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Postprandial paradoxical IGFBP-1 response in obese type 2 diabetes patients

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Abbreviations used in this paper: ANOVA, Analysis of variance; ALS, acid-labile subunit; Biostator, artificial pancreas; BMI, basal metabolic index; EDTA, ethylenediamine tetraacetate; GH, Growth hormone; IGF, Insulin-like growth factor; IGFBP, Insulin-like growth factor binding protein; RIA, radioimmunoassay

Keywords: Diabetes/IGF-1/IGFBP/glucagon/obesity

ABSTRACT

The insulin-like growth factors (IGFs), which in free form induce glucose and amino acid uptake, circulate bound to IGFBP which modulate the bioavailability and activity of IGF-1. The aim of this study was to examine the effect of a standard meal on the serum levels of IGFBP-1 in obese type 2 diabetes patients, non obese type 1 diabetes and healthy controls, during a mixed meal (550 kcal), using the artificial pancreas (Biostator®) to obtain a normal glycemic response to the meal. IGFBP-1 decreased 50 percent during two hours following the meal both in the controls and in type 1 diabetes patients with similar clearance rate, but no significant decline was seen in the type 2 diabetes group in spite of several fold increase in insulin levels. The type 2 diabetes patients were also studied during Sandostatin® infusion to decrease the inappropriate secretion of glucagon during the meal. During the 210 minutes somatostatin infusion the glucagon response was suppressed and the IGFBP-1 levels increased concomitantly with insulin peak without any significant decline after the meal. In conclusion the impaired IGFBP-1 response to meal related hyperinsulinemia in obese type 2 diabetes patients suggests a decreased availability of active IGF-1, leading to a decrease in glucose uptake during and after a meal in the obese type 2 diabetes patients. The stimulated meal response to glucagon which contributes to postprandial hyperglycemia could not explain the increase in serum IGFBP-1 in these obese type 2 diabetes patients.

INTRODUCTION

The insulin-like growth factors (IGFs) induce both glucose and amino acid uptake [1-4]. The amount of circulating IGFs are abundant and the combined potential biological effects are 100 folds that of insulin [5]. However, IGF-1 circulates in plasma bound to proteins (IGFBP 1-6) [6], which regulate the free biological forms. Free IGF-I has a half-life of ~ 5 min [2]. The binding to the IGFBPs ensures storage, and a prolonged effect, as well as inhibition of the hypoglycaemic effect of IGF-1 [2, 6]. The majority is bound to IGFBP-3 which together with an acid-labile subunit (ALS) forms a large ternary complex (150kD), that cannot leave the circulation, with a half life of > 15hrs [2, 7]. 10-20% of IGF is bound to the smaller binding proteins IGFBP-1-2 with a half-life in serum of 1-2 hrs [2]. Less than 1 % of the IGFs circulate in free form with a high affinity to one of these proteins, IGFBP-1 [8]. Moreover, IGFBP-1 although not the most predominant IGFBPs is the only binding protein to show rapid regulation *in vivo* and appears to be a reservoir from which IGF-1 can be dissociated into free forms and which stabilize circulating levels in serum [9, 10]. IGFBP-1 is also a

marker of free IGF-1 [11]. Regulation of circulating IGF-1 bioavailability is a key function of the IGFBNs [10]. IGFBN-1 has been suggested to modulate the short term bioavailability and activity of IGF-1 [9, 10] and plays a role for the glucose homeostasis [12, 13]. IGFBN-1 are secreted as phosphoprotein [14], and the affinity of phosphorylated human IGFBN-1 for IGF-1 is six fold higher than for the nonphosphorylated protein [14]. IGFBN-1 has been suggested to play a role in the acute regulation of IGF bioavailability by binding unbound IGFs, and regulation of its affinity by phosphorylation may be of important and versatile metabolic function [15]. Basal levels of IGFBN-1 can be low, normal or elevated in patients with type 2 diabetes [16-18], while they are several fold increased in type 1 diabetes patients [16, 19]. IGFBN-1 is mainly produced by the liver in humans with a high production rate [19, 20] and serum levels of IGFBN-1 display a diurnal rhythm [21], with the highest concentrations observed during the night and early morning; however, these are suppressed by meals [22]. Insulin regulates the production by inhibiting the IGFBN-1 production at the transcription level both *in vivo* and *in vitro* [18, 19, 23, 24]. Agents increasing intracellular levels of cAMP have been found to increase IGFBN-1 both at transcriptional and post-transcriptional levels [25-27]. Glucagon which mediates many of its cellular effects via cAMP has *in vivo* been found to increase levels of circulating IGFBN-1 [28] and *in vitro* increase IGFBN-1 mRNA level [29]. During insulin infusion IGFBN-1 is cleared from plasma with a half-life of 90-120 minutes both in type 1 and type 2 diabetes patients [16]. It is not known how meal induced hyperinsulinemia affects the serum levels of IGFBN-1 or the bioavailability of IGF-1 in type 2 diabetes patients. Recently, we have shown that the IGFBN-1 response to hyperinsulinemia during OGTT is reduced in obese type 2 diabetes as well as in patients with heredity for type 2 diabetes and that reduced first phase insulin response to OGTT is associated with low levels in serum IGF-1. [30, 31]. The postprandial hyperglycemia could therefore be caused by hepatic insulin resistance also involving IGFBN-1 and indirectly bioactive IGF-1. The aim of the present study was to evaluate the effect of a mixed meal on the serum levels of IGFBN-1 in obese type 2 diabetes patients, non obese type 1 diabetes patients and in healthy subjects. And to evaluate if elevated glucagon response to meal could explain the increase in serum IGFBN-1 in obese type 2 diabetes patients despite hyperinsulinemia. To obtain normoglycemia and discriminate the possibility that the results might have been somehow related to the hyperglycaemia and to achieve normoglycemic response to the meal in the diabetic patients we used a Biostator (artificial pancreas).

MATERIAL AND METHODS

Protocols

The study protocol was approved by the local ethics committee of Karolinska hospital, and written informed consent was given by the subjects asked to participate in the study. Nine obese patients with type 2 diabetes mellitus and poor metabolic control as reflected by their haemoglobin A_{1c} levels and six non obese type 1 diabetes patients participated and their results were compared with that of nine healthy subjects. Sex, age, BMI, duration of the disease and metabolic control of the participants are shown in Table 1. Renal function test was normal in all participants. No participants had signs of gastrointestinal disturbances or clinical evidence of neuropathy or proliferative retinopathy. The NPH insulin was interrupted 24 hours before the experiment, and the oral hypoglycemic agents were withdrawn three weeks before the onset of the study. Blood glucose was controlled by the use of subcutaneously injected regular insulin up to 16 hours before the experiments. All the subjects were studied after an overnight fast. At 07.30 a.m on the study day three cannulae were inserted; one cannula placed retrograde was connected to a Biostator® (artificial pancreas) for continuous blood withdrawal to monitor blood glucose and for delivering insulin, a second cannula was inserted in an antecubital vein for intermittent blood sampling for analyses of hormone levels, and was flushed with saline after use, and the third cannula was inserted in a contra lateral antecubital vein and used for all the infusions. At approximately 08.00 am the patients were connected to the Biostator in order to normalise the blood glucose levels. The standard Biostator controller algorithm was employed during feedback insulin infusion. The algorithms used in the Biostator have been described in detail [32]. Insulin infusion was started immediately after the first blood sample had been obtained. The meal induced glycemic responses in the diabetes patient were clamped to that of the healthy subjects, ~4-5 mM under basal condition and ~6-7 mM postprandially. Normoglycemia was then achieved within a 20- to 60-min period, and a standard lunch containing a total energy of 550 kcal (28% protein, 26% fat and 46 % carbohydrates) was served at time 0 min. After approximately 40 minutes normoglycemia was achieved. The healthy subjects were studied according to the same protocol but without the Biostator feedback insulin infusion. In addition the nine type 2 diabetes patients were studied as out-patients after an overnight fast during inhibition of glucagon secretion by continuous infusion with somatostatin at a constant rate of 6.7 ng/kg/min (Sandostatin®, Sandoz) from -30 min and throughout the study. Blood samples for determination of glucose, free-insulin, C-peptide, glucagon, GH, somatostatin and

IGFBP-1 were taken before the Biostator was connected and during normoglycemia at -30 minutes, ± 0 , +15, +30, +60, +90, +120, +150 and +180 minutes following the meal.

Assays

Blood samples were collected in plastic tubes containing EDTA (0.048 ml; 0.35M and Trasylol (1000 kallikrein inhibitor units; Bayer, Leverkusen, Germany) and immediately placed on ice. Samples were centrifuged at -4°C and plasma was frozen at -20°C . The blood glucose was measured by a glucose oxidase method [33]. Haemoglobin A_{1c} was measured by isoelectric method [34] and DCCT-aligned. GH levels were determined by RIA [35]. The intra- and interassay CVs for GH levels were 4% and 10% respectively, and the detection limit was 0.2 $\mu\text{g/L}$. The glucagon was measured by RIA, based on the method of Falcoona and Unger using the antibody 30K [36]. Insulin was analysed by RIA using antibodies raised in our laboratory [37]. For this analysis the inter-assay coefficient of variation (CV) was 5%. The intra- and interassay CV were 9 and 12% respectively. The detection limit was 9 $\mu\text{U/l}$. C-peptide was assayed with the use of a commercial kit (Novo Research Institute, Bagsvaerd, Denmark). Plasma concentrations of IGFBP-1 were measured with a RIA according to [38] using rabbit antiserum from animals injected with pure IGFBP-1 isolated from human amniotic fluid. Polyclonal antibody was used, measuring both phosphorylated and non-phosphorylated forms of IGFBP-1. The detection limit of the assay is 3ng/ml; the intra-assay CV was 4% and interassay CV 11%. The cross-reaction to other IGFbps is less than 0.5%. Somatostatin was extracted and concentrated from 2 ml plasma and analysed by a RIA method developed at our laboratory [39]. The detection limit of somatostatin was 0.5pg/assay tube. The intra- and interassay CVs were 13% (n=8), and 6% (n=12) respectively. All blood samples from each subject were analysed in duplicate in the same assay run.

Statistical analysis and calculations

Results are expressed as mean \pm SEM. Statistical significance was set at $P < 0.05$ and was assessed using ANOVA with repeated measures for interaction with time and treatment (Somatostatin vs. saline in the type 2 diabetic group). For paired data students paired t-test was carried out. For comparisons between groups of patients one way ANOVA was applied or ANOVA on Rank for non-normally distributed variables. The meal-related glucose and hormonal responses were calculated as the integrated increments above the basal value starting

30 minutes before the meal and up to 180 minutes after the meal. Insulin requirements for normalisation of blood glucose were calculated from Biostator readings. For the purpose of calculation, undetectable GH and somatostatin levels were assigned values of 0.2 µg/l and 2.5 µg/l, respectively.

RESULTS

Basal level

Basal fasting glucose levels were similar in type 1 diabetes and obese type 2 diabetes patients (15.2±2.5 mM vs. 13.7±1.6 mM) whereas the insulin levels were three fold increased in type 2 diabetes patients (30.9±4 mU/L) compared to type 1 diabetes patients (10.2±1 mU/L) ($P < 0.01$). The basal fasting IGFBP-1 levels were more than five fold increased in the type 1 diabetes (184.1±2 µg/L) than in the obese type 2 diabetes patients (36±7 µg/L) ($P < 0.001$) and controls (26±10 µg/L, $P < 0.001$). Basal C-peptide levels were 0.06±0.02 nmol/L in the type 1 diabetes, and 0.87±0.08 nmol/L in the obese type 2 diabetes patients ($P < 0.001$). The glucagon levels were not significantly different in the two diabetes groups; 291±85 pg/L in type 1 diabetes and 169 ± 31 pg/L in the type 2 diabetes, although there was a tendency to higher glucagon level in the type 1 diabetes patients. Neither were the somatostatin and GH levels significantly different between the groups although higher GH levels were seen in some of the type 1 diabetes patients (Table 2).

The effect of insulin infusion before the meal

The glycemic, hormonal, and binding protein responses to the first phase of insulin infusion (-30 min) are seen in Fig 1 A-D. Blood glucose decreased significantly in type 2 diabetes patients to 4.6±0.2 mM vs. 13.7±1.6 mM, $P < 0.001$ and in type 1 diabetes patients to 4.6±0.2 mM vs. 15.2±2.5 mM, $P < 0.01$. Circulating insulin levels increased from basal levels in type 2 diabetes patients, 30.9±4 mU/L to 68.7±17 mU/L, (ns) and in type 1 diabetes patients from 10.2±1 mU/L to 28.2±5 mU/L, ($p < 0.01$). The C-peptide levels decreased with 50 % in the type 2 diabetes, from 0.87±0.08 nM to 0.42±0.04 nM, $P < 0.0001$, *data not shown*. IGFBP-1 decreased significantly by 26 % in the type 1 diabetes, from 184±2 µg/L to 135±17 µg/L, ($P < 0.05$) and by 41 % in type 2 diabetes patients, from 36±7 µg/L to 21.2±3.2 µg/L, ($P < 0.05$). Glucagon and

somatostatin concentrations did not change significantly in either type 1 or type 2 diabetes groups (fig 1), with no significant changes seen for the GH levels in the type 1 or type 2 diabetes groups, *data not shown*.

Before the meal at ± 0 min the mean levels of GH, (*data not shown*) and glucagon were not significantly different between the groups. The glucose, C-peptide and IGFBP-1 levels were similar in the obese type 2 diabetes patients and the controls. IGFBP-1 was more than five fold increased in type 1 diabetes patients compared to the obese type 2 diabetes patients and healthy controls. The insulin levels were significantly increased in the type 2 diabetes compared to the controls 49.3 ± 11.6 mU/L vs 11.2 ± 1.4 mU/L, ($P < 0.001$), and type 1 diabetes patients 28.2 ± 5 mU/L, ($P < 0.01$).

Response to a standard meal

The peak glucose response (~ 6.5 mM in all groups) to the meal was seen 30 minutes after the start of the meal (Fig 1 A). The peak insulin levels seen at 30-60 minutes, were similar in type 1 diabetes patients (77 ± 29 mU/L) and controls (55 ± 3 mU/L) whereas they were more than three times higher in type 2 diabetes patients (196 ± 59 mU/L) than in the controls with a delay of ~ 30 min (Fig 1 B). The C-peptide levels increased two fold in the controls but not significantly in the type 2 diabetes patients, *data not shown*. IGFBP-1 decreased in the type 1 diabetes and healthy controls with a similar clearance rate, $T_{1/2}$ being ~ 120 min. No significant decline in IGFBP-1 was seen in the type 2 diabetes patients (Fig 1 C, Figure 1Cb). Glucagon increased significantly during the first 30 minutes in both type 1 and type 2 diabetes patients and within 90 minutes in the control group, with sustained but not significantly higher levels in type 2 diabetes (Fig 1 D). The somatostatin levels increased significantly within all study groups in response to the meal, with the highest levels in the control group (*data not shown*). The GH levels decreased in all subjects with a nadir at 60 minutes, where after a significant increase was seen only in the healthy controls, at the end of the study, *data not shown*

Response to the Somatostatin infusion

During the 210 minutes of somatostatin infusion in the nine obese type 2 diabetes patients, the somatostatin was increased to ~ 100 pg/ml (Fig 2 A), and the glucagon response to the meal was suppressed with a $\sim 150\%$ reduction of glucagon level (Fig 2 B). The glucose levels were significantly lower at +15 and +30 min during the somatostatin infusion, $P < 0.01$ (Fig 2 D). The insulin levels during the meal with somatostatin infusion was significantly lower at +30

minutes, 126 ± 14 mU/L vs 63 ± 6 mU/L, $P < 0.05$, where after there were no differences (Fig 2 C). The C-peptide levels were significantly lower from +15 min to +180 min (data not shown). The IGFBP-1 levels increased concomitantly with the insulin peak without any significant decline (fig 2 E). Moreover, both the glucose and IGFBP-1 peaks were shifted to the right. Somatostatin infusion during a mixed meal induced in the obese type 2 diabetes patient a significant increase at 90-180 min in IGFBP-1 ($P < 0.05$) in spite of several fold increased insulin levels and inhibited glucagon secretion.

DISCUSSION

The present study confirms the meal related insulin resistance in the obese patients with type 2 diabetes [39-41], and shows that patients with type 2 diabetes respond differently to meal related hyperinsulinemia with elevated glucagon response compared to patient with type 1 diabetes and healthy controls [28, 32, 42] although normoglycemic response during meal. It has been suggested that the inappropriate excessive secretion of glucagon in type 2 diabetes patients is mediated because of alpha-cell insulin resistance in type 2 diabetes [43]. Those data support our finding in type 2 diabetes patients showing a high postprandial glucagon in spite of several fold increase of insulin during the meal. However, also the type 1 diabetes patients had a high glucagon response in spite of hyperinsulinemia during meal which could be explained by insufficient insulin concentration locally in the islets. Moreover basal fasting glucagon level were higher in type 1 diabetes compared to type 2 diabetes patients, which may be due to insulin deficiency. A new finding in the obese type 2 diabetic patients is the impaired meal related response of IGFBP-1 with no suppression in spite of several fold increase in circulating insulin. Others and we have shown that fasting IGFBP-1 is inversely correlated to insulin concentrations in serum in healthy subjects and in patients with type 1 diabetes mellitus [12, 15, 16, 18, 44]. However, in type 2 diabetes patients with obesity there is no such correlation [44, 45]. Type 2 diabetes patients have basal insulin production, but reduced insulin response to glucose and a meal induced insulin resistance [39, 46]. The cause of this is not known, but impaired first phase insulin response [47] and abnormal glucagon response to a meal may be one explanation [39, 46]. The postprandial hyperglycemia in type 2 diabetes is an early event in the disease and is responsible for increased morbidity and mortality in cardiovascular disease [48]. Our findings suggest that obese patients with type 2 diabetes demonstrate a marked hepatic insulin resistance during the meal, encompassing both the insulin effect on gluconeogenesis and the glucose counter-regulatory hormone glucagon

[28] and on IGFBP-1 [49]. These findings also indirectly suggest that the postprandial hyperglycemia in obese type 2 diabetes is the effect of reduced bioavailable IGF-I as well as hepatic insulin resistance during the meal. Interestingly the IGFBP-1 response to insulin before the meal was adequate.

The fasting levels of insulin were 180 % higher in the obese type 2 patients compared to controls. Still the fasting IGFBP-1 was not significantly lowered. In healthy individuals and in patients with type 1 diabetes, circulating IGFBP-1 levels exhibit a characteristic diurnal pattern, with mealtime suppression related to increases in insulin concentrations and gradual increases during fasting [50-52]. This diurnal rhythm is absent in patients with Cushing's disease [53]. Thus the earlier shown inverse regression between fasting insulin and IGFBP-1 [21, 44, 54] was shifted to the right suggesting either a relative hepatic insulin resistance, a different or unknown factor in patients with type 2 diabetes that stimulates the production and/or degradation of IGFBP-1. The relative postprandial increase in IGFBP-1 and glucose in spite of high insulin levels may also suggest hepatic insulin resistance in obese type 2 patients [49]. The elevated glucagon levels seen in obese type 2 patients during the meal could have contributed to the augmented IGFBP-1 levels. In vitro studies have shown that glucagon stimulates release of IGFBP-1 in fetal liver cells and in vivo [28, 55]. However, suppression of the glucagon release with Sandostatin in our study did not blunt the increase in IGFBP-1. In fact somatostatin infusion further increased IGFBP-1 level compared to controls in spite of high circulating insulin levels and decreased level of glucagons after 15 min delay. The 15 min delay in glucose peak seen in our study could be secondary to an alternated gastric motility by sandostatin infusion, and also explain the delayed response of IGFBP-1 during sandostatin experiment. However, it could not explain the paradoxical postprandial increase in IGFBP-1. We have in an earlier study shown that hyperinsulinemia in healthy subjects and in type 1 diabetics cause a decrease in IGFBP-1, in spite of low C-peptide levels [20]. It is not excluded however that somatostatin may have a direct effect on IGFBP-1 [56]. Neither can we exclude that acute hyperinsulinemia causes a transient impaired hepatic insulin extraction [20]. It is also possible that other unidentified factor(s) possibly gastrointestinal peptide(s) released during the meal in patients with type 2 diabetes may be able to stimulate hepatic production of IGFBP-1. Theoretical alternative explanations are that there may be an activated liver cytokine pathway involved in patients with insulin resistance and obesity [57], which may contribute to a direct stimulation of IGFBP-1 [58] although still unproven. It has been shown that increased IGFBP-1 concentrations in the circulation reduce the bioavailability of IGF-I, inhibiting the hypoglycaemic effect of the IGF-I [9]. This hypothesis is supported by the present finding in obese type 2 diabetes patients of sustained increased glucose

levels for 3 hours after the meal in spite of high insulin level after the increase in IGFBP-1 (Fig 2). IGF-1 bioavailability was also low in patients with GH deficiency [59] and untreated thyrotoxicosis with signs of insulinresistance, but increased markedly after replacement therapy or as pharmacotherapy makes them euthyroid. The glucose level in the same study was significantly higher during hyperthyroidism with low IGF-1 bioavailability but normalized when patients got euthyroid [60]. In vivo studies in rats with recombinant IGFBP-1 (rIGFBP-1) have shown that it causes an increase in blood glucose and that rIGFBP-1 block the effect of IGF-I [61].

It has also been shown that treatment with IGF-I may reduce insulin resistance in type 2 diabetes patients and lowers blood glucose while at the same time lowering serum insulin levels in normal volunteers. This mechanism of achievement appears to be independent of activation of the insulin receptor [62]. Small-scale clinical trials have demonstrated the potential utility of rhIGF-1 in selected cases of severe insulin resistance [62] It is not excluded that the relative lowering of IGF-I bioavailability found in the present study may contribute to meal related insulin resistance in obese type 2 diabetes patients.

In summary, our study verify that obese type 2 diabetes patients have an impaired meal related suppression of IGFBP-1 in spite of several fold increase in circulating insulin, and this correlation seems not to be dependent on the meal related glucagon level suggesting postprandial hepatic insulin resistance in obese type 2 diabetes, which may explain the postprandial increase in glucose

Acknowledgement

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TABLE LEGENDS

Table 1. Characteristics of the participants.

	DM I	DM II	Healthy controls
Age (yr)	54±4.2	56.9±2.3	
	32.6±2.6		

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BMI (kg/m ²)	23.7±0.7***	36.4±2.3	
22.5±1.5***			
Sex	6M	5M/4F	4M/5F
Duration (yr)	21.2±4.0	9.4±1.2	-
HbA1c (%)	6.2±0.2***	8.5±1.1	< 4.9***

Clinical Characteristics of nine type 2 diabetic patients (DM II) and six type 1 diabetic patient (DM I) compared to the mean of nine healthy controls. All patients with type 1 diabetes were treated with insulin. Oral hypoglycaemic agents used by type 2 diabetes patients was removed three week's before the beginning of the study. Values are mean ±SEM. *** denotes $P < 0.001$ for difference vs. type 2 diabetic patients using Student's *t* test.

Table 2

Baseline characteristics of patients with type 1 and 2 diabetes before the standard Lunch and before they were connected to the Biostator

Group	Glucose (mmol/L)	Insulin (mU/L)	IGFBP-1 (µg/L)	Somatostatin (µg/L)	GH (µg/L)	C-peptide (µg/L)	Glucagon (nmol/L)
DM I	15.2±2.5	10.2±1**	184±2**	10.3±3.2	4.8±3.1	0.06±0.02***	291±85
DM II	13.7±1.6	30.9±4	36±7	6.5±1.1	0.5±0.2	0.87±0.08	169±31

Concentrations (mean± SEM) of blood glucose, serum insulin, IGFBP-1, somatostatin, GH, C-peptide and glucagon in the type1 diabetes (DM 1, 6M)and type 2 diabetes (DM 2, 5M/4F) By one way ANOVA DM 2 vs. DM 1 at corresponding time: **, $p < 0.01$; ***, $p < 0.001$.

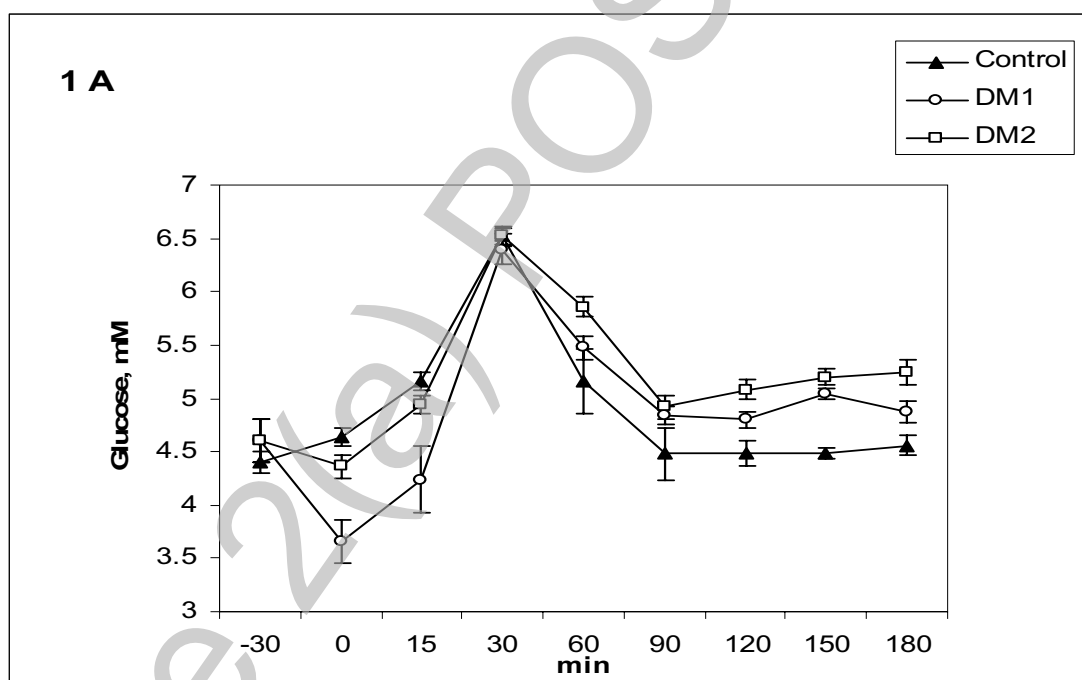
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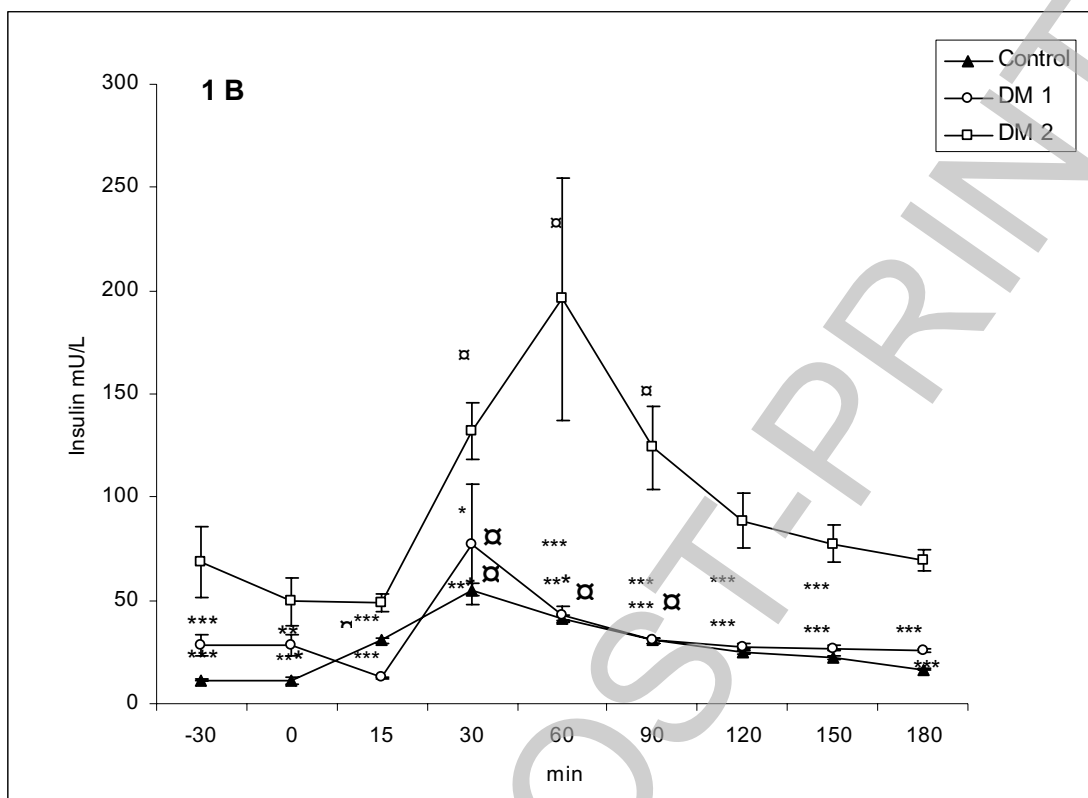
Figure 1 A-D. Mean serum concentrations (\pm SEM) before and during a standard meal containing a total energy of 550 kcal (28% protein, 26% fat and 46 % carbohydrates). Figures of glucose, insulin, IGFBP-1, GH and glucagon, in type 2 diabetic patients (n=9), type 1 diabetic patients (n=6), and healthy controls (n=9). By one way ANOVA; Type 1 diabetic patients and control group vs. type 2 diabetic patients at corresponding time: *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$.

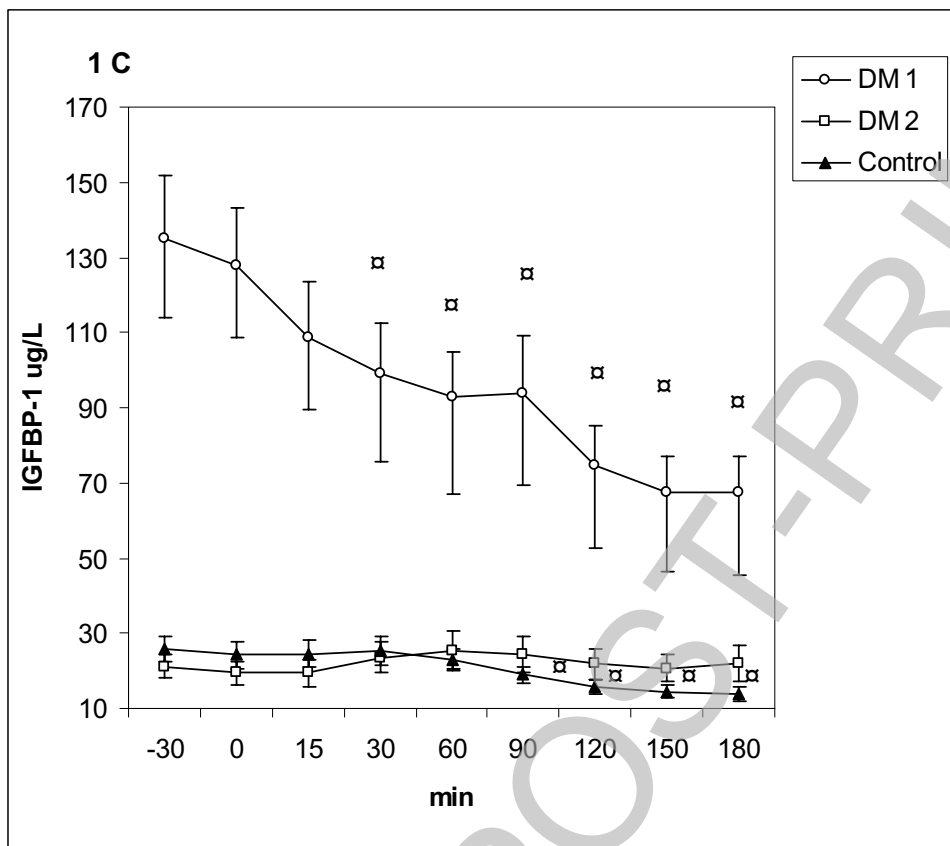
By ANOVA with repeated measures for time; ± 180 , ± 150 , ± 120 , ± 90 , ± 60 , ± 30 , ± 15 , -30 min vs ± 0 min: $p < 0.05$. **Figure 1Cb.** % difference of IGFBP-1 compared with time 0 min.

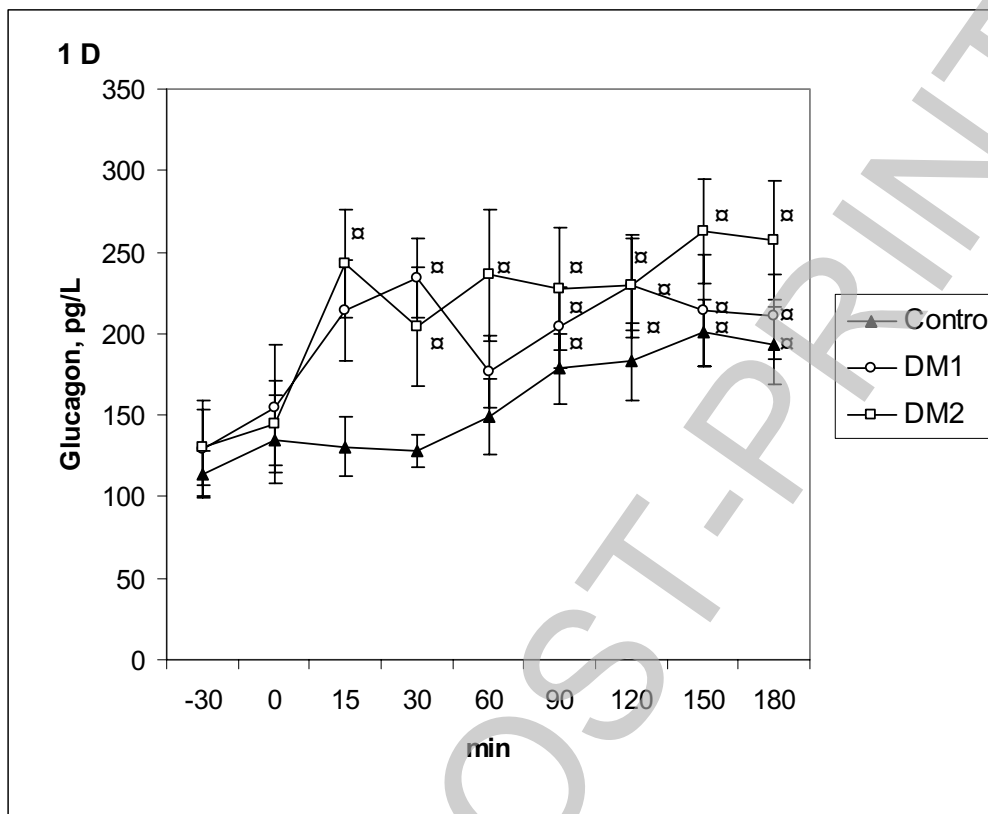
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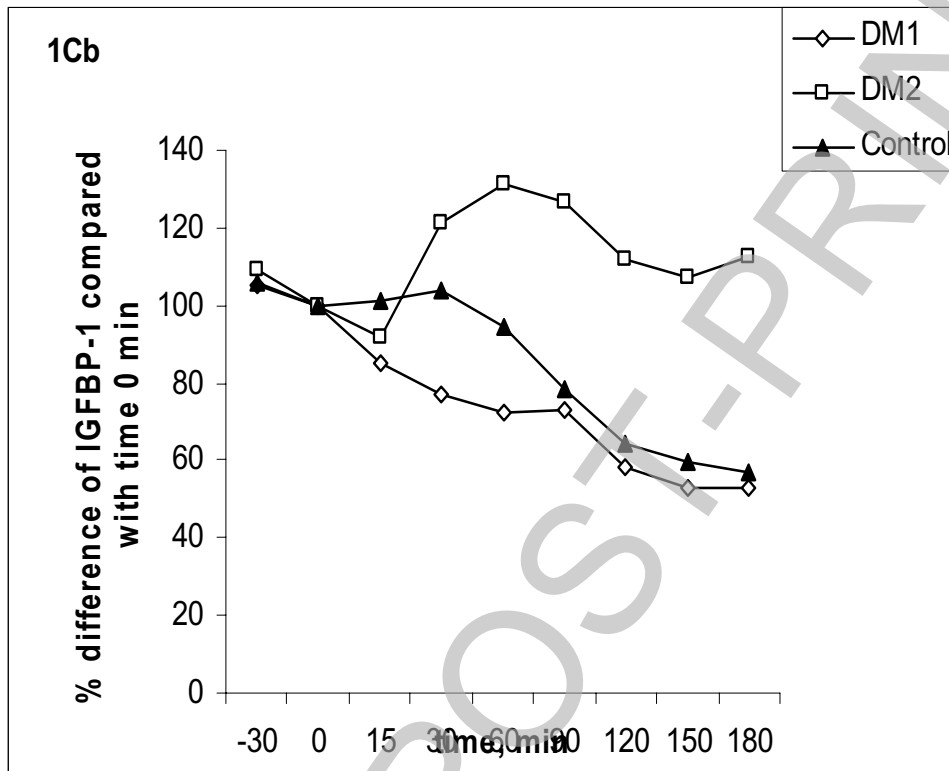
Figure 2 A-E. Mean serum concentration (\pm SEM) before and during a standard meal containing a total energy of 550 kcal (28% protein, 26% fat and 46 % carbohydrates). Figures of glucose, insulin, IGFBP-1, GH, glucagon, C-peptide and somatostatin in type 2 diabetic patients (n=9) after a somatostatin infusion (6.7 ng/kg/min) from -30 min to \pm 180 min, and under control conditions. By one way ANOVA, somatostatin treated type 2 diabetic patients vs type 2 diabetic patients under control conditions at corresponding time; *, $p < 0.05$, **, $p < 0.01$, ***, $p < 0.001$. By ANOVA with repeated measures for time; $\pm 180, \pm 150, \pm 120, \pm 90, \pm 60, \pm 30, \pm 15, -30$ min vs. ± 0 min in somatostatin treated type 2 diabetic patients vs. type 2 diabetic patients under control conditions: \square , $p < 0.05$.

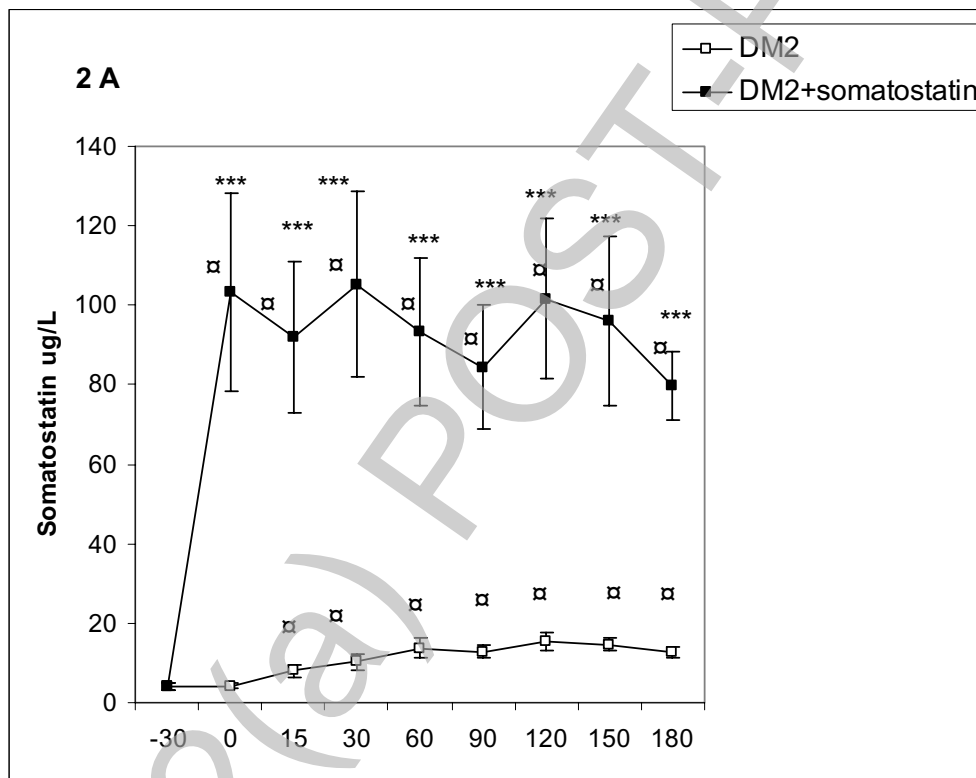


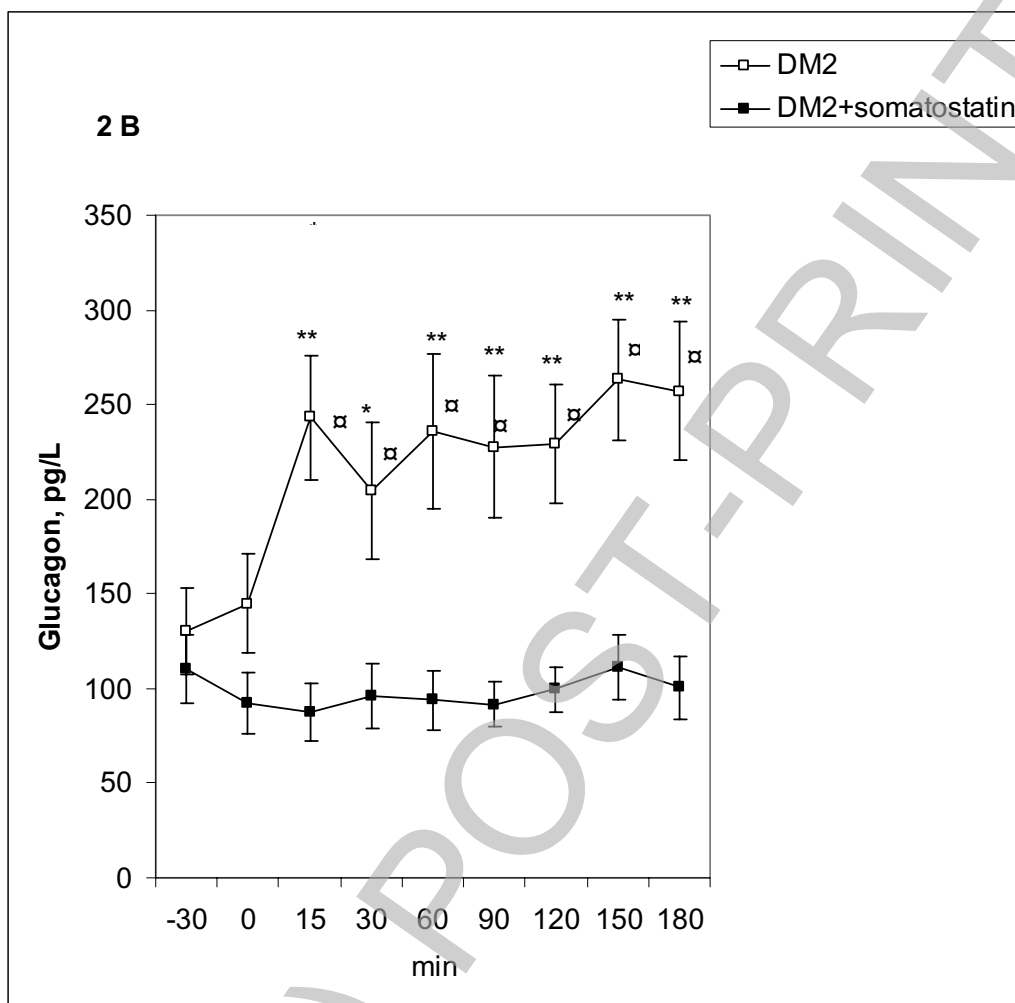


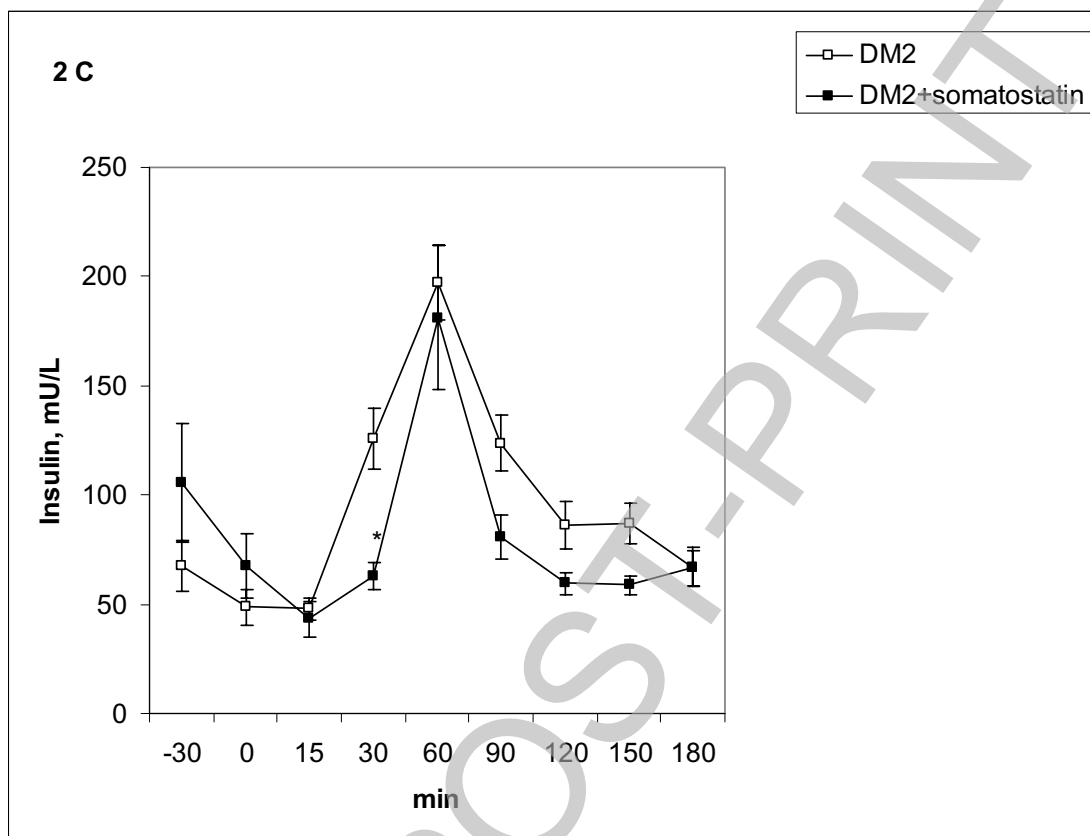


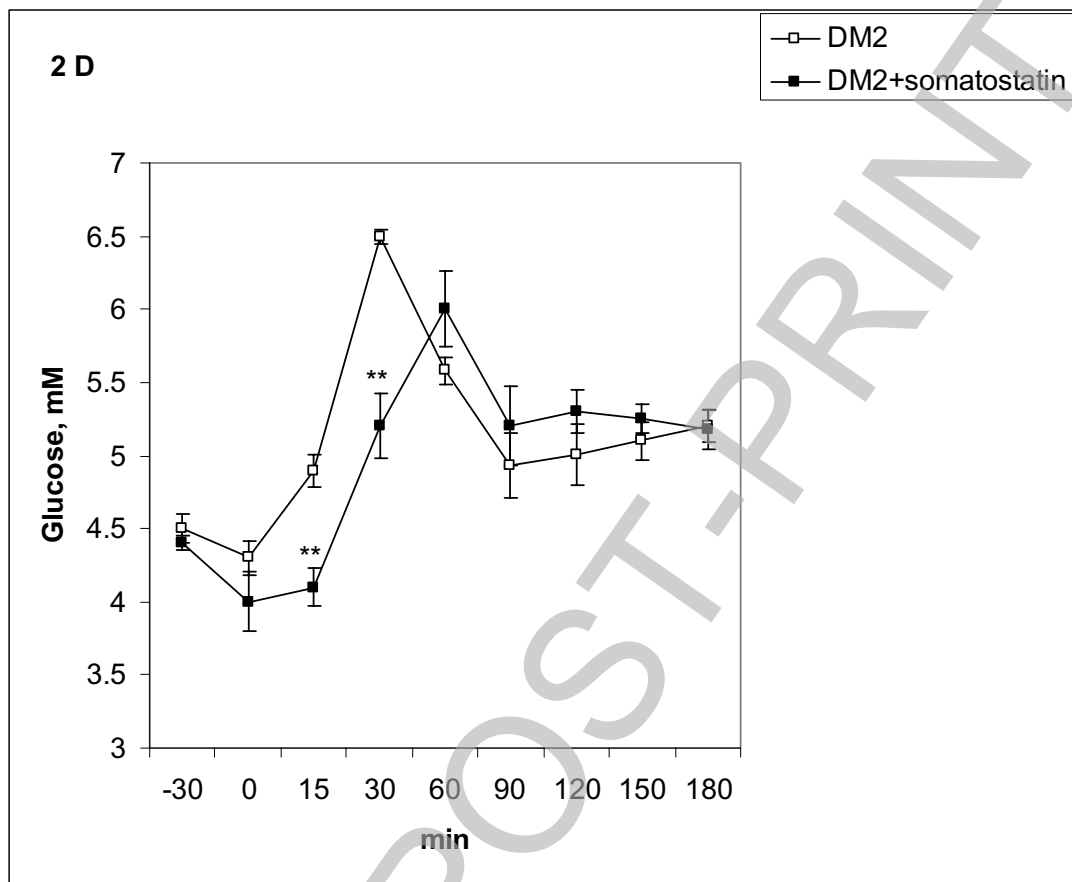


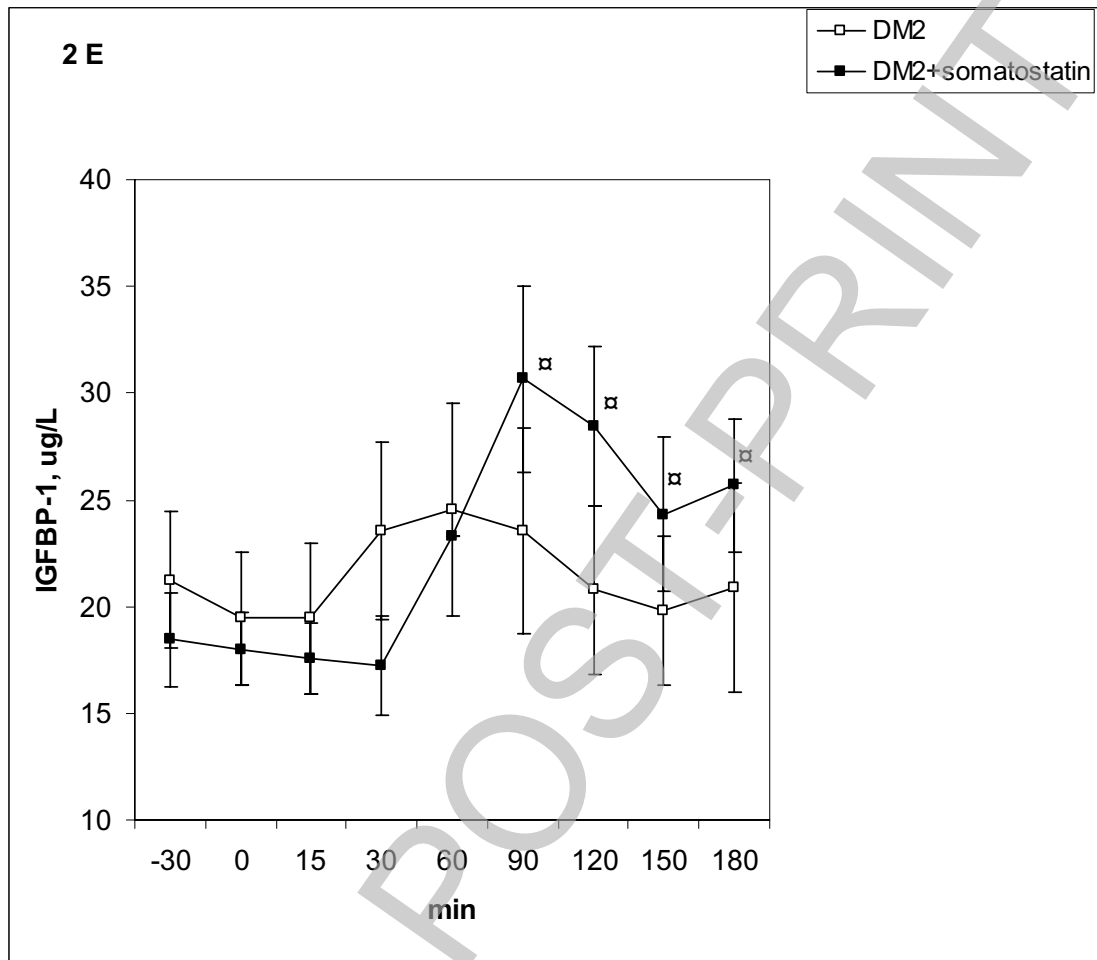












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