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**Differential lipid profile and hormonal response in type 2 diabetes by
exogenous insulin aspart versus the insulin secretagogue repaglinide, at the
same glycaemic control**

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Abstract

To study, at the same glycaemic control, how treatment with either the insulin secretagogue repaglinide or exogenous insulin aspart affects endogenous insulin secretion, plasma insulin and IAPP (islet amyloid polypeptide) levels, GH-IGF (growth hormone - insulin-like growth factor) axis and plasma lipoprotein concentrations in patients with type 2 diabetes. Five patients, age 65.0 ± 4.1 years (mean \pm SE), body weight 82.5 ± 5.0 kg, BMI (body mass index) 27.7 ± 1.5 kg/m² were treated for 10 weeks with repaglinide or insulin aspart in a randomized, cross-over study. At the end of each treatment a 24-h metabolic profile was performed. Blood glucose, C-peptide, free human insulin, free total (human and analogue) insulin, proinsulin, IAPP, IGF-I, IGFBP-1 (IGF binding protein-1), GHBP (growth hormone binding protein) and plasma lipoprotein concentrations were measured. Similar 24-h blood glucose profiles were obtained with repaglinide and insulin aspart treatment. During the repaglinide treatment the meal related peaks of C-peptide and free human insulin were about twofold higher than during treatment with insulin aspart. Proinsulin, GHBP were higher and IAPP levels tended to be higher during repaglinide compared to insulin aspart. Postprandial plasma total cholesterol, triglycerides and apolipoprotein B concentrations were higher on repaglinide than on insulin aspart treatment. Our results show that, at the same glycaemic control, treatment with exogenous insulin aspart in comparison with the insulin secretagogue repaglinide result in a lower endogenous insulin secretion, and a tendency towards a less atherogenic postprandial lipid profile.

Keywords. Insulin secretagogue, insulin-like growth factor, lipoprotein

Abbreviations. IGF-I (insulin-like growth factor-I), GH-IGF (growth hormone-insulin-like growth factor axis), IGFBP-1 (insulin-like growth factor binding protein-1), GHBP (growth hormone binding protein) and IAPP (islet amyloid polypeptide).

Introduction

Cardiovascular diseases are the leading cause of morbidity and mortality in patients with type 2 diabetes and lipid disturbances are of great importance for development of these complications [1, 2]. Dyslipidaemia, associated with type 2 diabetes, is characterized by increased levels of triglycerides (TG), reduced levels of high-density lipoprotein (HDL) cholesterol, while total cholesterol (TC) and low-density lipoprotein (LDL) cholesterol may be either normal or elevated [3]. Postprandial lipaemia is a characteristic feature of diabetic dyslipidaemia and a risk factor for premature atherosclerosis [4, 5].

Treatment of type 2 diabetes entails, besides life style changes, oral hypoglycaemic agents and when needed exogenous insulin with the goal to maintain glycaemic levels as close as possible to the nondiabetic range [6]. Insulin aspart and the insulin secretagogue repaglinide have been developed to have an onset and duration of action that closely matches the postprandial blood glucose peak [7-11].

Insulin and the liver have central roles in lipid metabolism [12]. Improving glycaemic control by exogenous insulin in patients with type 2 diabetes and insufficient glycaemic control on treatment with oral hypoglycaemic agent alters the lipoprotein profile towards a less atherogenic pattern [13, 14]. Independent of glycaemic control the mode of treatment, insulin secretagogue versus exogenous insulin, may alter the lipoprotein profile possibly by affecting portal insulin delivery to the liver [15-17]. This can also have importance for the GH-IGF axis [18], since insulin up regulates the GHR and GHBP level in the liver and down regulates the production of IGFBP-1 [19-22].

To test the role of the pharmacological properties of an insulin secretagogue and an insulin analogue (repaglinide) in patients with type 2 diabetes we studied, at the same glycaemic control, the lipoprotein profile and GH-IGF axis.

Research Design and Methods

Patients

Five patients (two men and three women), age 65.0 ± 4.1 years (mean \pm SE) (range 52-77 years), body weight 82.5 ± 5.0 kg, BMI 27.7 ± 1.5 kg/m² and known diabetes duration for 5.0 ± 1.6 (range 2-9) years took part in this study. Two patients had well-controlled hypertension while no patients had a history of cardiovascular disease. All patients had normal renal function and no retinopathy. Previous treatment with oral antihyperglycaemic agents before the study were only metformin in two patients, sulphonylureas only in one patient and combination treatment of metformin and sulphonylureas in two patients. None of the patients were treated with lipid lowering agents.

Study design

Patients with type 2 diabetes mellitus treated with oral hypoglycaemic agents (sulphonylurea and/or metformin) were invited to take part in this open label, randomised, cross-over study. The patients were randomized to start with either insulin aspart (Novorapid[®] U-100, Novo Nordisk, Denmark) or repaglinide (Novonorm[®], Novo Nordisk, Denmark) and during the study other oral hypoglycaemic agents were withdrawn. The treatment was given for a period of 10 weeks and all patients were then switched to the alternative treatment for another 10 weeks. The patients were instructed to monitor blood glucose frequently, before and 1.5-2h after the main meals and at bedtime, for adjusting therapy. Insulin aspart or repaglinide were administered immediately before the main meals. Adjustment of insulin and repaglinide doses was done in cooperation with the staff of the diabetes unit. Target pre-prandial plasma glucose concentrations were 4–7 mmol/l and post-prandial (1.5–2h after a main meal) below 10 mmol/l. The protocol allowed addition of intermediate acting NPH insulin given during the evening if acceptable fasting blood glucose

control was not achieved. During the last 4 weeks of each treatment period the doses were not changed.

At the end of each 10-week period, a 24-h profile with frequent blood sampling was performed for glucose, C-peptide, free human insulin and free total insulin. All patients arrived at 16:00 to the clinic. The patients had dinner at 17:00, breakfast at 07:00 and lunch at 12:00. The total caloric intake during the profile days was 1852 kcal consisting of 52% carbohydrates, 22% proteins, 25% fat and 1% alcohol. Between 17:00-19:00 the blood samples were drawn every 30 minutes and thereafter every 2 hours until 06:50 in the morning. In the morning the blood samples were taken every 10 minutes from 06:50 until 08:00, and thereafter every hour until 16:00. To determine the lipoprotein profile, blood samples were drawn fasting at 06:50 and nonfasting at 11:00 and 15:00. Fasting blood samples were drawn to determine IGF-I and GHBP. Fasting and postprandial blood samples were used to determine proinsulin, IAPP and IGFBP-1 (times are indicated in the figures).

The study was performed according to the recommendations of the Declaration of Helsinki and the local ethical committee approved the protocol. All patients gave their informed consent.

Biochemical analysis

Blood glucose was analysed by the HemoCue[®] (Hemocue Inc., Mission Viejo, CA, USA). A1c (reference range: 3.2 – 5.4%) was analyzed with reverse-phase partition chromatography on a cation exchanger using high-performance liquid chromatography (HPLC; Auto A1C HA 8110, Boehringer Mannheim). C-peptide was measured with an enzyme-linked immunosorbent assay (ELISA) from DakoCytomation (DakoCytomation Ltd., Cambridgeshire, UK), based on two monoclonal antibodies against C-peptide. Plasma free insulin was measured after removal of insulin antibodies and antibody-bound insulin by polyethylene-glycol (PEG) precipitation [23].

Free human insulin was measured by Mercodia Insulin ELISA (Mercodia AB, Uppsala, Sweden) using a two-site enzyme immunosorbent assay containing two monoclonal antibodies against human insulin, with no cross-reactivity with insulin aspart [24]. Human insulin was used for the standard curve. Plasma free total insulin was measured by Mercodia Iso-Insulin ELISA (Mercodia AB, Uppsala, Sweden) using a two-site enzyme immunoassay containing two monoclonal antibodies cross-reacting equally with human insulin and insulin aspart [24]. The plasma proinsulin was analyzed by an ELISA two-site enzyme immunosorbent assay (Mercodia AB, Uppsala, Sweden). Total serum IGF-I was measured by a one-step enzyme-linked immunosorbent assay (ELISA) after acid-ethanol-extraction from its binding protein with a commercial kit from Diagnostic System Laboratories (Webster, Texas, USA). The assay was performed according to the manufacturer's protocol. Plasma IGFBP-1 was determined by a two-step enzyme-linked immunosorbent assay using a kit from Diagnostic System Laboratories (Webster, Texas, USA). Serum human IAPP was measured by a monoclonal antibody-based sandwich immunosorbent assay (Linco Research Inc, USA). The capture antibody recognizes IAPP, IAPP acid (deamidated IAPP), a 1-20 fragment of IAPP, but not reduced IAPP. The detection antibody binds to reduced or unreduced human IAPP but not IAPP acid, and is complexed with streptavidin-alkaline phosphatase.

In brief, determination of lipoproteins and apolipoprotein A-1 and B, were performed as describe below. Plasma very low density lipoprotein (VLDL) was separated from low (LDL) and high (HDL) density lipoproteins with preparative ultracentrifugation at $d = 1.006$. Apolipoprotein B-containing lipoproteins were precipitated in the infranatant using phosphotungstic acid/magnesium chloride leaving HDL in solution. LDL cholesterol concentrations were calculated by subtraction of values of the infranatant after precipitation from the values before

precipitation. Cholesterol was determined by Monotest CHOD-PAP and triglycerides GPO-PAP (Boehringer Mannheim). Apolipoproteins A-1 and B were determined by electroimmunoassay [25, 26]. Free fatty acids (FFA) were analysed according to Ho [27].

Statistical analysis

Statistical comparisons were made with SPSS program (SPSS Inc. Headquarters, Chicago, Illinois, USA). The results are presented as means \pm SE. Paired-samples t-test was used for comparisons and when the values did not have a Gaussian distribution the two related samples nonparametric test was used. A p-value less than 0.05 were considered statistically significant.

Results**Glycaemic control, C-peptide, free human insulin, free total insulin, proinsulin and IAPP during repaglinide and insulin aspart treatment**

After dose titration all patients on repaglinide treatment received 12 mg daily (4mg before each main meal) while the daily dose of insulin aspart varied from 13 U to 46 U (4-20 U at breakfast, 5-15 U at lunch and 4-15 U at dinner). Due to the insufficient glycaemic control in the morning, one patient received Insulatard[®] 16 U during repaglinide and 22 U during insulin aspart treatment, at bedtime (22:00). There were no differences in the 24-h blood glucose profiles (figure 1a) or the 24-h area under the curve (AUC) 17.1 ± 1.4 during treatment with repaglinide vs. 16.0 ± 1.2 insulin aspart (NS). The corresponding glycated haemoglobin A1c values were 6.1 ± 0.4 % at the end of repaglinide therapy and 5.9 ± 0.3 % at the end of insulin aspart therapy (NS).

C-peptide concentrations were significantly higher during repaglinide treatment compared to insulin aspart treatment (AUC 2453 ± 502 vs. 1153 ± 250 ; $p = 0.02$) (figure 1b). Calculated during 0-2h intervals after the main meal AUC (AUC_(0-2h)) for C-peptide was higher with repaglinide than with insulin aspart after breakfast (AUC_{breakfast (0-2h)} 2909 ± 554 vs. 1506 ± 371 ; $p = 0.01$) and lunch (AUC_{lunch (0-2h)} 3025 ± 587 vs. 1110 ± 296 ; $p = 0.02$).

Free human insulin levels (figure 1c) were significantly higher on repaglinide than insulin as part therapy (AUC 215 ± 61 vs. 128 ± 30 ; $p < 0.05$). AUC for free human insulin calculated during 0-2h post meal showed higher values after breakfast (AUC_{breakfast (0-2h)} 397 ± 92 vs. 232 ± 55 ; $p = 0.04$) and lunch (AUC_{lunch (0-2h)} 300 ± 86 vs. 145 ± 35 ; $p = 0.04$) during repaglinide than insulin aspart treatment.

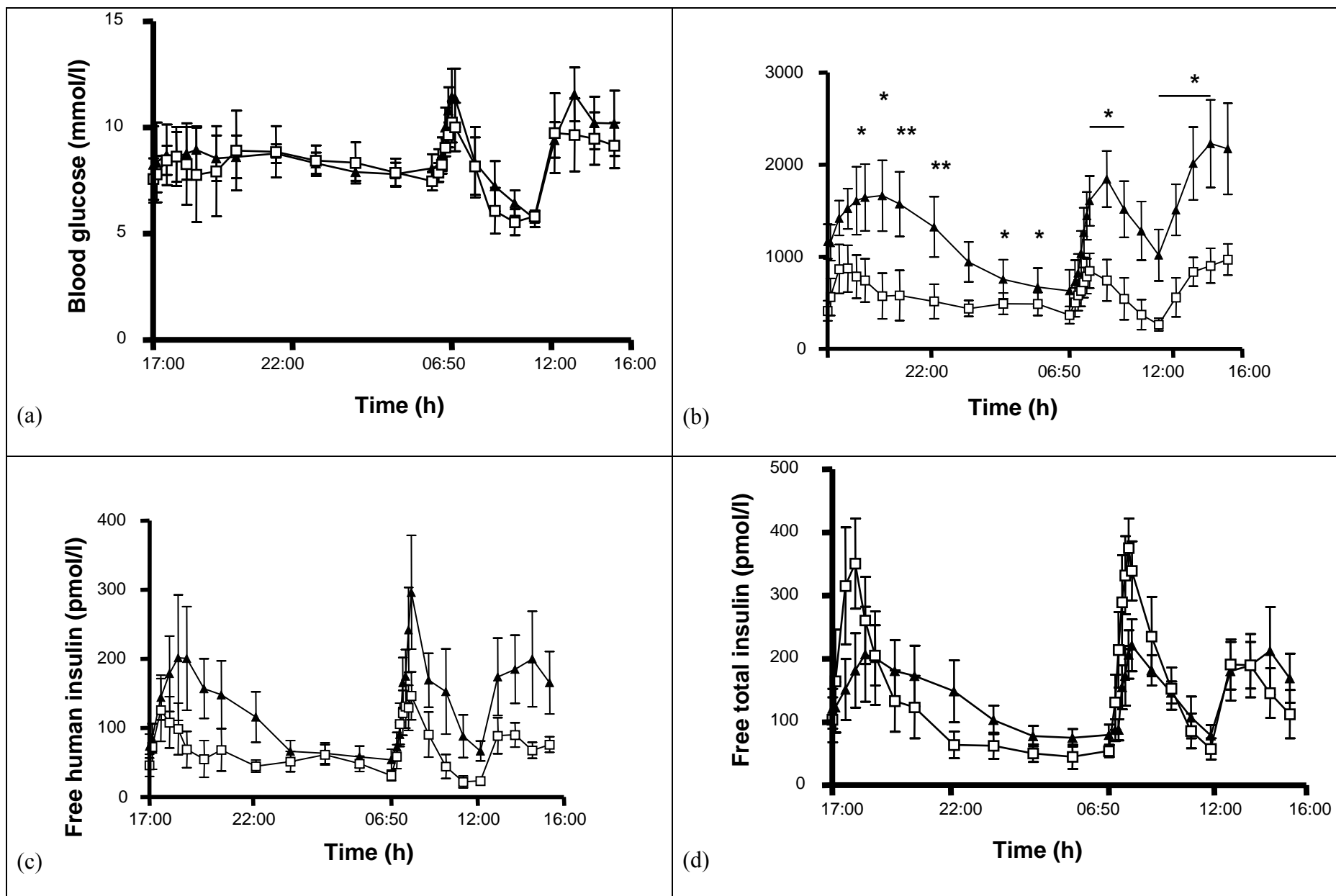


Figure 1. 24-h profiles of (a) blood glucose, (b) plasma C-peptide, (c) plasma free human insulin and (d) plasma free total insulin concentration (mean \pm SE) in 5 patients with type 2 diabetes treated with insulin aspart (\square) and repaglinide (\blacktriangle); (* signify $p < 0.05$, ** signify $p < 0.01$).

AUC of 24-h free total insulin (which measures both endogenous free human insulin and insulin aspart) levels were not significantly different between repaglinide and insulin aspart treatment (figure 1d). Insulin aspart give higher AUC of free total insulin calculated 0-2h after each main meal showed higher values than repaglinide treatment did ($AUC_{\text{breakfast (0-2h)}} 569 \pm 90$ vs. 344 ± 58 ; $p = 0.04$).

Proinsulin levels were higher when measured during repaglinide treatment than during treatment with insulin aspart, respectively (figure 2a).

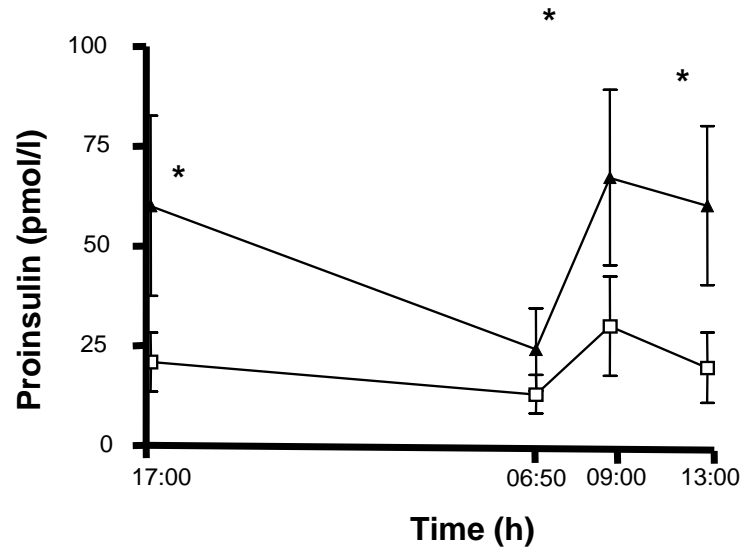
IAPP levels tended to be higher during repaglinide compared to insulin aspart treatment (NS). In comparison with the fasting state, higher IAPP levels were found postprandially during both treatments ($p = 0.03$) (figure 2b).

IGF-I, IGFBP-1 and GHBP

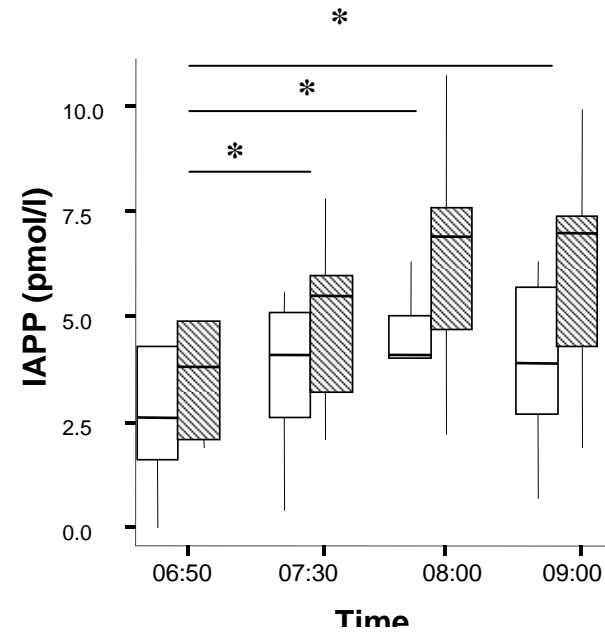
Fasting plasma IGF-I concentration was 220 ± 19 ng/ml during treatment with insulin aspart and 226 ± 15 ng/ml during treatment with repaglinide (NS). Compared to fasting levels the IGFBP-1 levels were lower during repaglinide ($p < 0.05$), but not during insulin aspart treatment (NS) (figure 2c). Repaglinide treatment increased plasma GHBP concentration compared with insulin aspart (1094 ± 112 pmol/l vs. 942 ± 143 pmol/l; $p = 0.02$).

Lipoprotein concentrations

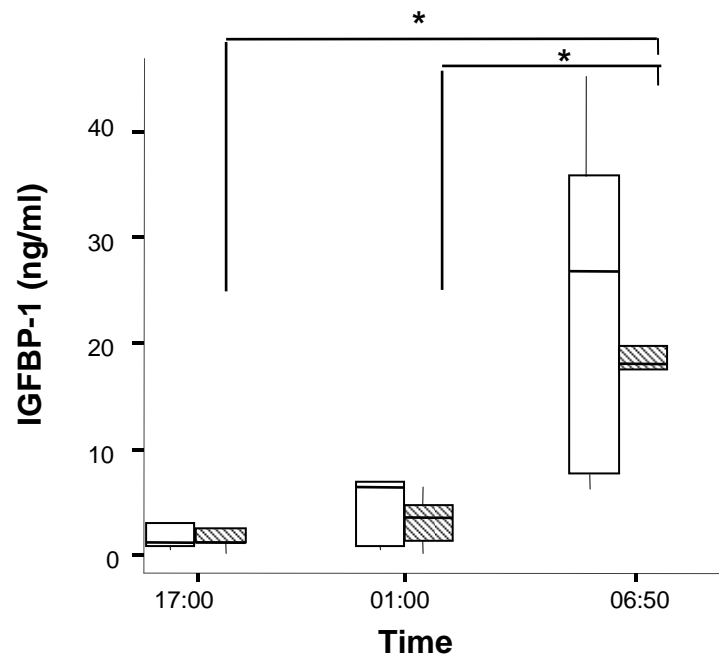
The mean plasma lipoprotein concentrations were within normal limits when determined at the end of the run-in period. Insulin aspart treatment resulted in lower postprandial levels of total triglycerides at 11:00 ($p = 0.001$) and at 15:00 ($p = 0.03$) than repaglinide treatment (table 1). Insulin aspart also decreased the postprandial levels of total cholesterol at 11:00 ($p = 0.02$) when compared with repaglinide treatment (table 1). No significant difference was observed in LDL



(a)



(b)



(c)

Figure 2. (a) Plasma proinsulin (mean \pm SE) concentration for patients treated with insulin aspart (\square) and repaglinide (\blacktriangle); and (b) plasma IAPP (median \pm IQR) and (c) plasma IGFBP-1 (median \pm IQR) concentration in 5 patients with type 2 diabetes treated with insulin aspart (white box) and repaglinide (dash box), (* signify $p < 0.05$).

cholesterol or in HDL cholesterol concentration between insulin aspart and repaglinide treatment (table 1).

Table 1. Plasma fasting (06:50) and postprandial (11:00 and 15:00) lipoproteins concentration (mean \pm SE) in 5 patients with type 2 diabetes treated with insulin aspart and repaglinide, and respectively P-values. * signify $p < 0.05$ calculated by pair-test when compared fasting to postprandial lipoproteins levels during insulin aspart or repaglinide treatment.

		insulin aspart	repaglinide	p-value
		mean \pm SE	mean \pm SE	
Total triglycerides	06:50	1.46 \pm 0.22	1.52 \pm 0.23	0.16
(mmol/L)	11:00	1.77 \pm 0.37	2.30 \pm 0.38 [†]	0.001
	15:00	2.28 \pm 0.64	2.85 \pm 0.53 [†]	0.03
Total cholesterol	06:50	4.40 \pm 0.38	4.55 \pm 0.39	0.29
(mmol/L)	11:00	4.52 \pm 0.36	4.79 \pm 0.39 [†]	0.02
	15:00	4.47 \pm 0.44	4.77 \pm 0.41	0.16
LDL cholesterol	06:50	2.78 \pm 0.32	2.90 \pm 0.36	0.33
(mmol/L)	11:00	2.72 \pm 0.26	2.77 \pm 0.31	0.57
	15:00	2.47 \pm 0.29	2.57 \pm 0.29 [†]	0.63
HDL cholesterol	06:50	0.98 \pm 0.13	0.95 \pm 0.10	0.52
(mmol/L)	11:00	0.99 \pm 0.10	0.98 \pm 0.11	0.74
	15:00	0.96 \pm 0.11	0.92 \pm 0.95	0.51
Apolipoprotein A-1	06:50	1.08 \pm 0.05	1.08 \pm 0.02	0.91
(g/l)	11:00	1.13 \pm 0.04	1.15 \pm 0.03 [†]	0.77
	15:00	1.13 \pm 0.03	1.14 \pm 0.04	0.87
Apolipoprotein B	06:50	0.85 \pm 0.09	0.91 \pm 0.11	0.20
(g/l)	11:00	0.89 \pm 0.97	0.98 \pm 0.10 [†]	0.13
	15:00	0.89 \pm 0.10 [†]	0.98 \pm 0.11	0.04
Apolipoprotein B/ Apolipoprotein A-1	06:50	0.80 \pm 0.09	0.85 \pm 0.12	0.48
	11:00	0.79 \pm 0.10	0.86 \pm 0.10	0.37
	15:00	0.79 \pm 0.10	0.87 \pm 0.11	0.24
Free fatty acids	06:50	0.55 \pm 0.06	0.45 \pm 0.05	0.22
(g/l)	11:00	0.38 \pm 0.03	0.46 \pm 0.04	0.06
	15:00	0.26 \pm 0.06 [†]	0.35 \pm 0.03	0.23

The apolipoprotein B levels were lower during insulin aspart therapy than during repaglinide therapy ($p = 0.04$) at 15:00 (table 1). There was a tendency towards lower postprandial FFA levels at 11:00 during insulin aspart than during repaglinide treatment ($p = 0.06$). The ratio between apolipoprotein B and apolipoprotein A-1 (apolipoprotein B/ apolipoprotein A-1) was similar during repaglinide and insulin aspart treatment (table 1).

During repaglinide treatment, the 4-h postprandial levels of total triglycerides ($p = 0.02$), total cholesterol ($p = 0.04$), apolipoprotein A-1 ($p = 0.01$) and apolipoprotein B ($p = 0.005$) were higher than in the fasting state. Also during repaglinide treatment, at 8-h from the fasting state, total triglycerides level ($p = 0.04$) was higher, whereas LDL cholesterol level ($p = 0.03$) was lower. During insulin aspart the 8-h postprandial level of apolipoprotein B ($p = 0.04$) was higher, whereas FFA level ($p = 0.04$) were lower when compared to the fasting state (table 1).

Discussion

Different approaches can be used to control hyperglycaemia in patients with type 2 diabetes [6]. In this study we compared the effects of two rapid-acting treatments that give pronounced effects on the postprandial metabolism by increasing endogenous insulin secretion or by administration of a rapid acting exogenous insulin analogue, respectively. We aimed, at similar glucose control, to be able to compare these treatment principles without being influenced by differences in glycaemia. This goal was achieved as the 24-h blood glucose profiles obtained were similar and there was no significant difference in A1c. While a few studies have been performed comparing effects of insulin treatment with oral hypoglycaemic agents during similar glycaemic control [15, 16], this is the first study comparing these rapid-acting treatments with main effects in the postprandial phase. We found differences in a number of variables including lipid metabolism and the IGF-system.

Repaglinide stimulated the secretion of endogenous insulin more than insulin aspart as shown by increased circulating levels of C-peptide, human insulin and proinsulin. These results are in accordance with previous studies showing enhanced insulin secretion after treatment with other insulin secretagogue as sulfonylureas [28, 29] and metiglinides [30, 31]. In this study we were able to determine the contribution of human insulin and aspart insulin separately by using antibodies able to interact with human insulin, but not cross-reacting with insulin aspart [24]. We found that administration of insulin aspart tended to accentuate total (endogenous + aspart) insulin peaks and lower basal (overnight) total insulin levels compared with repaglinide therapy. Exogenous insulin administration lowers endogenous insulin secretion, an effect that seems to be due mainly to the reduction of blood glucose concentration and probably not by a negative feedback on endogenous insulin secretion by insulin itself [1, 13].

Proinsulin and pro-IAPP are converted by the same endopeptidases to insulin, C-peptide, and IAPP, respectively, and co-secreted by pancreatic β -cells thereafter [32, 33]. In our study the levels of IAPP increased at breakfast and tended to be higher when the patients were treated with repaglinide compared to insulin aspart in agreement with previous studies of treatment with other insulin secretagogues in comparison with insulin [34, 35]. A growing body of evidence suggests that islet amyloid deposits may play an important role in the loss of β -cells and the progressive decline in insulin secretion characteristic of type 2 diabetes [33] and the degree of amyloid deposition correlates with severity of the disease in humans [36-38].

Increased proinsulin levels are associated with increased cardiovascular risk factors in both subjects without [39] and with type 2 diabetes [40], but it is debatable whether proinsulin is just a marker of compensatory increase of insulin secretion in insulin resistant individuals or if proinsulin has a mechanistic effect by itself in this respect.

To see if the changes in endogenous insulin secretion i.e. portal insulin level affected the IGF-system we determined IGF-I, IGFBP-1 and GHBP. The significant change in the GHBP level between repaglinide and aspart treatment might reflect an alteration in GH receptors similar to what previously has been shown in type 1 diabetes [21, 22, 41, 42]. There is evidence that insulin down regulates the production of IGFBP-1 [19-22]. We obtained a tendency to lower IGFBP-1 levels with repaglinide compared to aspart, which might reflect that the higher portal insulin levels during treatment with repaglinide suppress hepatic production of IGFBP-1 [29]. Gibson et al found that treatment with sulfonylurea depresses both fasting and circadian levels of IGFBP-1 when compared with multiple insulin injections, which support this hypothesis [29]. IGF-I, which has been shown to be low in patients with type 2 diabetes treated with insulin due to secondary failure [43], showed no difference between the treatments in our study.

When glycaemic control is improved in patients with type 2 diabetes concomitant changes of the lipoprotein concentrations are found with marked reductions of triglyceride-rich lipoproteins and also increased HDL cholesterol concentrations [4]. In our study an aim was to investigate how treatment with a rapid-acting insulin analogue affects the lipoprotein profile in comparison with a short-acting insulin secretagogue with similar glycaemic control in order to minimize the influence of glycaemia per se. We emphasized measurements of postprandial lipoprotein levels as both treatments have the most pronounced effect in this phase. During insulin treatment lowering of postprandial triglyceride levels and of apolipoprotein B was found when compared to treatment with repaglinide. While there is no previous study that has compared fasting and postprandial lipaemia between insulin aspart and repaglinide, the effect by insulin and sulphonylurea on fasting lipoprotein levels were studied by Romano and et al [15]. They found lower fasting triglycerides and higher HDL₂ cholesterol during insulin therapy than during treatment with glyburide and related these differences to lower production of large VLDL1 particles and a lowered activity of hepatic lipase during insulin treatment [15]. Although VLDL subfractions and hepatic lipase activity were not measured in our study, it seems reasonable that similar changes explain the differences we found in postprandial lipaemia between insulin aspart and repaglinide treatment as both repaglinide and sulphonylureas are considered to exclusively act as insulin secretagogues [44]. In a study by Ruotolo et al intraperitoneal insulin administration to patients with type 1 diabetes increased hepatic lipase activity, which is in support of this concept [45]. Our results showed a tendency towards lower postprandial FFA levels during insulin aspart than during repaglinide treatment, which suggest a decrease in FFA flux to the liver causing a lower hepatic TG production.

There were no differences in the fasting lipoprotein profile which is coherent with the short action of both therapies used as they were both administered during day-time with the last dose given at dinner. In a previous study [34] we found lower fasting triglycerides but higher LDL cholesterol concentrations during insulin treatment than during treatment with insulin in combination with glyburide, which is a more long-acting insulin secretagogue than repaglinide [46]. Data on repaglinide effects on the lipid profile are divergent which might possibly be explained by that mostly fasting measurements of lipoprotein concentrations have been performed. One short-term (20 days) study on 25 patients of repaglinide stated that the drug produced statistically significant decreases from baseline in both total cholesterol and triglycerides but did not provide specific data [47]. A larger, long-term study, found no significant changes from baseline in total cholesterol, HDL cholesterol, LDL cholesterol or total triglyceride levels [46]. In a third study, repaglinide significantly increased HDL cholesterol, LDL cholesterol and total cholesterol from baseline, but did not significantly affect triglycerides [48].

In summary, at the same glycaemic control, treatment with exogenous insulin aspart in comparison with the insulin secretagogue repaglinide results in a much lower endogenous insulin secretion, and a tendency towards a less atherogenic postprandial lipid profile.

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