### 6.1. EFFECT OF INSULIN THERAPY ON LIVER FAT CONTENT AND HEPATIC INSULIN SENSITIVITY IN PATIENTS WITH TYPE 2 DIABETES (I)

Before the initiation of insulin treatment, LFAT correlated significantly with S-ALT concentrations ( $r=0.77$ ,  $p<0.01$ ), with the amount of intra-abdominal fat ( $r=0.74$ ,  $p<0.01$ ) but not with the amount of subcutaneous fat (NS) (**Fig. 5**) and with fasting insulin ( $r=0.57$ ,  $p<0.05$ ) (**Fig. 6**). The volume of intra-abdominal fat also correlated with S-ALT concentrations ( $r=0.77$ ,  $p<0.01$ ) (**Fig. 5**).



**Figure 5.** *Left panels:* The relationship between liver fat content, and intra-abdominal fat ( $p < 0.001$ ,  $r = 0.76$ , top left) and subcutaneous fat ( $p = \text{NS}$ , bottom left). *Right panels:* The relationship between intra-abdominal fat and S-ALT ( $p < 0.001$ ,  $r = 0.89$ , top right), and the relationship between liver fat and S-ALT ( $p < 0.001$ ,  $r = 0.70$ , bottom right), before insulin therapy. Adapted from *Juurinen et al. Am J Physiol Endocrinol Metab* 292:E829-E853, 2007 (Study I), reproduced with permission of the copyright holder.



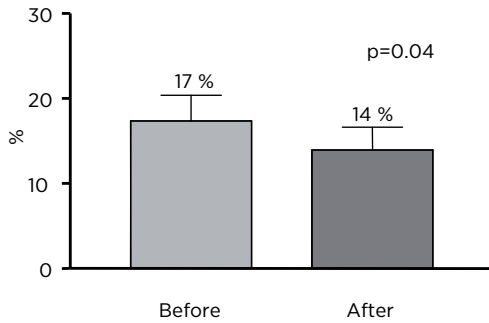
**Figure 6.** Liver fat correlated with fasting insulin. Adapted from *Juurinen et al. Am J Physiol Endocrinol Metab* 292:E829-E853, 2007 (Study I), reproduced with permission of the copyright holder.

Effects of insulin therapy on metabolic parameters and body composition are shown in **Table 3**. The insulin dose averaged  $75 \pm 10$  IU/day ( $0.69 \pm 0.08$  IU/kg, range 24–132 IU/day). HbA<sub>1c</sub> decreased from  $8.9 \pm 0.3$  % to  $7.4 \pm 0.2$  %,  $p=0.001$ . FPG decreased from  $10.7 \pm 0.7$  mmol/l to  $8.4 \pm 0.6$  mmol/l,  $p=0.001$ . Body weight increased by  $3.0 \pm 1.1$  kg ( $p<0.05$ ) from  $101 \pm 6$  kg to  $104 \pm 6$  kg. This was mainly fat-free mass, which increased by  $2.5 \pm 0.7$  kg (**Table 7**).

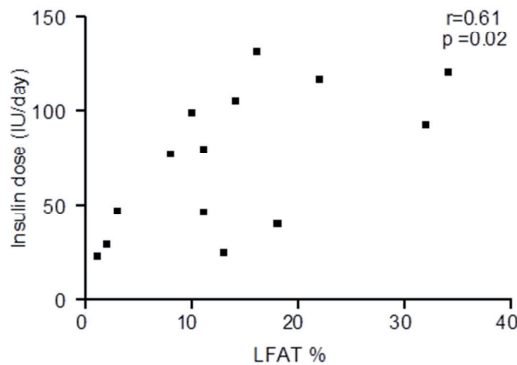
	Before	After	P-value
HbA <sub>1c</sub> (%)	$8.9 \pm 0.3$	$7.4 \pm 0.2$	0.001
S-ALT (U/l)	$51 \pm 10$	$40 \pm 5$	NS
Body mass index (kg/m <sup>2</sup> )	$33.1 \pm 1.4$	$34.0 \pm 1.4$	0.02
Waist-to-hip ratio	$1.01 \pm 0.02$	$1.02 \pm 0.02$	NS
Fat-free mass (kg)	$69.5 \pm 3.8$	$72.0 \pm 4.0$	0.004
Fat mass (kg)	$31.8 \pm 2.6$	$32.3 \pm 2.6$	NS
Intra-abdominal fat (dm <sup>3</sup> )	$3.5 \pm 0.3$	$3.5 \pm 0.4$	NS
Abdominal subcutaneous fat (dm <sup>3</sup> )	$5.3 \pm 0.5$	$5.4 \pm 0.6$	NS
S-adiponectin (mg/l)	$4.6 \pm 0.5$	$4.2 \pm 0.4$	NS

**Table 7.** Physical and biochemical characteristics of the patients with type 2 diabetes before and after 7 months of insulin therapy. Data are mean  $\pm$  SEM. NS, not significant. Adapted from *Juurinen et al. Am J Physiol Endocrinol Metab* 292:E829–E853, 2007 (Study1), reproduced with permission of the copyright holder.

LFAT decreased slightly but significantly by 20 % from  $17 \pm 3$  to  $14 \pm 3$  % ( $p=0.04$ ) (**Fig. 7**). LFAT and the insulin dose at 7 months were significantly correlated ( $r=0.63$ ,  $p<0.05$ ) (**Fig. 8**). LFAT correlated significantly with S-ALT concentrations ( $r=0.77$ ,  $p<0.01$ ).



**Figure 7.** Liver fat content before and after 7 months insulin therapy. Adapted from *Juurinen et al. Am J Physiol Endocrinol Metab* 292:E829-E853, 2007 (Study I), reproduced with permission of the copyright holder.

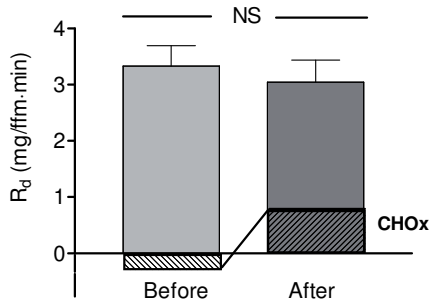


**Figure 8.** The relationship between insulin dose at 7 months and liver fat content Adapted from *Juurinen et al Am J Physiol Endocrinol Metab* 292:E829-E853, 2007 (Study I), copyright holder.

### The changes in hepatic and peripheral insulin sensitivity, FFA concentrations, and substrate oxidation rates

Basal glucose appearance ( $R_a$ ) as a measure of basal hepatic glucose production decreased from  $3.42 \pm 0.20$  to  $2.66 \pm 0.33$  mg/kg ffm  $\cdot$  min ( $p < 0.05$ ) and basal glucose disappearance ( $R_d$ ) from  $3.28 \pm 0.27$  to  $2.40 \pm 0.31$  mg/kg ffm  $\cdot$  min ( $p < 0.01$ ) before and after insulin therapy.

Insulin-stimulated  $R_d$  remained unchanged ( $3.3 \pm 0.4$  vs.  $3.2 \pm 0.4$  mg/kg ffm  $\cdot$  min, NS), but the fraction of glucose  $R_d$  directed toward oxidation increased significantly from  $-7 \pm 6$  % ( $-0.40 \pm 0.28$  mg/kg ffm  $\cdot$  min) to  $30 \pm 3$  % ( $1.24 \pm 0.12$  mg/kg ffm  $\cdot$  min,  $p < 0.001$ ) (**Fig. 9**).



**Figure 9.** Glucose  $R_d$  and the rate of glucose oxidation measured by indirect calorimetry during hyperinsulinemia before and after insulin therapy.  $CHO_x$ =rate of carbohydrate oxidation, which increased significantly ( $p<0.02$ ). Insulin-stimulated  $R_d$  remained unchanged ( $p=NS$ ), but the fraction of glucose  $R_d$  directed toward oxidation increased significantly. Adapted from *Juurinen et al. Am J Physiol Endocrinol Metab* 292:E829-E853, 2007 (Study I), reproduced with permission of the copyright holder.

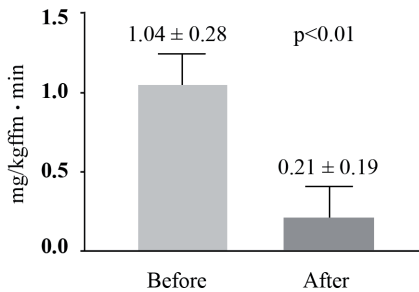
Whole body insulin sensitivity increased from  $2.2\pm 0.4$  to  $3.1\pm 0.4$  mg/kg ffm·min ( $p<0.05$ ). This improvement was due to enhanced hepatic insulin sensitivity, since HGP was better suppressed by insulin after than before insulin therapy ( $0.21\pm 0.19$  vs.  $1.04\pm 0.28$  mg/kg ffm·min,  $p<0.01$ ) (**Fig. 10**). The percent suppression of HGP by insulin increased from  $72\pm 8$  to  $105\pm 11$  % ( $p=0.001$ ) (**Fig. 11**). The change in LFAT was significantly correlated with the change in HGP suppression by insulin (difference in mg/kg ffm·min,  $r=0.56$ ,  $p<0.05$ ).

Fasting S-FFA decreased from  $970\pm 75$  to  $751\pm 51$   $\mu\text{mol/l}$  ( $p<0.05$ ) and S-triglyceride concentrations from  $2.7\pm 0.4$  to  $2.0\pm 0.2$  mmol/l ( $p<0.05$ ). S-LDL and S-HDL cholesterol concentrations remained unchanged. There was no significant change in serum adiponectin concentration before and after insulin therapy ( $4.6\pm 0.5$  mg/l vs.  $4.2\pm 0.4$  mg/l,  $p=NS$ ).

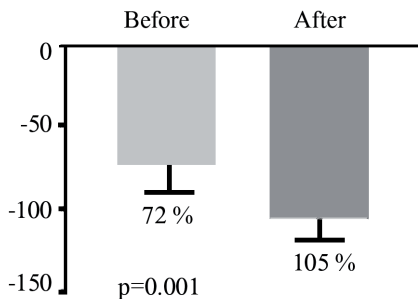
S-FFA concentrations during the insulin infusion were significantly lower after ( $439\pm 42$   $\mu\text{mol/l}$ ) than before ( $563\pm 66$   $\mu\text{mol/l}$ ,  $P<0.05$ ) insulin therapy. The percent suppression of S-FFA by insulin remained unchanged ( $42\pm 12$  vs.  $42\pm 17$  %, NS).

Rates of substrate oxidation in the basal state showed a trend to change toward less lipid oxidation ( $1.56\pm 0.14$  vs.  $1.24\pm 0.20$  mg/kg ffm·min,  $p=0.15$ ) and increased carbohydrate oxidation ( $0.49\pm 0.20$  vs.  $1.28\pm 0.47$  mg/kg ffm·min,  $p=0.09$ ). The rate of protein oxidation remained unchanged ( $0.058\pm 0.004$  vs.  $0.063\pm 0.006$  mg/kg ffm·min, NS).

The rate of lipid oxidation during the insulin infusion decreased significantly from  $1.88\pm 0.15$  to  $1.09\pm 0.06$  mg/kg ffm·min ( $p<0.001$ ). Rates of total energy expenditure remained unchanged.



**Figure 10.** Endogenous hepatic glucose production during hyperinsulinemia before and after insulin therapy. Adapted from *Juurinen et al. Am J Physiol Endocrinol Metab* 292:E829-E853, 2007 (Study I), reproduced with permission of the copyright holder.



**Figure 11.** The percent suppression of endogenous glucose  $R_g$  during the last 2 hours by insulin was used as a measure of HGP, i.e. the sensitivity of endogenous glucose production to insulin (percent suppression of endogenous  $R_g$ ) before and after insulin therapy. Adapted from *Juurinen et al. Am J Physiol Endocrinol Metab* 292:E829-E853, 2007 (Study I), reproduced with permission of the copyright holder.

## 6.2. LIVER FAT CONTENT AND HEPATIC INSULIN SENSITIVITY AFTER COMBINATION OF ROSIGLITAZONE TO METFORMIN AND INSULIN THERAPY (II)

Clinical and biochemical characteristics of the patients before and after 8 months of additional treatment with rosiglitazone are shown in **Table 8**. HbA<sub>1c</sub> and insulin dose decreased significantly. FPG decreased from 9.1±0.6 mmol/l to 7.9±0.5 mmol/l (P=NS). Fasting serum free insulin decreased by 48 % (**Table 8**). Body weight increased by 3.2±0.9 kg, which was due to increases in fat-free (1.7±0.7 kg) and fat mass (1.6±0.7 kg). Subcutaneous but not intra-abdominal fat increased significantly when measured with MRI.

Liver fat content, S-ALT and S-ALP concentrations also decreased significantly (**Table 8**). The percent change in liver fat weakly correlated with the percent changes in S-AST ( $r=0.63$ ,  $p=0.02$ ) but not in S-ALT. The percent change in liver fat content

correlated positively with the percent change in insulin dose ( $r=0.66$ ,  $p=0.014$ ), and with the percent change in the suppression of endogenous glucose production (EGP) ( $r=0.76$ ,  $p=0.003$ ) as a direct measure of hepatic insulin sensitivity.

**Table 8.** Clinical and biochemical characteristics of the patients with type 2 diabetes before and after 8 months of additional treatment with rosiglitazone (II).

	Before	After	P-value
Body weight	111±4	114±4	0.002
BMI (kg/m <sup>2</sup> )	36.7±1.1	37.8±1.1	0.002
Waist (cm)	120±3	125±3	NS
HbA <sub>1c</sub> (%)	8.9±0.4	7.8±0.3	0.007
Liver fat (%)	20±3	11±3	0.0002
IA fat (cm <sup>3</sup> )	3900±340	4200±360	NS
SC fat (cm <sup>3</sup> )	5500±540	6000±530	0.0002
Insulin dose (IU)	218±22	129±20	0.002
Insulin dose	1.98±0.2	1.11±0.15	0.001
fS-insulin (mU/l)	62±25	32±6	NS
fS-FFA (μmol/l)	716±52	597±50	0.04
S-ALT (U/l)	46±6	32±4	0.02
S-ALP (U/L)	108±14	67±7	0.02

All data are shown as mean ± SEM. Adapted from *Juurinen et al. J Clin Endocrinol Metab* 2008;93:118-124 (Study II), reproduced with permission of the copyright holder.

### **Measurements of substrate oxidation rates**

Energy expenditure in the basal state decreased significantly by rosiglitazone from 83.2±3.2 J/kg ffm·min to 78.2±2.1 J/kg ffm·min ( $p=0.05$ ) and energy expenditure in the insulin-stimulated state decreased significantly from 80.1±3.0 J/kg ffm·min to 75.1±1.9 J/kg ffm·min ( $p=0.017$ ). The rate of lipid oxidation during insulin stimulation decreased significantly from 1.81±0.12 mg/kg ffm·min to 1.51±0.12 mg/kg ffm·min ( $p=0.04$ ) by rosiglitazone treatment. There was no significant change in rates of carbohydrate and protein oxidation.

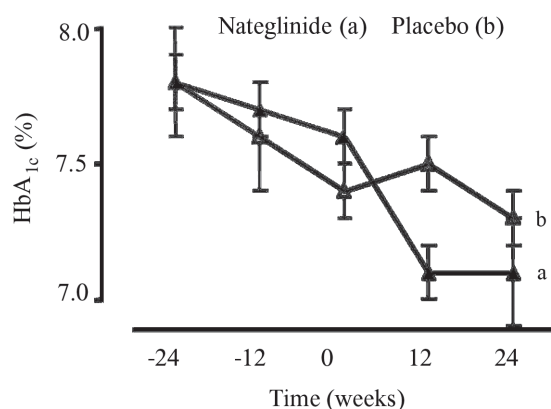
## **6.3. POSTPRANDIAL HYPERGLYCEMIA AFTER COMBINATION OF NATEGLINIDE TO METFORMIN AND INSULIN THERAPY (III)**

From total of 88 eligible patients were randomized and 81 continued to the treatment phase. Reasons for discontinuation during the optimization period included: acute myocardial infarction ( $n=1$ ), unwillingness to continue within four weeks after screening visit ( $n=3$ ), poor compliance, no home-monitoring data ( $n=2$ ), and a too high HbA<sub>1c</sub>

at visit 0 (n=1). There were no drop-outs during the treatment period. Baseline characteristics of the study groups were comparable (Table 6).

### Optimization period (-24 to 0 weeks): HbA<sub>1c</sub>, insulin doses and hypoglycemia

During the optimization period HbA<sub>1c</sub> and insulin doses did not change in the nateglinide and placebo groups ( $-0.3\pm 0.1$  vs.  $-0.4\pm 0.2$  % (NS) and 10.0 IU (2.0–32.0) IU [0.09 IU/kg (0.02–0.34 IU/kg)] vs. 10.0 IU (0.0–19.0 IU) [0.11 IU/kg (0.0–0.25 IU/kg)] (p=NS), respectively. There was no significant difference in symptomatic, confirmed hypoglycemia or biochemical or nocturnal hypoglycemia between the nateglinide and placebo groups during the optimization period.



**Figure 12.** During the optimization period (weeks -24–0) HbA<sub>1c</sub> decreased by  $-0.3\pm 0.1$  and  $-0.4\pm 0.2$  % (NS). During the treatment period (weeks 0–24) HbA<sub>1c</sub> decreased from  $7.5\pm 0.1$  to  $7.1\pm 0.2$  % in the nateglinide and from  $7.4\pm 0.1$  to  $7.3\pm 0.1$  % in the placebo group (p=0.001). p<0.0001 nateglinide vs. placebo at week 12. ▲ nateglinide (a) △ placebo (b). Adapted from Juurinen *et al. Diabetic Medicine*, 2009; 26, 409–415 (Study III) reproduced with permission of copyright holder.

### Treatment period (1 to 24 weeks): Glycemic control, weight, lipids, and liver enzymes

During the treatment period, insulin doses increased in the placebo group (0.02 IU/kg ( $-0.01$ – $0.10$  IU/kg)) but remained unchanged in the nateglinide group (0.0 IU/kg ( $-0.5$ – $0.05$  IU/kg)), p=0.013 for repeated measurements ANOVA). The number of tablets/day was  $2.8\pm 0.1$  vs.  $2.8\pm 0.1$  at week 16 and  $2.8\pm 0.1$  vs.  $2.8\pm 0.1$  at week 24 in the nateglinide vs. placebo groups (p=NS). HbA<sub>1c</sub> decreased from  $7.5\pm 0.1$  to  $7.1\pm 0.2$  % in the nateglinide and from  $7.4\pm 0.1$  to  $7.3\pm 0.1$  % in the placebo group (p=0.001). The change in HbA<sub>1c</sub> averaged  $-0.41\pm 0.12$  % in the nateglinide group and  $-0.04\pm 0.12$  % in the placebo group (p=0.023) (Fig. 12). There were no differences between the groups with respect to changes in weight, fS-triglycerides, or fS-HDL and fS-LDL cholesterol during the optimization or treatment period.

**Table 9. Results after treatment phase.**

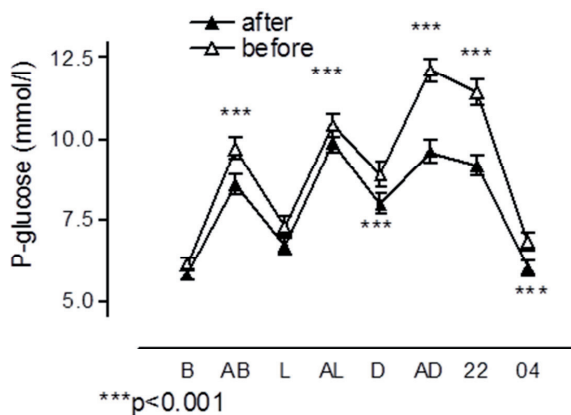
Characteristics	Nateglinide	Placebo	P-value
Weight change	1.2±0.5	1.0±0.4	NS
HbA <sub>1c</sub> (%)	7.1±0.2	7.3±0.1	NS
Change in HbA <sub>1c</sub>	-0.4±0.2	-0.04±0.1	<0.05
FPG (mmol/l)	5.6±0.3	5.9±0.3	NS
PPGE (mmol/l)	2.5±0.2	3.1±0.2	<0.02

Adapted from *Juurinen et al. Diabetic Medicine*, 2009; 26, 409–415 (Study III) reproduced with permission of copyright holder.

### ***Postprandial glucose excursions and diurnal glucose profiles***

The patients performed over 90 % of the recommended home-glucose measurements (91 % and 94 %, n=6126 and n=6484 for nateglinide and placebo groups, weeks 1–24). After the 24 week treatment phase, FPGs were similar between the the nateglinide and placebo groups and averaged 5.6 mmol/l [5.0–6.7 mmol/l] and 5.9 mmol/l [5.1–6.9 mmol/l], respectively (NS). PPGE, the primary endpoint, was lower in the nateglinide than in the placebo group (**Table 9**).

Postprandial glucose (mean of all post-meal glucoses during weeks 20–24) decreased significantly from baseline (mean glucose during weeks -2 and 0) in the nateglinide group (**Fig. 13**) and remained unchanged in the placebo group (10.6±0.4 to 9.0±0.3 mmol/l, and 10.1±0.3 to 10.0±0.3 mmol/l, p=0.001). Plasma glucose concentrations after dinner and at 10 p.m. and 4 a.m. also decreased significantly by nateglinide as compared to placebo. There were no changes in plasma glucose concentrations between the groups at baseline and at weeks 20–24 before breakfast, after breakfast, before lunch, after lunch, and before dinner.



**Fig 13.** Mean diurnal glucose profiles before (weeks -2 and 0) and during nateglinide therapy (weeks 20–24). Adapted from *Juurinen et al. Diabetic Medicine*, 2009; 26, 409–415 (Study III) reproduced with permission of copyright holder.

### Hypoglycemia

The number of episodes of symptomatic, confirmed hypoglycemia and the number of grade 2 hypoglycemia per patient was higher in the nateglinide than the placebo group. The episodes of biochemical hypoglycemia, nocturnal, confirmed hypoglycemia were similar in both groups (**Table 10**). The home monitored glucose at the time of hypoglycemic symptoms during the optimization period averaged  $3.4 \pm 0.06$  vs.  $3.1 \pm 0.06$  mmol/l in nateglinide and placebo group and during the treatment phase  $3.2 \pm 0.05$  vs.  $3.3 \pm 0.06$  mmol/l, respectively.

**Table 10.** Treatment period: rates of hypoglycemia (events/pt-year), (% of patients).

	Nateglinide	Placebo	P-value
Symptomatic, confirmed, plasma glucose <4.0 mmol/l	7.7 (75 %)	4.7 (54 %)	<0.05
Symptomatic, confirmed, plasma glucose <3.1 mmol/l	3.4 (50 %)	1.6 (24 %)	<0.05
Nocturnal, confirmed	1.6 (33 %)	0.4 (17 %)	NS
Daytime, confirmed	6.1 (68 %)	4.2 (49 %)	NS

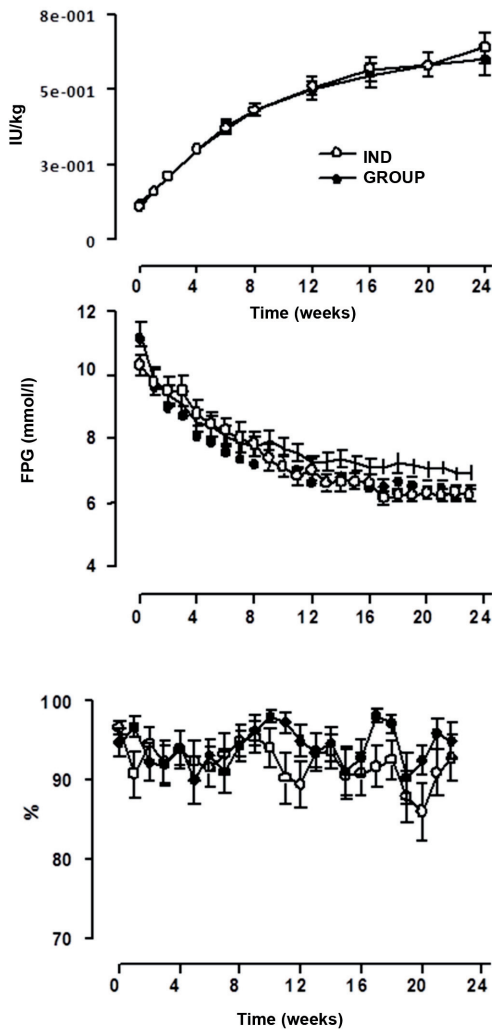
Adapted from *Juurinen et al. Diabetic Medicine*, 2009; 26, 409–415 (Study III) reproduced with permission of copyright holder.

#### 6.4. INITIATION OF INSULIN THERAPY INDIVIDUALLY OR IN GROUPS (IV)

Eligible patients (n=128) were recruited at randomization visit. Seven patients dropped out during the recruitment phase while waiting for the initiation of insulin therapy (metastasis of papillary thyroid carcinoma, retinal neovascularization, and unwillingness to continue: n=2; group formation took too long: n=1; other: n=2). Intention-to-treat population, a total of 121 patients started insulin therapy. Five dropped out from the individual education arm (poor compliance, n=2; hypoglycemia, n=1; protocol violation, n=1; new adverse event, n=1). No patients dropped out from the group education arm. The mean group size was 5.3 individuals. Of scheduled visits there was no difference in attendance between the educational groups: 95.6 % vs. 90.3 %, individual education vs. group education, p=NS.

##### *Glycemic control*

HbA<sub>1c</sub> decreased from 8.65±0.18 % to 6.89±0.14 % in patients individually treated (p<0.001) and from 8.79±0.20 % to 6.81±0.12 % in patients treated in the group education arm (p<0.001). There was no difference between the two arms. FPG decreased from 9.0±0.1 mmol/l to 6.3±0.1 mmol/l in the individual education arm. In the group treatment patients FPG decreased from 8.7±0.1 mmol/l to 6.4±0.1 mmol/l (**Fig. 14**). No difference was found between the study arms in the percentage of patients achieving target (mean FPG: range 4.0–5.5 mmol/l): 66 % in the group treatment arm and 70 % in the individual treatment arm (p=NS).



**Figure 14.** Insulin dose, FPG and days with available FPGs (%) by week did not differ significantly between study arms. Adapted by *Juurinen L.* from *Yki-Järvinen et al. Diabetes Care*, 2007;30:1364-1369 (Study IV) reproduced with permission of copyright holder.

### **Hypoglycemia**

The number of episodes of symptomatic hypoglycemia in the individual education arm was 3.5 episodes/patient year and 3.1 episodes/patient year in the group treatment arm ( $p=NS$ ). The percentage of symptomatic hypoglycemia was similar in the individual and group treatment arms 44 and 40 %, ( $p=NS$ ). The percentage of nocturnal symptomatic hypoglycemia was 13 % in the individual and 10% in group treatment arms ( $p=NS$ ). There were no episodes of severe hypoglycemia.

***Insulin dose and other clinical characteristics at week 24***

The insulin doses did not differ significantly between study arms:  $62 \pm 5$  IU/day in the individual arm and  $56 \pm 5$  IU/day in group treatment arm ( $p=NS$ ) ( $0.64 \pm 0.05$  IU/kg/day and  $0.60 \pm 0.05$  IU/kg/day,  $p=NS$ ) (**Fig. 13**). Weight gain was lower in the individual arm ( $2.2 \pm 0.4$  kg) than in the group arm ( $3.7 \pm 0.6$  kg),  $p < 0.02$ . There were no differences in S-triglycerides, S-HDL cholesterol, or S-LDL cholesterol concentrations between the study groups.

***Treatment satisfaction and time spent on patient education***

The treatment satisfaction outcome improved significantly in both education arms. No difference was found in the responses to the individual questions of the DTSQ between study groups. The total time spent starting insulin was 48 % lower in the group than in the individual education arm. However, there was no correlation between total time spent in education and the  $HbA_{1c}$ .

The total (scheduled in-class or over-the-phone and extra time for the group arm was  $2.3 \pm 0.1$  h ( $n=58$ ) and  $4.4 \pm 0.2$  h ( $n=63$ ) in the individual arm. In the group arm:  $1.6 \pm 0.1$  h for scheduled visits,  $0.59 \pm 0.03$  h for scheduled phone calls, and  $0.73 \pm 0.24$  h for extra visits ( $n=10$ ) and  $0.17 \pm 0.05$  h for extra phone calls ( $n=11$ ). The corresponding times in the individual arm:  $3.6 \pm 0.2$  h for scheduled visits,  $0.59 \pm 0.03$  h for scheduled phone calls,  $0.59 \pm 0.2$  h for extra visits ( $n=14$ ) and  $0.14 \pm 0.03$  h for extra phone calls ( $n=11$ ).

## 7. DISCUSSION

All of the patients in Studies I-IV were obese, middle-aged patients with type 2 diabetes. BMI varied between 31 to 37 kg/m<sup>2</sup> and duration of diabetes varied from 7 to 12 years. The glycemic control was suboptimal, mean HbA<sub>1c</sub> in the studies ranged from 7.4 % to 8.9 %. In I and IV patients were insulin-naïve and basal insulin therapy started as recommended by the current ADA/EASD treatment guideline (6). The additional OAD, rosiglitazone (II) or nateglinide (III) was combined with basal insulin therapy and MET.

### ***Effects of insulin therapy on liver fat and hepatic insulin sensitivity (I)***

LFAT decreased during chronic insulin therapy. The 20 % decrease in LFAT is less than that of PPAR $\gamma$  agonists have shown to decrease in studies of patients with type 2 diabetes (mean 40 %, range 32–51 %) (10). A moderate weight loss of 7 kg has decreased LFAT also more than insulin therapy did in the present study (53). Since the normal value for liver fat is maximally 5 % (20), (217), LFAT was still high and liver was insulin resistant at the end of insulin therapy.

Our result was confirmed later by *Lingvay et al* (218), (219). Liver fat content measured by <sup>1</sup>H-MRS decreased 45 % by insulin therapy combined with MET in treatment-naïve patients with type 2 diabetes.

We found insulin therapy to enhance hepatic insulin sensitivity as measured from insulin suppression of HGP. Effects of insulin therapy on insulin peripheral sensitivity have previously been examined in 18 studies. In most of these studies, a significant improvement in whole body or peripheral glucose uptake was found (220), (221), (222), (223), (224), (225), (226), (227) (228), (229), (230), (231), (232), (233), (74) and the metabolic clearance rate of glucose improved significantly by insulin therapy in two studies (233), (234). We used lower insulin infusion rate than all these studies except two of them (224), (235) and our finding that insulin-stimulated glucose uptake remained unchanged is consistent with these two studies (224), (235).

The changes in LFAT correlated significantly with hepatic insulin sensitivity. We have previously made a similar finding in patients with type 2 diabetes (7) and in healthy men (2). A significant positive correlation was found between the fasting insulin concentration before insulin therapy and liver fat. Thus hepatic insulin resistance may increase the need for endogenous insulin secretion to control HGP. Consistent with these data, our result found a correlation between LFAT and the insulin dose.

Total serum adiponectin levels remained unchanged by insulin therapy and thus unlikely contributed to decreases in LFAT or enhancement of hepatic insulin sensitivity. On the other hand, S-FFA concentrations decreased possibly due to inhibition of lipolysis by insulin. This is a likely mechanism underlying reduced liver fat since FFA are the main source of intrahepatocellular triglycerides both basally and postprandially

(77). FFA also induce hepatic insulin resistance (93). A significant proportion of liver triglycerides originates from de novo lipogenesis in patients with NAFLD (77). Insulin therapy induces hyperinsulinemia, when diurnal insulin profiles are measured before and during basal insulin therapy (68). This would be expected to stimulate rather than inhibit lipogenesis in the liver (9). However, it seems that inhibition of peripheral lipolysis, as measured from the decrease in S-FFA is more predominant in determining the direction of change in LFAT since the latter decreased significantly. Lowering of plasma glucose concentrations by insulin therapy also reduces the substrate for de novo lipogenesis and thereby also LFAT although de novo lipogenesis was not directly measured in the present study. *Ryysy et al* found that LFAT correlated with insulin requirements in type 2 diabetic patients, independent of body weight (7). S-ALT also predicts insulin requirements independent of body weight (8).

### ***Effects of addition of a PPAR $\gamma$ agonist on liver fat and hepatic insulin sensitivity (II)***

We have previously shown that the degree of hepatic steatosis is the key determinant of insulin requirements (7) and that the PPAR $\gamma$  agonist rosiglitazone markedly decreases liver fat content compared to MET (10). We therefore now tested the hypothesis that rosiglitazone decreases insulin requirements in type 2 diabetic patients treated with high doses of insulin. Addition of a PPAR $\gamma$  agonist (rosiglitazone) to basal insulin therapy for 8 months decreased HbA $_{1c}$  significantly despite a 41 % reduction in the insulin dose. LFAT decreased by 46 %, and hepatic insulin sensitivity increased significantly. The decrease in LFAT correlated with a decrease in insulin requirements and an increase in hepatic insulin sensitivity. In previous studies, PPAR $\gamma$  agonists when combined with insulin therapy, decreased insulin doses averaged 13 IU/d (236), (237), (238), (69), (70), (71), (72), (73). In the present study, insulin requirements decreased by 90 IU/d. Patients with the highest baseline insulin doses at baseline showed the greatest relative reduction of insulin doses (238), (74).

Peripheral insulin sensitivity remained unchanged during rosiglitazone treatment. This was an expected finding, since low physiological insulin infusion rates are optimal for assessment of hepatic rather than peripheral insulin sensitivity (239), (23), (24), (45).

Weight gain (3.2 kg) was greater than would be expected from improvement in glycemic control [1.5–2.0 kg per 1 % decrease in HbA $_{1c}$  (240)]. The finding is similar as previous study by *Balas B et al.* (241). PPAR $\gamma$  agonists increase mainly fat mass (241). Weight gain in Study II was found to be due to an increase in fat free mass and fat mass.

Hepatic insulin sensitivity was measured by the euglycemic, hyperinsulinemic clamp technique, which is the golden standard of the method to quantitate peripheral and hepatic insulin sensitivity. However, this method is expensive and invasive. Thus, it was not feasible to study a control group. This is a potential limitation (I, II) and

a time effect independent of insulin (I) or rosiglitazone (II) could contribute to the results. On the other hand, we have shown that addition of MET alone has no effect on LFAT and that rosiglitazone alone compared to MET decreases LFAT by 50 % in patients with type 2 diabetes (10).

***Effect of a short-acting insulin secretagogue on postprandial glycemia in patients treated with basal insulin and metformin (III)***

Studies III and IV of the present thesis were undertaken to improve treatment of type 2 diabetic patients. In study III, we combined a short-acting insulin secretagogue, nateglinide to basal insulin with MET to improve postprandial hyperglycemia. In previous studies in patients treated with oral agents only, decreases in HbA<sub>1c</sub> by nateglinide as compared to placebo have averaged 0.5 % (161), 0.55 % (162), 0.6 % (163), 0.39 % (160), 0.7 % (165), 0.31 % (164) and 0.8 % (242). In the present study HbA<sub>1c</sub> averaged 7.4–7.5 % before addition of nateglinide or placebo to basal insulin MET combination therapy. HbA<sub>1c</sub> decreased by 0.4 %, which is comparable to that found in studies using nateglinide in patients not treated with insulin.

Regarding studies on effects of nateglinide in previously insulin-treated patients, *Dashora et al* (158) compared added nateglinide or placebo to previous insulin therapy. In this study 55 type 2 diabetic patients were recruited. These patients initially used mixed insulin regimens. Insulin therapy was changed to insulin glargine for one month before addition of either nateglinide or placebo for 16 weeks (158). Before addition of nateglinide or placebo, HbA<sub>1c</sub> averaged 8.2 % in the nateglinide and 8.2 % in the placebo group. The baseline-adjusted mean decrease in HbA<sub>1c</sub> by nateglinide compared to placebo averaged 0.43 % at the end of treatment. This was very similar to that in our study. This difference in HbA<sub>1c</sub> was not statistically significant (158). The decrease in HbA<sub>1c</sub> in study III by nateglinide as compared to placebo reached statistical significance most likely because of a larger sample size.

The frequency of hypoglycemia in study III was significantly higher in the nateglinide than the placebo group. Almost half of the patients in the nateglinide group had at least one episode of symptomatic confirmed hypoglycemia. The frequencies of hypoglycemia were greater than in the study of *Dashora et al*. (158). In our study home-glucose monitoring was extensive as was the recording of hypoglycemia. Glycemic control was also significantly better in our study with nateglinide (HbA<sub>1c</sub> 7.1 %) than in the study of *Dashora et al* (158) (HbA<sub>1c</sub> 7.8%). This could also have contributed to the higher rate of hypoglycemia in the present study than in that of *Dashora et al* (158), since the rate of hypoglycemia is inversely proportional to glycemic control (14).

### ***Initiation of insulin (IV)***

In the last study of the present thesis, we tested whether basal insulin therapy can be started in groups as well as individually in patients with type 2 diabetes. We compared, in a randomized fashion, initiation of insulin therapy by adding basal insulin to existing oral agents individually and in groups. Both methods of insulin initiation seemed equally effective in improving glycemic control. The primary end point was difference in HbA<sub>1c</sub> between the education programs. We found that starting insulin in type 2 diabetes in groups gives as good glycemic control as individual initiation, and that excellent glycemic control can be achieved using both models. There were no differences in insulin doses, symptomatic or biochemical hypoglycemia or treatment satisfaction between the groups. However, the amount time the nurse spent in initiating insulin individually was twice that spent in initiating insulin in groups. Basal insulin therapy is possible to start with the education in groups as well as with the individual education using the simple principles of the present study. The patient education in groups is a considerable option because this saves time and resources.

We chose to start insulin therapy by adding a single injection of basal insulin to existing oral agents. This means that only one measurement of glucose was needed for adjustment of the insulin dose. In addition, basal insulin therapy combined with OADs is associated with less weight gain and hypoglycemia than multiple insulin injection regimens (243). Glucose values were sent to the treatment center electronically, which allowed the nurse to easily follow fasting plasma glucoses over time. We achieved one of the best glycemic controls in any insulin treatment study in type 2 diabetes by the year 2007, when the study was published. The HbA<sub>1c</sub> in the group education arm averaged 6.8 % (244), (245), (202), (8), (15), (198). Body weight increased 3.7 kg. HbA<sub>1c</sub> decreased by 2 % units in both study groups. We have previously shown that for every 1 % decrease in HbA<sub>1c</sub>, body weight increases by 2 kg. Thus the average predicted weight gain was a 4 kg, which was observed. Weight gain was slightly higher in the group than the individual education arm. The patients may have received more dietary advice during the individual education sessions than in the group. This could have contributed to the difference in weight gain between the groups. Treatment satisfaction improved similarly in both educational groups. This was observed although participation in the group as compared to the individual arm required less time of the patient.

This thesis is based on studies published between year 2007 and 2009. Since then, newer OADs, DPP4-inhibitors and GLP-1 agonists, have been increasingly used instead of SUs, glinides and TZDs. Rosiglitazone was withdrawn due to an increase in cardiovascular events, especially heart failure (169). However, there are no data to demonstrate superiority of these newer agents. Indeed, in two cardiovascular outcome studies, the effects of alogliptin (246) and saxagliptin (247) were modest (0.3 % decrease in HbA<sub>1c</sub> compared to placebo) and there was no benefit with respect cardiovascular disease, the main complication of type 2 diabetes. In the other

study, the incidence of heart failure increased by 30 % in the DPP-4 as compared to the placebo group (247).

Although use of TZDs has decreased, recent studies have shown pioglitazone to have beneficial effects on NASH (61), (66), (248). Thus, although use of TZDs were initially restricted to patients with normal liver enzymes, because of the fear of liver toxicity after troglitazone, which caused liver failure (168), TZDs are now recommended to be used in patients with NASH (249).

Altogether, basal insulin therapy remains one of the basic options to treat hyperglycemia in patients with type 2 diabetes due to the proceeding beta-cell failure and hepatic insulin resistance.

## 8. SUMMARY AND CONCLUSIONS

The first two studies of the present thesis (I and II) were undertaken to understand hepatic effects of insulin and a PPAR $\gamma$  agonist in patients with type 2 diabetes. It was shown that insulin therapy increases hepatic insulin sensitivity and significantly decreases liver fat content in obese type 2 diabetic patients. These changes were associated with decreases in circulating FFA, the predominant substrate of intrahepatocellular triglycerides synthesis. Decreases in glucose might also have contributed to the decrease in liver intrahepatocellular triglycerides, although we did not measure the rate of de novo lipogenesis. These data show that subcutaneous insulin therapy has beneficial effect on the liver.

In study II, it was shown that rosiglitazone decreased insulin requirements by 41 % and liver fat content by 47 %. It also significantly improved hepatic insulin sensitivity and glycemic control in type 2 diabetic patients who were poorly controlled despite using unusually high doses of insulin. However, this improvement was obtained at the expense of weight gain.

Studies III and IV of the present thesis were undertaken to improve treatment of type 2 diabetic patients. We hypothesized that addition of nateglinide might improve postprandial hyperglycemia in patients treated with basal insulin. We found that addition of nateglinide to basal insulin with MET lowered postprandial glucose excursions significantly compared to placebo and improved average glycemic control (HbA $_{1c}$ ) by 0.4 %. This relatively small improvement was obtained at the expense of increased hypoglycemia and necessitated administration of three daily doses of nateglinide, which is cumbersome.

In the last study of the present thesis, we tested whether basal insulin therapy can be started in groups as well as individually in patients with type 2 diabetes. We found that starting insulin in type 2 diabetes in groups gives as good glycemic control as individual initiation, and that excellent glycemic control can be achieved using both models.

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## ORIGINAL PUBLICATIONS

- I *Juurinen Leena, Tiikkainen Mirja, Häkkinen Anna-Maija, Hakkarainen Antti, Yki-Järvinen Hannele*  
**Effects of insulin therapy on liver fat content and hepatic insulin sensitivity in patients with type 2 diabetes.** *American Journal of Physiology, Endocrinology and Metabolism* 292: E829–E835, 2007.
- II *Juurinen Leena, Kotronen Anna, Granér Marit, Yki-Järvinen Hannele*  
**Rosiglitazone reduces liver fat and insulin requirements and improves hepatic insulin sensitivity and glycemic control in patients with type 2 diabetes requiring high insulin doses.** *Journal of Clinical Endocrinology and Metabolism* 93: 118–124, 2008.
- III *Juurinen Leena, Tiikkainen Mirja, Saltevo Juha, Nikkilä Katriina, Lanki, Helena, Leppävuori Eeva, Kock Tuula, Teikari-Myyrä Tarja, Kauppinen-Mäkelin Ritva, Kotronen Anna, Yki-Järvinen Hannele*  
**Nateglinide combination therapy with basal insulin and metformin in patients with type 2 diabetes.** *Diabetic Medicine* 26: 409–415, 2009.
- IV *Yki-Järvinen Hannele, Juurinen Leena, Alvarsson Michael, Bystedt Tord, Caldwell Ian, Davies Melanie, Lahdenperä Sanni, Nijpels Gil, Vähätalo Markku*  
**Initiate Insulin by Aggressive Titration and Education (INITIATE): A randomized study to compare initiation of insulin combination therapy in type 2 diabetic patients individually and in groups.** *Diabetes Care* 30: 1364–1369, 2007.

Publication II has also been included in the doctoral thesis of *Anna Kotronen* entitled **Liver fat in the metabolic syndrome and type 2 diabetes**, University of Helsinki, 2008.