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Altered response of insulin-like growth factor–binding protein 1 to nutritional deprivation in type 2 diabetes mellitus

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Abstract

Circulating insulin-like growth factor–binding protein 1 (IGFBP-1) normally has a close inverse relationship to insulin secretion, which results in a characteristic diurnal variation. However, in type 2 diabetes the correlation with insulin may be lost and IGFBP-1 concentrations relatively increased. The aim of this study was to determine the effect of nutritional deprivation on the diurnal patterns of IGFBP-1 regulation in type 2 diabetes mellitus. After a baseline assessment period, food intake was reduced over 48 hours to 627.6 kJ/d (150 kcal/d) for 72 hours and increased again over 24 hours to baseline (refeeding). Blood samples were taken at 2-hour intervals, for 24 hours in the baseline period, 48 hours during nutritional deprivation, and 24 hours during refeeding. Six individuals with type 2 diabetes were compared with 2 groups that were selected for normal fasting glucose and insulin levels and comprised 6 obese and 6 lean subjects. During energy (caloric) restriction, fasting insulin levels decreased to a similar extent in each study group. At baseline, IGFBP-1 concentrations were similar in each of the study groups and at the end of the period of energy (caloric) restriction the 6:00 AM fasting levels had increased by 144% in the obese control group and by 245% in the lean individuals (each $P < .001$). In the patients with type 2 diabetes there was a blunted increase in IGFBP-1 concentrations with nutritional deprivation by 33% compared with baseline. During refeeding after nutritional deprivation the IGFBP-1 response to insulin was restored in the individuals with diabetes. In conclusion, patients with type 2 diabetes mellitus have altered IGFBP-1 regulation, relating to impaired hepatic insulin sensitivity, which improves after a period of energy (caloric) restriction.

1. Introduction

The insulin-like growth factor–binding proteins (IGFBP-1 to -6) determine the availability of IGF-I and -II from the circulation to the peripheral tissues [1]. Circulating concentrations of IGFBP-1 are dynamically regulated by nutritional variables in a way that suggests it is a counter-regulator of IGF availability for glucose homeostasis [2]. Insulin-like growth factor–binding protein-1 is secreted from liver in human beings as a phosphorylated protein, which is under hormonal control [3]. It is generally regarded as a reliable marker of hepatic insulin action because insulin clearly inhibits human IGFBP-1 synthesis in vitro [4,5] and in vivo [6,7]. In healthy individuals, fasting IGFBP-1 and insulin concentrations are closely inversely related [8]. This close correlation is lost in a number of conditions, for example, in catabolic states, resulting in "inappropriately" elevated IGFBP-1 concentrations [9]. Although these observations may be a consequence of reduced hepatic insulin action, increased activity of direct stimulators of IGFBP-1 may contribute. Insulin-like growth factor–binding protein-1 is increased in vivo by glucose counter-regulatory hormones, such glucagon [10], catecholamines [11], and glucocorticoids [12]. A number of other pathways have been identified that may contribute to a direct stimulation of IGFBP-1 including the activity of cytokines [13] and adenosine monophosphate–activated protein kinase [14]. Elevated levels of IGFBP-1 in relation to insulin can also be seen in type 2 diabetes [15,16].

Circulating IGFBP-1 levels exhibit a characteristic diurnal pattern in normal individuals, with mealtime suppression related to increases in insulin concentrations and gradual increases during fasting [17-21]. These fluctuations are

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suppressed in Cushing's disease [22], but preserved in type 1 diabetes mellitus [20,23,24] and during moderate (50%) energy (caloric) restriction [25]. We have reported the effect of marked energy (caloric) restriction (<7% of baseline intake) on the mean daily IGFBP-1 concentration and observed a blunted response in type 2 diabetes compared with controls [26]. The aim of this study was to explore further the effect of marked energy (caloric) restriction on the diurnal pattern of IGFBP-1 levels and its relationship to insulin in this group of patients with type 2 diabetes.

2. Materials and methods

2.1. Subjects

This study was approved by the local ethical authority of the Karolinska Hospital. The 3 men and 3 women with type 2 diabetes had hemoglobin A_{1c} concentrations of 5.4% to 10.1% (reference range, <6.3%). Usual treatment with a sulfonylurea (3 subjects on glibenclamide 7 mg bid) or insulin (1 subject on 40 U NPH insulin nocte, 1 subject on regular insulin before meals) was withdrawn one day before, and during, the study. The control group consisted of 6 overweight subjects (2 men, 4 women) (body mass index [BMI] $\geq$25 kg/m^2) of similar age with normal fasting glucose (4.6 $\pm$ 0.2 mmol/L) and normal insulin values. A second study group of 6 lean individuals (2 men, 4 women) with normal fasting glucose (4.2 $\pm$ 0.2 mmol/L) and insulin values were also recruited. The patients with type 2 diabetes mellitus and the healthy obese subjects were similarly moderately overweight (BMI 31.1 $\pm$ 1.3 and 31.4 $\pm$ 1.9 kg/m^2, respectively) and were of similar age (47.0 $\pm$ 5.9 and 40.7 $\pm$ 4.0 years, respectively). Compared to both of these groups the healthy lean individuals had a lower BMI of 22.1 $\pm$ 1.1 kg/m^2 and were younger (28.5 $\pm$ 1.7 years) [26]. Changes in weight, nitrogen balance, growth hormone (GH), IGF-I, IGF-II, and IGFBP-3 concentrations during the study have previously been reported in these 3 groups [26]. The mean IGFBP-1, glucose, and insulin values were also reported at that time, but not the diurnal patterns of secretion, which are reported here.

2.2. Study design

The study design is illustrated in Fig. 1. The entire study lasted 9 days with admissions for supervision and sampling during the indicated periods. After the basal assessment, the energy (caloric) intake was reduced over 48 hours to 627.6 kJ/d (150 kcal/d) and consisted of carbohydrate in the form of fruit and vegetable juices taken at the times described below. Participants were instructed in, or provided with 5 meals or snacks, consumed at 8:00 AM, 12:00 PM, 2:00 PM, 5:00 PM, and 7:30 PM. On sampling days, subjects were admitted at 9:00 AM, an indwelling venous cannula was inserted, and the sampling period commenced at 12:00 PM. Blood glucose concentrations and serum samples were taken at 2-hour intervals for 24 hours in the basal period, 48 hours during nutritional deprivation, and 24 hours during refeeding. Thus, the first blood sample was taken at least 39 hours after cessation of any insulin or oral hypoglycemic therapy. Samples taken at 6:00 AM were taken in the fasting state, before the morning energy (caloric) intake.

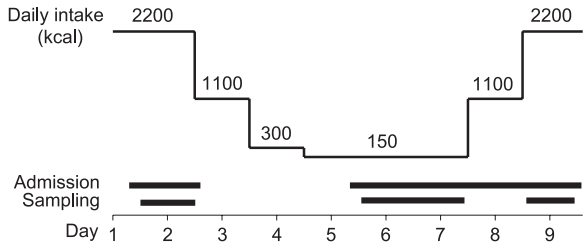

Fig. 1. Study design. The nutritional intake, hospital admissions for supervision, and sampling periods are shown for the 9-day study duration as described in Materials and Methods. The sampling periods commenced at 12:00 PM.

2.3. Assays

These assay methods have been described in detail previously [26] Serum IGFBP-1 concentrations were measured by radioimmunoassay (RIA), with intra- and inter-assay coefficients of variation (CVs) of 3% and 11%, respectively, and a detection limit of 3 ng/mL. Serum insulin was measured by RIA with a detection limit of 56 pmol/L and intra- and inter-assay CVs of 5% and 10%, respectively. Blood glucose was measured at the bedside using a Reflolux (Boehringer Mannheim, Germany) and was documented to have an interobserver variation of less than 10%. Cortisol was measured by RIA (Farmos Diagnostica, Turku, Finland).

2.4. Statistical analysis

Concentrations below the level of sensitivity of the assay were assigned the level of sensitivity. Insulin and IGFBP-1 values were $\log_e$ (ln)-transformed before statistical analysis. The ln-transformed values were then raised exponentially to show the geometric mean and confidence intervals (CIs) in Table 1 and Fig. 3. The responses were analyzed by 1-way or 2-way analysis of variance, with repeated measures, as appropriate, followed by a multiple comparison procedure (SigmaStat, SSPS Science, Chicago, Ill). Statistical significance was set at $P < .05$.

3. Results

In Fig. 2 we show the glucose concentrations, measured every 2 hours, throughout the study. During energy (caloric) deprivation, concentrations declined by 50% in the patients with diabetes and in the obese control group. Similar responses were observed in the lean individuals. The insulin levels showed a 30% decline during the period of energy (caloric) restriction (data not shown), and a few of the values fell below the level of assay sensitivity in the obese and lean groups. We have previously reported that the mean

Table 1
Diurnal variation in IGFBP-1 concentrations and response to nutritional deprivation

	Nutritional period	Fasting insulin, pmol/L, mean (95% CI)	Fasting IGFBP-1, ng/mL, mean (95% CI)	Change in fasting IGFBP-1, % mean (95% CI)	Within-subject IGFBP-1 variation, CV, % (95% CI)	Diurnal range IGFBP-1, mean (95% CI)
Diabetes	Basal	142 (56-363)	32 (18-56)		34 (21-47)	20 (10-38)
	T1 restriction	97 (46-205)*	41 (23-73)	127 (100-160)‡,¶	33 (23-44)	26 (12-54)
	T2 restriction	93 (43-197)*	43 (25-73)*,‡	133 (110-161)‡‡‡,¶¶	28 (19-37)	24 (13-43)‡‡
	Refeeding	124 (52-296)	29 (18-45)††	67 (59-74)‡	32 (22-42)	23 (14-37)
Obese controls	Basal	104 (82-133)	25 (18-34)		31 (16-47)	16 (10-28)
	T1 restriction	72 (59-89)*	63 (34-116)***	255 (163-399)	45 (28-62)	47 (23-97)
	T2 restriction	69 (62-77)*	60 (37-97)***,‡	244 (191-313)	35 (23-46)	48 (25-91)‡
	Refeeding	92 (79-106)	26 (19-35)‡‡‡	43 (31-60)	42 (26-58)	26 (14-48)
Lean subjects	Basal	100 (85-117)	36 (27-48)		47 (30-64)	28 (23-35)
	T1 restriction	74 (63-87)*	82 (63-107)***	227 (189-271)	41 (34-48)	76 (53-109)***
	T2 restriction	71 (62-82)*	125 (81-194)***	345 (269-441)	48 (34-61)	126 (77-205)***
	Refeeding	92 (79-107)	42 (27-65)†††	34 (24-48)	51 (34-67)	36 (21-61)†††

Serum samples were obtained from 6 individuals with diabetes mellitus , 6 subjects matched for age and weight (obese controls), and 6 lean individuals. Blood was taken at 2-hour intervals for 24 hours: during a basal assessment period, the final two 24-hour periods of energy (caloric) restriction (T1 and T2), and for 24 hours during refeeding. Fasting insulin (pmol/L) and fasting IGFBP-1 (ng/mL) are the 6:00 AM values. The change in fasting IGFBP-1 levels during each period of energy (caloric) restriction was compared with basal value and the change with refeeding was compared with restriction T2. The within-subject variability is expressed as the CV. The range was calculated as the difference between the maximum and minimum IGFBP-1 concentration in each nutritional period. Results are expressed as the geometric mean and 95% CIs. Analysis of variance was performed on ln-transformed values.

* $P < .05$, compared to basal assessment, within a subject group.
*** $P < .001$, compared to basal assessment, within a subject group.
†† $P < .01$, compared to food restriction (T2), within a subject group.
††† $P < .001$, compared to food restriction (T2), within a subject group.
‡ $P < .05$, compared to lean, within a nutritional period.
‡‡ $P < .01$, compared to lean, within a nutritional period.
‡‡‡ $P < .001$, compared to lean, within a nutritional period.
¶ $P < .05$, compared to obese, within a nutritional period.
¶¶ $P < .01$, compared to obese, within a nutritional period.

glucose and insulin concentrations were higher in the diabetic patients throughout the study compared with both the obese and lean groups [26]. The 6:00 AM fasting insulin values, none of which fell below the level of assay sensitivity, are shown in Table 1.

Diurnal IGFBP-1 concentrations for the patients with type 2 diabetes are compared with the obese controls and shown alongside the responses in lean subjects in Fig. 3 and analyzed in Table 1. At baseline, the fasting IGFBP-1 concentration, within-subject variability, and diurnal range of IGFBP-1 values were not significantly different between the 3 groups despite a tendency toward lower levels in the obese individuals. Fasting (6:00 AM) IGFBP-1 levels increased with energy (caloric) restriction and decreased

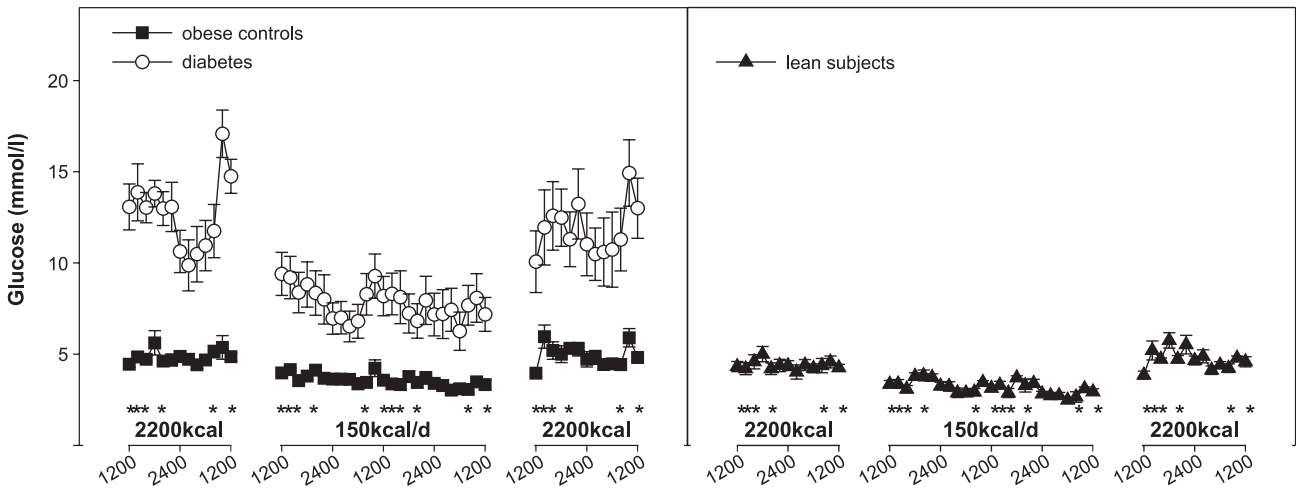

Fig. 2. Glucose concentrations during nutritional deprivation in patients with type 2 diabetes mellitus and healthy obese controls (left panel) and lean individuals (right panel). Whole blood samples were obtained at 2-hour intervals during a 24-hour 9204.8 kJ/d (2200 kcal/d) basal assessment period, during 48 hours on 627.6 kJ/d (150 kcal/d), and during a 24-hour 9204.8 kJ/d (2200 kcal/d) refeeding period. Meal times are indicated by the asterisks. Values are the mean ± SE for 6 subjects. Glucose concentrations fell with nutritional deprivation (analysis of variance; $P < .001$ for each group).

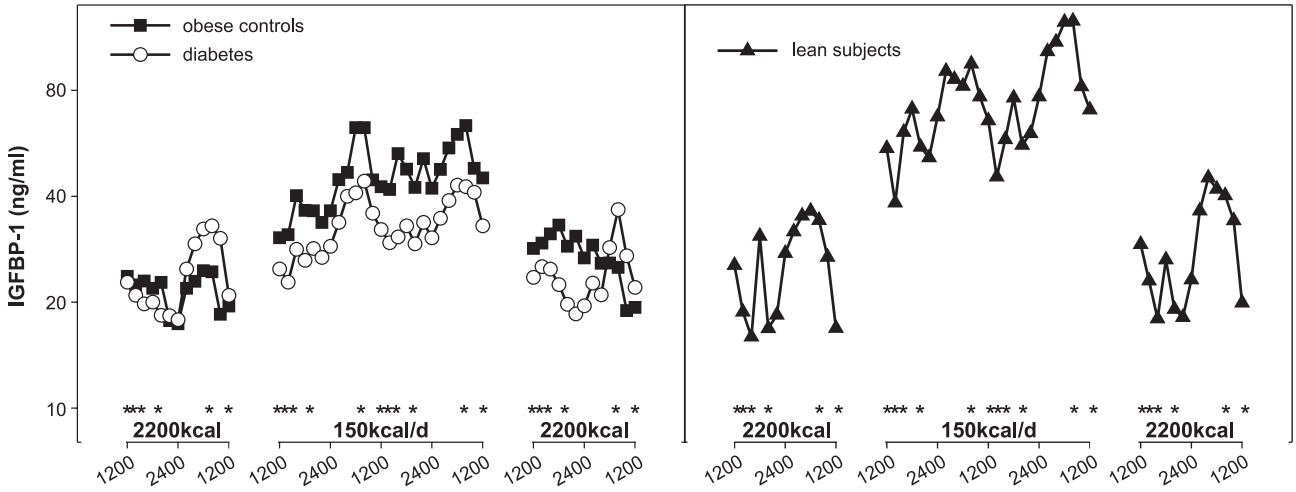

Fig. 3. Insulin-like growth factor–binding protein 1 concentrations and diurnal variation during nutritional deprivation in patients with type 2 diabetes mellitus and healthy obese controls (left panel) and lean individuals (right panel). Serum samples were obtained at 2-hour intervals during a 24-hour 9204.8 kJ/d (2200 kcal/d) basal assessment period, during 48 hours on 627.6 kJ/d (150 kcal/d), and during a 24-hour 9204.8 kJ/d (2200 kcal/d) refeeding period. Meal times are indicated by the asterisks. Values are the geometric mean for 6 subjects in each group.

with refeeding in all 3 groups. However, the increase was only 30% in the diabetic individuals, significantly less than the 150%-200% increments seen in the obese controls and lean subjects (each $P < .001$; during the second 24-hour sampling period, T2). The variability in IGFBP-1 within subjects, taking into account the mean IGFBP-1 level (CV), was similar in each group and in each period of nutritional intake. Only the lean individuals showed clear increases in the diurnal range of absolute IGFBP-1 concentrations.

We further explored the relationship between fasting insulin and IGFBP-1 concentrations during each 24-hour study period using the 6:00 AM fasting values. As shown in Table 1, during energy (caloric) restriction, there was little change in IGFBP-1 concentrations in the subjects with diabetes despite an approximately 30% decline in insulin levels. In contrast, there were clear increases in the IGFBP-1 concentrations in both the obese controls and in the group of lean individuals, concomitant with similar, 30% falls in insulin levels. These results (the relationship between the change in IGFBP-1 and the change in insulin) can be expressed as slopes, determined by $k = (\ln[\text{IGFBP-1}_{T2}] - \ln[\text{IGFBP-1}_{\text{basal or refed}}])/(\ln[\text{insulin}_{\text{basal or refed}}] - \ln[\text{insulin}_{T2}])$. The k value for the response to fasting was less in the diabetic subjects than in the obese controls (0.679 [95% CI, 0.362-0.996] vs 2.793 [95% CI, 1.090-4.496]; $P = 0.038$). In 5 of the 6 diabetic individuals the k value increased during the refeeding period and did not differ significantly from obese controls (2.964 [0.389-5.540] vs 3.953 [1.115-6.790]). The k values for the lean subjects were not significantly different compared with the obese group and were 4.926 (1.955-7.897) and 4.759 (2.698-6.819) for the fasting and refeeding periods, respectively. There was no relationship between any changes in IGFBP-1 in relation to insulin and the HbA1c level (data not shown).

In view of the blunted IGFBP-1 response, despite declining peripheral insulin concentrations during energy (caloric) restriction in type 2 diabetes, we also considered the possibility that other hormonal responses to nutritional deprivation might contribute to the patterns of IGFBP-1 observed. We measured cortisol concentrations. The mean cortisol values for each study period increased significantly during nutritional deprivation ($P < .02$), with no differences between the 3 clinical groups. There was no difference in the diurnal pattern of cortisol secretion between the 3 groups (data not shown).

4. Discussion

In this study we confirmed that IGFBP-1 concentrations in relation to insulin are inappropriately increased in type 2 diabetes mellitus [15,16] and make the novel observation that the response to energy (caloric) restriction is altered in these individuals. A group of patients with type 2 diabetes and hyperinsulinemia were matched with a control group with normal fasting insulin and glucose levels. Both groups were moderately obese to a similar degree. At baseline, despite higher insulin levels in the diabetic group, the mean IGFBP-1 concentrations were the same as in the obese subjects. During marked energy (caloric) deprivation, insulin and glucose concentrations declined approximately 50% and 30%, respectively, in both groups. Compared with the substantial changes in IGFBP-1 seen in the obese controls, a blunted increase in the fasting 6:00 AM IGFBP-1 concentration was observed in the patients with type 2 diabetes. The increment in IGFBP-1 in the obese group was similar to that seen in a second study group of healthy lean individuals of younger age. The changes in concentrations and diurnal patterns of cortisol failed account for these differences.

The increases in the diurnal range of IGFBP-1 concentrations with short-term nutritional deprivation in both lean and obese normal individuals were a striking feature of this study and contrast to the blunted variation seen with prolonged complete fasting [27]. The changes that we observed were related to the intake of small (~30 kcal) carbohydrate drinks. It seems likely that the low insulin concentrations, perhaps combined with greater sensitivity to small changes in portal insulin, are responsible for these observations.

It has been reported that IGFBP-1 concentrations are low in obesity, usually in association with hyperinsulinemia, and may be responsible for the documented increase in free IGF-I concentrations [28]. In obese children, low IGFBP-1 concentrations may be seen in the presence of normal peripheral insulin levels [29], suggesting that IGFBP-1 is a sensitive marker of portal insulin action. In our group of obese adults without fasting hyperinsulinemia, the 6:00 AM (fasting) and diurnal range of IGFBP-1 at basal assessment were not different to the lean group. However, we did observe that, with nutritional deprivation, the range of IGFBP-1 levels did not increase significantly in the obese subjects compared with the lean individuals. This observation is consistent with the presence of higher portal insulin levels in the obese group than observed in the lean group during the period of energy (caloric) restriction. It is also possible that relative hepatic insulin resistance improved during energy (caloric) restriction so that there was a more appropriate response to the prevailing insulin concentrations.

Previous studies in patients with type 2 diabetes mellitus have shown that fasting IGFBP-1 concentrations may be low [30] or elevated [6]. In our type 2 diabetic subjects, despite hyperinsulinemia, the baseline fasting and range of IGFBP-1 concentrations were no different to either obese or lean groups with a similar diurnal pattern. The diabetic subjects were studied off any hypoglycemic treatment, choice of which has been shown to influence the diurnal IGFBP-1 variation [16]. Reduced energy (caloric) intake was associated with a decline in insulin concentrations in each group. Appropriate to a concomitant fall in portal insulin concentrations, IGFBP-1 concentrations rose in both the lean subjects and in the obese control group. In contrast, in the diabetic subjects, there was little change in the secretion of IGFBP-1. We considered a number of factors that might be responsible. Other counter-regulatory hormones that increase with fasting may directly alter hepatic IGFBP-1 secretion. Cortisol, which has been shown stimulate IGFBP-1 in human beings [12], did not vary in its pattern of secretion between the study groups. Growth hormone has been suggested to be an independent inhibitor of IGFBP-1 in short-term in vivo studies [31-33] and in vitro [34]. In our earlier study, the mean GH secretion did not account for the observed changes in IGFBP-1 [26] although the sampling interval of 2 hours precluded a detailed analysis of GH pulse frequency and amplitude. There are sex differences in fasting concentrations of IGFBP-1, but there is a similar response to carbohydrate intake in males and females [19]. Our sample size was not large enough to evaluate any sex difference. Age is an important variable in the determination of IGFBP-1 concentrations [35]. Although in our study many of the patterns of IGFBP-1 in the obese group were similar to a group of lean individuals, the latter group was younger and therefore cannot be regarded as a true control group.

Why is there a blunted IGFBP-1 response during nutritional deprivation in type 2 diabetes? We speculate that in this group there is significant hepatic insulin resistance with respect to IGFBP-1 synthesis in the normally fed state. With reduced energy (caloric) intake an improvement in hepatic insulin sensitivity would lead to an improved inhibitory effect of insulin, opposing the expected increase in IGFBP-1 concentrations. Improved hepatic insulin sensitivity would also explain the normalization of the IGFBP-1 response to the increase in insulin levels during refeeding. Theoretical alternative explanations are that there may be an unidentified factor(s) that stimulates IGFBP-1 in type 2 diabetes, and which decreases during fasting in parallel to the fall in insulin, or that IGFBP-1 clearance is altered with nutritional status. Whether relative hepatic insulin resistance, opposing stimulators induced early in the postprandial period, or altered IGFBP-1 clearance contributes to the lack of response in this group is the subject of ongoing studies.

Acknowledgments

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