available at www.sciencedirect.com

ScienceDirect

www.metabolismjournal.com

Effect of a high-protein diet on ghrelin, growth hormone, and insulin-like growth factor–I and binding proteins 1 and 3 in subjects with type 2 diabetes mellitus

Mary Carol Gannon[a,b,c,*], *Frank Quentin Nuttall*[a,b]

[a] *Section of Endocrinology, Metabolism and Nutrition and the Metabolic Research Laboratory, Minneapolis VA Medical Center, Minneapolis, MN 55417, USA*
[b] *Department of Medicine, University of Minnesota, Minneapolis, MN 55455, USA*
[c] *Department of Food Science and Nutrition, University of Minnesota, Minneapolis, MN 55108, USA*

ARTICLE INFO

Article history:
Received 10 December 2010
Accepted 29 January 2011

ABSTRACT

We have developed a diet that over 5 weeks dramatically lowers plasma glucose in people with type 2 diabetes mellitus. This diet consists of 30% carbohydrate, 30% protein, and 40% fat and is referred to as a *Low Biologically Available Glucose* (LoBAG) diet. The diet also resulted in an approximately 30% increase in fasting insulin-like growth factor–I (IGF-I). Thus, we were interested in determining if the IGF-I elevation was due to an increase in ghrelin and growth hormone (GH) or to a change in IGF-I binding proteins (IGFBPs). Eight men with type 2 diabetes mellitus ingested a control diet (15% protein, 55% carbohydrate, and 30% fat) and a $LoBAG_{30}$ diet for 5 weeks in a randomized crossover design with a washout period in between. Before and after each 5-week period, subjects had blood drawn for total glycated hemoglobin and, at several time points over 24 hours, for GH, IGF-I, IGFBP-1, IGFBP-3, ghrelin, glucose, and insulin. Fasting and 24-hour glucose concentrations and total glycated hemoglobin were decreased, as expected (all *P*s < .05). Fasting IGF-I increased by approximately 30% (*P* = .05) and remained unchanged throughout 24 hours. Ghrelin, GH, IGFBP-1, IGFBP-3, and insulin were not different between diets. Insulin and IGFBP-1 concentrations were reciprocal, as expected. Insulin-like growth factor–I binding protein 1 decreased as insulin increased to greater than approximately 30 to 40 μU/mL. Ingestion of a $LoBAG_{30}$ diet by weight-stable subjects with type 2 diabetes mellitus resulted in an increase in total IGF-I without an increase in ghrelin, GH, and IGFBP-3 or a change in IGFBP-1 regulation. The mechanism remains to be determined.

Published by Elsevier Inc.

1. Introduction

For the past several years, we have been interested in improving glucose control without weight loss in people with type 2 diabetes mellitus by increasing the protein and fat content of the diet and decreasing the carbohydrate content, particularly the starch sources. We refer to the diets we designed as *Low Biologically Available Glucose* (LoBAG) diets.

The study is filed in ClinicalTrials.gov (NCT001058225).

Author contributions: The authors were equally responsible for funding acquisition, study design, data analysis and interpretation, and manuscript writing.

* *Corresponding author*. Metabolic Research Laboratory, VA Medical Center, Minneapolis, MN 55417, USA. Tel.: +1 612 467 2895; fax: +1 612 725 2273.
E-mail address: ganno004@umn.edu (M.C. Gannon).

0026-0495/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.metabol.2011.01.016

A subscript indicates the percentage of carbohydrate, that is, $LoBAG_{30}$ refers to a 30% carbohydrate diet. The protein content of these diets always is 30% of total food energy. Dietary fat, 10% of which is saturated, makes up the remainder of food energy. All of the studies resulted in an improvement in 24-hour integrated glucose concentration and in glycated hemoglobin (HbA_{1c}) [1-4]. The subjects were weight stable in all studies. In addition, in all of these studies, we noted an increase in the fasting insulin-like growth factor–I (IGF-I) concentration, presumably due to the increased protein content.

The present study was designed to determine the effect of the increased protein content in one of these diets, $LoBAG_{30}$, on the circulating growth hormone (GH) concentration and several GH- or IGF-I–related metabolic effectors in subjects with untreated type 2 diabetes mellitus.

2. Research design and methods

Eight male subjects with type 2 diabetes mellitus were recruited, screened, and found to be free of hematologic abnormalities, liver disease, kidney disease, macroalbuminuria (>300 mg/24 h), untreated thyroid disease, congestive heart failure, angina, life-threatening malignancies, proliferative retinopathy, diabetic neuropathy, peripheral vascular disease, and serious psychological disorders. All subjects weighed less than 136 kg (300 lb). The weight limit was set for practical considerations regarding the amount of food to purchase and prepare to keep an individual weight stable throughout the study.

The participants met the National Diabetes Data Group criteria for the diagnosis of type 2 diabetes mellitus [5] and were not being treated with oral hypoglycemic agents during the study. Seven of the subjects had been treated with oral hypoglycemic agents before enrollment in the study. These medications were discontinued. The subjects then were followed biweekly for varying periods until the HbA_{1c} stabilized. Once the HbA_{1c} was stable for approximately 6 weeks, the diet intervention was begun. None of the subjects had been treated with insulin or other injectable glucose-lowering agents.

Informed consent was obtained from all subjects. The study was approved by the Department of Veterans Affairs Medical Center and the University of Minnesota Committees on Human Subjects. Specifically, subjects verified that they were aware that their blood glucose concentration would increase when they discontinued their oral hypoglycemic medications. All subjects also obtained the approval of their primary care provider before discontinuing their diabetes medications (metformin and/or a sulfonylurea).

Other medications being taken by the subjects were continued and remained unchanged during the entire study. The subjects were instructed to maintain their current activity level and medication dosage throughout the study. Two weeks before beginning the study, the participants completed a 3-day food questionnaire, with one of the days being a Saturday or a Sunday. This information was used to calculate the total food energy necessary to maintain body weight. Patient characteristics are listed in Table 1. The study is filed in ClinicalTrials.gov (NCT001058225).

This is a randomized, crossover design study in which subjects are provided with isocaloric diets that are calculated to keep each individual weight stable throughout the study. A washout period of 5 to 12 weeks between diets was designed to allow the total glycated hemoglobin (%tGHb) to return to pre–diet intervention levels. The control diet was designed according to the recommendations of the American Heart Association [6], the United States Department of Agriculture [7], and the American Cancer Society [8]. It consisted of 55% carbohydrate, 15% protein, and 30% fat (10% monounsaturated, 10% polyunsaturated, and 10% saturated fat). The test diet consists of 30% carbohydrate, 30% protein, and 40% fat (~10 % saturated fat), that is, a $LoBAG_{30}$ diet. Six-day rotating menus were designed. Examples of each diet have been published previously [9].

Subjects were randomized to begin the study with either the $LoBAG_{30}$ or the control diet, as determined by a flip of a coin. Four subjects began the study on the control diet; 4 subjects began the study on the $LoBAG_{30}$ diet. Participants were admitted to the Special Diagnostic and Treatment Unit (SDTU), similar to a General Clinical Research Center, on the evening before the study.

The next day, while in the SDTU, standardized meals containing 55% carbohydrate, 30% fat, and 15% protein were given for breakfast (8:00 AM), lunch (12:00 PM), and dinner (6:00 PM). A snack was given at 8:00 PM. Fasting blood samples were obtained at 7:30, 7:45, and 8:00 AM. Samples then were collected every 15 minutes for the first hour after meals, every

Table 1 – Patient characteristics

	Age (y)	Ht (in)	Ht (cm)	Wt (lb)	Wt (kg)	BMI	tGHb %	Concomitant diseases	Medications
	52	68	173	206	94	31	8.3	MS, HT, hypercholesterolemia	Glatiramer, nifedipine
	64	74	187	206	94	27	8.2	HT, dyslipidemia	Atenolol, Lipitor, lisinopril
	61	74	188	218	99	28	7.1	HT, depression	Lisinopril, metoprolol, amitriptyline
	64	70	178	211	96	30	10.7	HT, dyslipidemia	Ezetimibe, fosinopril, metoprolol
	62	70	178	237	107	34	7.4	HT, lumbar pain	Hydrochlorothiazide, codeine, gabapentin
	58	71	180	237	108	33	8.5	Dyslipidemia	Lisinopril, simvastatin
	53	68	173	188	85	29	9.0	HT, dyslipidemia	Lisinopril, simvastatin
	70	67	170	211	99	33	11.4	Depression	Mirtazapine
Mean	61	70	178	214	97	31	8.8		
SEM	2.1	0.9	2.3	5.8	2.6	0.9	0.5		

BMI indicates body mass index; MS, multiple sclerosis; HT, hypertension.

30 minutes for the next 2 hours, and then hourly until the next meal. Additional blood samples were obtained at 20-minute intervals during the night, specifically for determining the GH concentrations because most of GH is secreted during the night. Thus, while in the SDTU, blood was drawn for glucose, insulin, %tGHb, GH, IGF-1, IGFBP-1, IGFBP-3, and ghrelin. During the day, subjects were encouraged to drink water to ensure adequate urine output. Subjects were asked to remain in the SDTU during the study period with minimal activity.

After this 24-hour test period, the subjects were sent home with all food prepared for the next 2 to 3 days as appropriate for the diet to which they were randomized. Subjects then returned to the SDTU twice a week to pick up food and meet with the study dietitian and study coordinator. At that time, the subjects provided a urine specimen for analysis of creatinine and urea to determine dietary compliance. They also were weighed and had blood pressure and a blood glucose concentration determined. If their body weight decreased or increased on 2 successive occasions, the total food energy of the meals was increased or decreased as appropriate in an effort to maintain a stable weight throughout the study. In addition, subjects were interviewed regarding dietary compliance during each visit and food preferences were discussed. Thus, weekly, on an outpatient basis, urine urea and creatinine, as well as blood glucose, %tGHb, weight, and blood pressure, were determined.

At the end of the 5-week period, the subjects again were admitted to the SDTU and blood was drawn as described earlier. At this time, subjects were given the meals appropriate for that day, that is, either the $LoBAG_{30}$ or control diet to which they had been randomized. Thus, the subjects were admitted to the SDTU on 4 occasions to have blood drawn during a 24-hour period, at the beginning and end of the 5-week control diet period, and at the beginning and end of the 5-week LoBAG diet period.

Serum glucose concentrations were determined using a hexokinase method on an Abbott Architect analyzer (Abbott Laboratories, Abbott Park, IL). Serum immunoreactive insulin was determined using an automated chemiluminescent assay on a DPC IMMULITE platform (Diagnostic Products, Los Angeles, CA). Growth hormone was determined on a Siemens Immulite platform (Munich, Germany) using an enzyme-linked immunosorbent assay method in the laboratory of Dr Ali Iranmanesh (Salem, VA) (reference range = 1 ng/mL or less for men). Insulin-like growth factor–I (reference range = 91-443 ng/mL, median = 240 ng/mL), IGFBP-1 (reference range not available), and IGFBP-3 (reference range = 1500-5580 ng/mL) all were determined using enzyme-linked immunosorbent assay kits manufactured by Diagnostic Systems Laboratories (DSL), a Beckman Coulter company (Webster, TX).

Total glycated hemoglobin was measured by boronate affinity high-performance liquid chromatography (Biorad Variant; Biorad Laboratories, Hercules, CA). This is a highly specific and precise method for determination of glycation (fructosyl derivatization) at all potential glycation sites in the 4 polypeptide chains (2 α and 2 β polypeptides) that make up the globin protein in hemoglobin.

With the HbA_{1c} cation-exchange chromatography methods, generally, what is actually measured is loss of a charge when a fructosyl derivatization occurs on the N-terminal ends of the 2 β chains; that is, HbA_{1c} represents only derivatization of the N-terminal amino acids on the 2 β chains. When direct comparisons of total glycohemoglobin and HbA_{1c} were done, the total glycohemoglobin values were higher, as expected [10]. However, the methods yield similar results at present because of national standardization of all methods currently in use.

Both %tGHb and HbA_{1c} provide an index of the average blood glucose concentration over the past approximately 3 months (the average life span of the red blood cells containing hemoglobin).

Fructosamine measures glycated serum proteins (albumin mostly, not globin in hemoglobin). The albumin molecule turns over approximately every 2 to 3 weeks. Therefore, the fructosamine assay is often used to reflect rapid changes in average blood glucose concentrations, but is not as well standardized as globin glycation methods. We have been able to demonstrate a statistically significant decrease in %tGHb after 2 to 3 weeks [2], and it is known that 50% of the final decrease in tGHb occurs at approximately 34 days [11].

The total integrated 24-hour area responses were calculated, using zero as baseline, with a computer program based on the trapezoid rule [12]. Statistics were determined using repeated-measures analysis of variance (ANOVA) with Prism 4 software for the Macintosh by GraphPad (La Jolla, CA). This was followed by Student t test for paired variates. A P value $< .05$ was the criterion for significance. Data are presented as means ± SEM.

3. Results

3.1. *Body weight*

The average body weight at the beginning of the control diet was 98 ± 3 kg (216 ± 6 lb). It was essentially unchanged at the end of the 5-week period (97 ± 3 kg; 214 ± 6 lb). Similarly, the average body weights at the beginning and end of the $LoBAG_{30}$ diet were identical at 98 ± 3 kg (215 ± 6 lb). Thus, body weights were stable throughout the study (data not shown).

3.2. *Urea to creatinine ratio*

The mean urea to creatinine ratio was 7.5 ± 0.5 and 7.8 ± 0.4 at the beginning and end of the control diet period, respectively. During the 5 weeks while ingesting the control diet, the mean ratio was 7.1. The mean urea to creatinine ratio was 8.7 ± 0.6 and 10.3 ± 0.5 before and at the end of the $LoBAG_{30}$ diet period, respectively (Table 2). The mean baseline value before beginning the $LoBAG_{30}$ diet is significantly higher than the mean baseline value before beginning the control diet ($P = .04$). This is due to one individual whose ratio was 12.1 during that 24-hour period. We have no explanation for the elevated value. When the 12.1 value is excluded, the mean ratio is 8.2 ± 0.3, and the baselines are no longer statistically different ($P = .27$). During the 5 weeks of ingesting the $LoBAG_{30}$ diet, the mean ratio was 11.1. These ratios indicate good compliance with the dietary regimen.

3.3. *Glucose*

The glucose excursions following meals were similar before and after the control diet, as were the glucose area responses (Fig. 1, top). In contrast, the fasting glucose

Table 2 – Urea to creatinine ratios

	Week 0 inpatient	Week 1	Week 2	Week 3	Week 4	Week 5 inpatient
Control	7.5 ± 0.5	6.6 ± 0.5	7.0 ± 0.5	7.2 ± 0.5	7.7 ± 0.8	7.8 ± 0.4
$LoBAG_{30}$	8.7 ± 0.6[a]	11.1 ± 0.4	10.5 ± 0.8	10.4 ± 0.6	12.2 ± 0.8	10.3 ± 0.5

The mean ratios for week 0 and week 5 are based on 24-hour urine collections while the subjects were in the SDTU. The ratios for weeks 1 to 4 are based on random specimens the subjects brought to the facility when they came in for their routine visits.

[a] The mean baseline value for the $LoBAG_{30}$ is significantly higher than the baseline value for the control ($P = .04$). It is due to one individual whose ratio was 12.1 during that 24-hour period. We have no explanation for the elevated value. The control meals ingested at baseline were identical for the 2 arms of the study. Thus, there must be residual urea excreted from the prior day(s). We instructed the subjects to return to their usual diets and eating habits during the washout period, but we did not monitor the diets during that time. The mean ratio when the 12.1 value is excluded is 8.2 ± 0.3, and the baselines are no longer statistically different ($P = .27$).

concentration and the postmeal glucose excursions were markedly attenuated following 5 weeks on the $LoBAG_{30}$ diet when compared with the response before the diet (Fig. 1, bottom). As a result, the total area response following the $LoBAG_{30}$ diet was significantly less compared with the areas before and after the control diet and before the $LoBAG_{30}$ diet (ANOVA, $P = .0001$; $P < .05$ for $LoBAG_{30}$ compared with all others). These data were as expected based on our previous observations [3,4].

3.4. *Insulin*

The insulin concentrations were similar regardless of the dietary regimen (Fig. 2). The total insulin areas also were

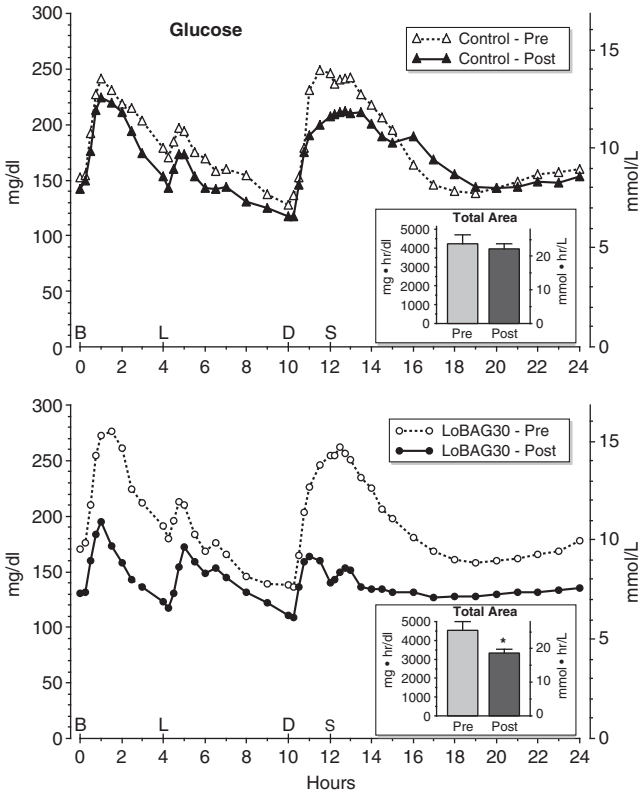

Fig. 1 – Twenty-four–hour serum glucose response (top, control diet; bottom, $LoBAG_{30}$ diet). The open triangles and broken line represent the mean glucose response at the beginning of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed triangles and solid line represent the glucose response after 5 weeks of ingesting a control diet. The open circles and broken line represent the glucose response at the beginning of the test arm of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed circles and solid line represents the glucose response after 5 weeks of ingesting a $LoBAG_{30}$ diet consisting of 30% carbohydrate, 30% protein, and 40% fat. The insets indicate the mean total 24-hour integrated glucose area response pre and post 5 weeks after ingesting a control (top) or $LoBAG_{30}$ diet (bottom), using zero as a baseline. *Statistical significance. B = breakfast, L = lunch, D = dinner, S = snack.

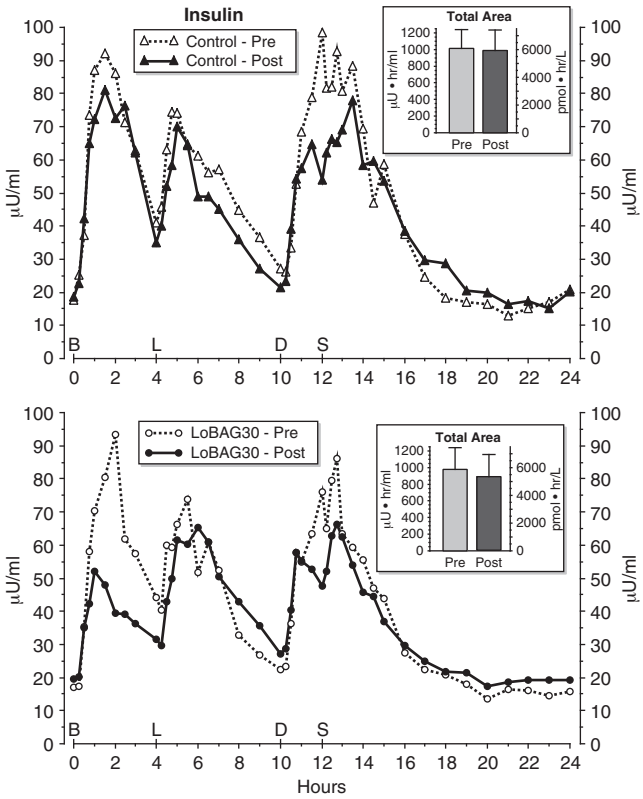

Fig. 2 – Twenty-four–hour serum insulin response (top, control diet; bottom, LoBAG$_{30}$ diet). The open triangles and broken line represent the mean insulin response at the beginning of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed triangles and solid line represent the insulin response after 5 weeks of ingesting a control diet. The open circles and broken line represent the insulin response at the beginning of the test arm of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed circles and solid line represent the insulin response after 5 weeks of ingesting a LoBAG$_{30}$ diet consisting of 30% carbohydrate, 30% protein, and 40% fat. The insets indicate the mean total 24-hour integrated insulin area response pre and post 5 weeks after ingesting a control (top) or LoBAG$_{30}$ diet (bottom), using zero as a baseline.

similar and not significantly different from one another (P = .245), as observed previously [3,4].

3.5. *Percentage of total glycated hemoglobin*

The %tGHb decreased from 8.6% ± 0.5% to 8.2% ± 0.4% (Δ = 0.4) during the 5 weeks on the control diet. The decrease during the 5 weeks on the LoBAG$_{30}$ diet was greater (8.7% ± 0.5% to 7.5% ± 0.4%; Δ = 1.2). The difference between the decrease at 5 weeks on the control vs 5 weeks on the LoBAG$_{30}$ diet was statistically significantly different (P = .03), also as observed in our previous studies [3,4].

Glucose, insulin, and %tGHb were measured to confirm our previous observations.

3.6. *Ghrelin*

The ghrelin concentration decreased following meals, as expected. However, there was no difference in response whether subjects were ingesting a control diet or a LoBAG diet (Fig. 3).

3.7. *Growth hormone*

Growth hormone is an anabolic hormone and is largely secreted at night. It regulates synthesis of IGF-I, IGFBP-3, and the acid-labile subunit. The mean fasting GH concentration was 0.27 ± 0.1 and 0.55 ± 0.2 ng/mL at the beginning and end of the control diet, respectively (Fig. 4, top). It was 0.19 ± 0.1 ng/mL before starting the LoBAG$_{30}$ diet and 0.37 ± 0.2 ng/mL 5 weeks after ingesting the LoBAG$_{30}$ diet (Fig. 4, bottom). These differences in fasting baseline concentration were not statistically significantly different (P = .11). The GH concentrations during the nighttime hours increased in a pulsatile fashion, as expected. The 24-hour total area responses were 9.2 ± 2.1, 11.4 ± 2.7, 9.8 ± 1.6, and 12.0 ± 3.5 ng·h/mL before and after 5 weeks on the control diet and before and 5 weeks after ingesting the LoBAG$_{30}$ diet, respectively (Fig. 4, insets). The differences were not statistically significantly different (P = .51).

3.8. *Insulin-like growth factor–I*

The mean fasting IGF-I concentrations were similar (260 ± 57 and 248 ± 45 ng/L), as were the mean total IGF-I area

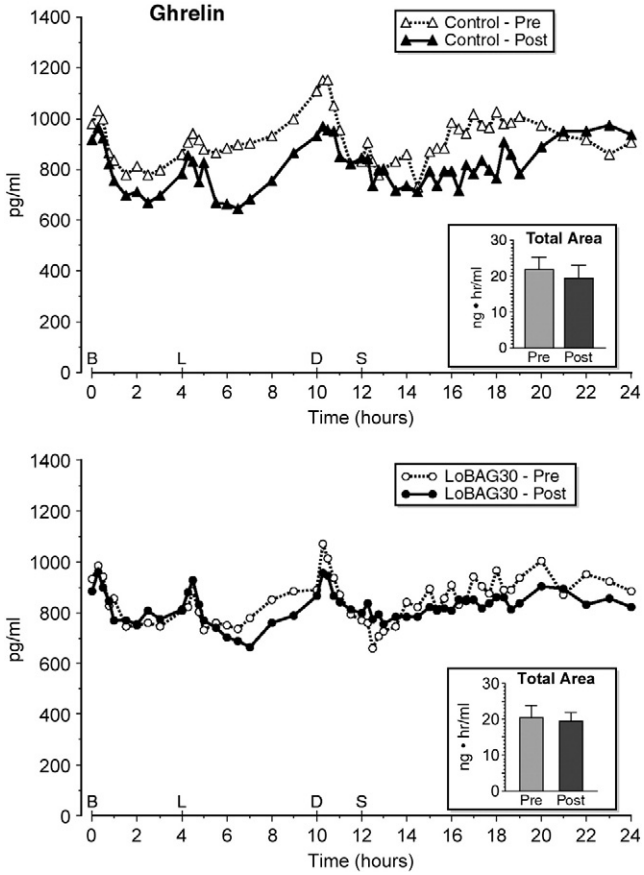

Fig. 3 – Twenty-four–hour serum ghrelin (top, control diet; bottom, LoBAG$_{30}$ diet). The open triangles and broken line represent the mean ghrelin response at the beginning of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed triangles and solid line represent the ghrelin response after 5 weeks of ingesting a control diet. The open circles and broken line represent the ghrelin response at the beginning of the test arm of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed circles and solid line represent the ghrelin response after 5 weeks of ingesting a LoBAG$_{30}$ diet consisting of 30% carbohydrate, 30% protein, and 40% fat. The insets indicate the mean total 24-hour integrated ghrelin area response pre and post 5 weeks after ingesting a control (top) or LoBAG$_{30}$ diet (bottom), using zero as a baseline.

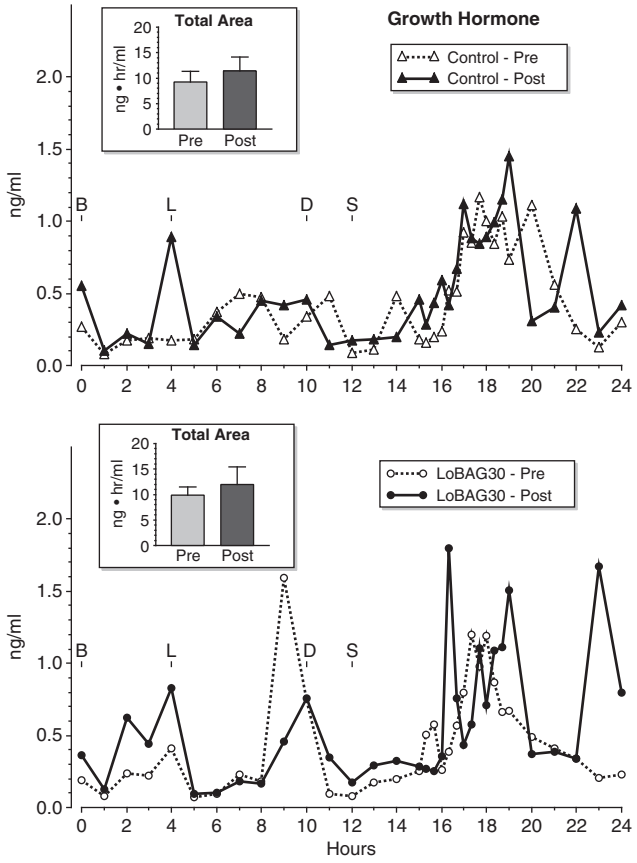

Fig. 4 – Twenty-four–hour serum GH response (top, control diet; bottom, LoBAG$_{30}$ diet). The open triangles and broken line represent the mean GH response at the beginning of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed triangles and solid line represent the GH response after 5 weeks of ingesting a control diet. The open circles and broken line represent the GH response at the beginning of the test arm of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed circles and solid line represent the GH response after 5 weeks of ingesting a LoBAG$_{30}$ diet consisting of 30% carbohydrate, 30% protein, 40% fat. The insets indicate the mean total 24-hour integrated GH area response pre and post 5 weeks after ingesting a control (top) or LoBAG$_{30}$ diet (bottom), using zero as a baseline.

responses (6.1 ± 1.4 and 5.9 ± 1.1 μg·h/mL) before and after the control diet, respectively (Fig. 5, top). The mean fasting IGF-I concentration before beginning the LoBAG$_{30}$ arm of the study was 221 ± 48 ng/mL. This was statistically significantly increased to 289 ± 52 mg/mL after 5 weeks on the LoBAG$_{30}$ diet (ANOVA = 0.044, P = .05) (Fig. 5, bottom). The reason the pre-LoBAG$_{30}$ result is lower than before or after being on the control diet is unknown. Nevertheless, when comparing the difference between the pre- and post-LoBAG$_{30}$ (baseline control vs high protein) diet, statistical significance was observed (P = .05) using Student paired 2-tail t test. The mean 24-hour integrated total IGF-I area response was significantly increased from the control following 5 weeks on the LoBAG$_{30}$ diet (5.3 ± 1.0 vs 6.5 ± 1.3 μg·h/mL) (P < .05).

3.9. *Insulin-like growth factor binding protein 3*

The mean fasting IGFBP-3 concentrations and the mean 24-hour area responses were similar before and after the control diet (Fig. 6, top) and before and after 5 weeks on the LoBAG$_{30}$ diet, respectively (Fig. 6, bottom). Consequently, the mean total area responses also were similar and were not statistically significantly different (P = .08).

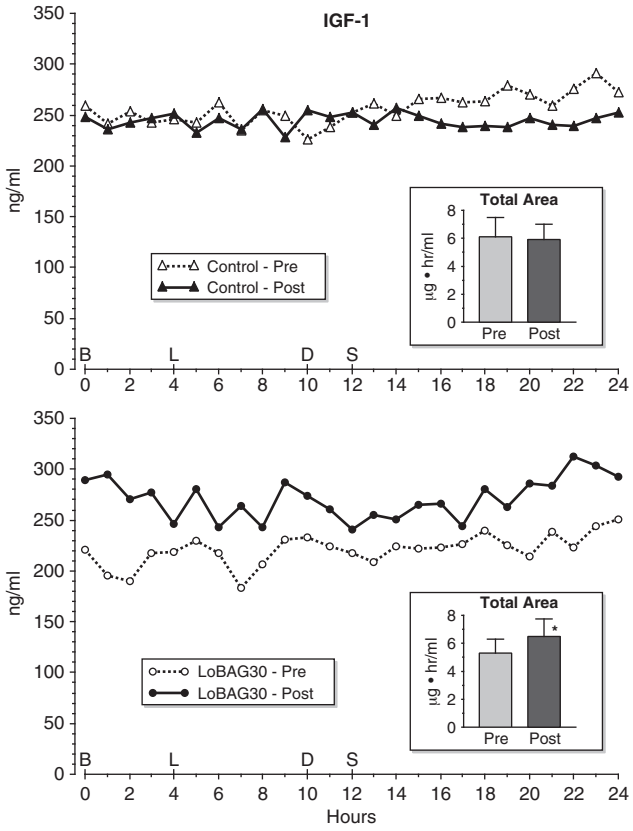

Fig. 5 – Twenty-four–hour serum IGF-I response (top, control diet; bottom, LoBAG$_{30}$ diet). The open triangles and broken line represent the mean IGF-I response at the beginning of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed triangles and solid line represent the IGF-I response after 5 weeks of ingesting a control diet. The open circles and broken line represent the IGF-I response at the beginning of the test arm of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed circles and solid line represent the IGF-I response after 5 weeks of ingesting a LoBAG$_{30}$ diet consisting of 30% carbohydrate, 30% protein, and 40% fat. The insets indicate the mean total 24-hour integrated IGF-I area response pre and post 5 weeks after ingesting a control (top) or LoBAG$_{30}$ diet (bottom), using zero as a baseline. *Statistical significance.

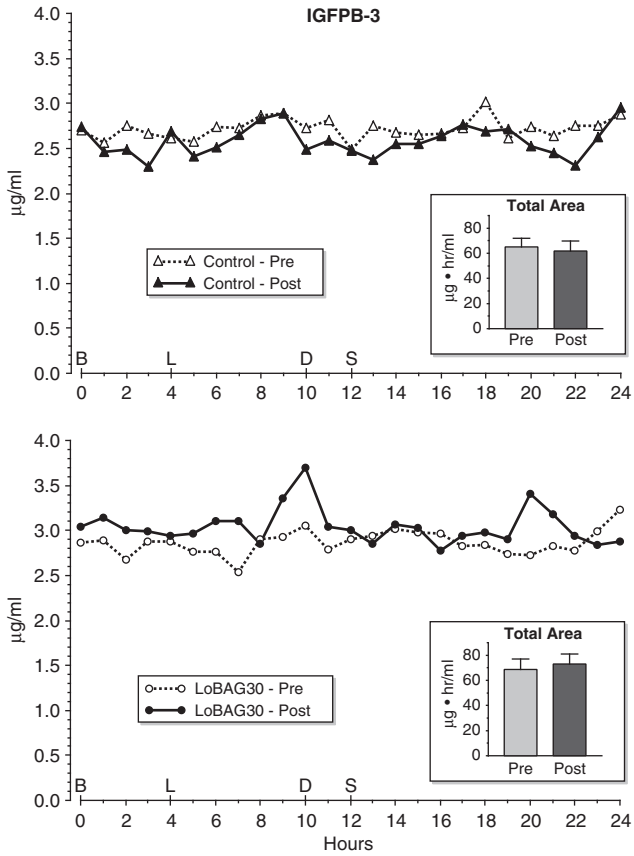

Fig. 6 – Twenty-four–hour serum IGFBP-3 response (top, control diet; bottom, LoBAG$_{30}$ diet). The open triangles and broken line represent the mean IGFBP-3 response at the beginning of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed triangles and solid line represent the IGFBP-3 response after 5 weeks of ingesting a control diet. The open circles and broken line represent the IGFBP-3 response at the beginning of the test arm of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed circles and solid line represent the IGFBP-3 response after 5 weeks of ingesting a LoBAG$_{30}$ diet consisting of 30% carbohydrate, 30% protein, and 40% fat. The insets indicate the mean total 24-hour integrated IGFBP-3 area response pre and post 5 weeks after ingesting a control (top) or LoBAG$_{30}$ diet (bottom), using zero as a baseline.

3.10. *Insulin-like growth factor binding protein 1*

The IGFBP-1 concentrations began to decrease within 1 hour after ingestion of breakfast, from a fasting concentration of 10 to 14 ng/mL to a low of approximately 2 ng/mL just before lunch (Fig. 7). This decrease occurred regardless of whether the subjects had ingested the control diet the day of the test, the control diet for 5 weeks, or the LoBAG$_{30}$ diet for 5 weeks. The IGFBP-1 concentrations remained low until near dinnertime when an increase to approximately 50% of the fasting value was observed. Approximately 1 hour after dinner, the IGFBP-1 concentrations decreased to approximately 2 ng/mL and remained low for 2 to 3 hours. Subsequently, the concentrations increased continually until the next morning. The total 24-hour integrated area responses before and after 5 weeks on the control diet or before and after 5 weeks on the LoBAG$_{30}$ diet were similar.

3.11. *Relationship between IGFBP-1 and insulin*

Because insulin is known to be a major regulator of IGFBP-1, we have included a figure demonstrating the dynamics between the insulin concentration and the IGFBP-1 concentration (Fig. 8). We realize that an inverse relationship has been recognized, but to our knowledge, 24-hour data have not been published in people with type 2

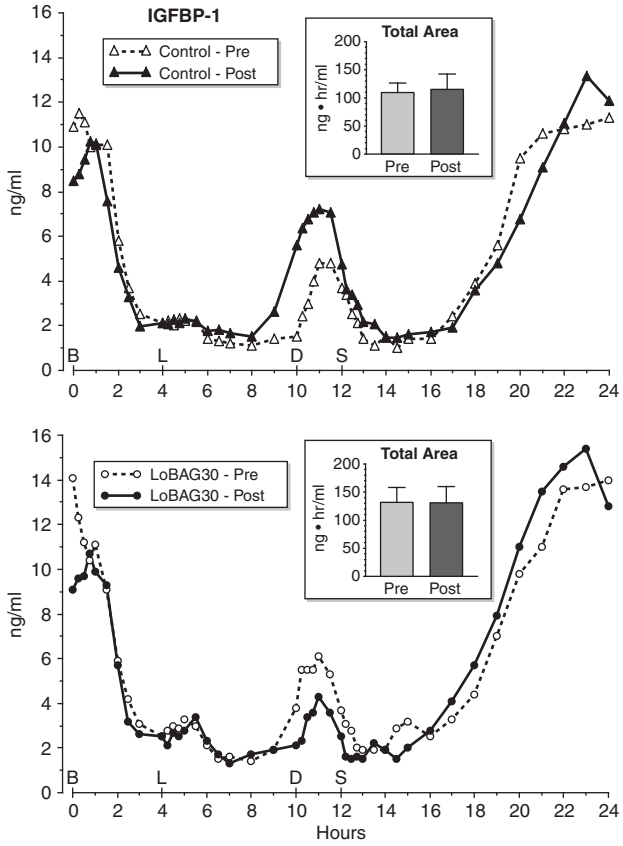

Fig. 7 – Twenty-four–hour serum IGFBP-1 response (top, control diet; bottom, LoBAG$_{30}$ diet). The open triangles and broken line represent the mean IGFBP-1 response at the beginning of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed triangles and solid line represent the IGFBP-1 response after 5 weeks of ingesting a control diet. The open circles and broken line represent the IGFBP-1 response at the beginning of the test arm of the study while ingesting a control diet of 55% carbohydrate, 15% protein, and 30% fat. The closed circles and solid line represent the IGFBP-1 response after 5 weeks of ingesting a LoBAG$_{30}$ diet consisting of 30% carbohydrate, 30% protein, and 40% fat. The insets indicate the mean total 24-hour integrated IGFBP-1 area response pre and post 5 weeks after ingesting a control (top) or LoBAG$_{30}$ diet (bottom), using zero as a baseline.

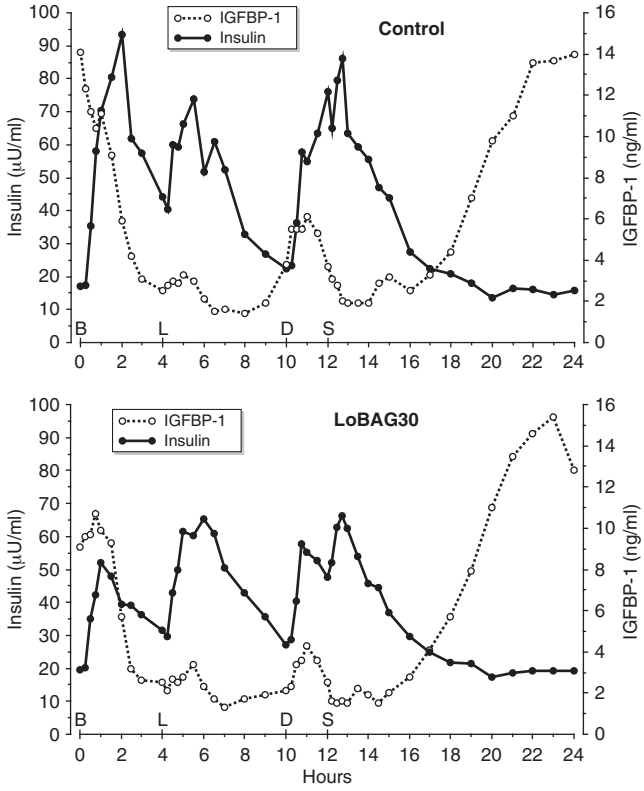

Fig. 8 – Relationship between circulating IGFBP-1 and insulin concentrations. The mean 24-hour serum IGFBP-1 response (open circles, broken line) and the mean 24-hour serum insulin response (closed circles, solid line) following 5 weeks on a control diet (top) or following 5 weeks on a LoBAG$_{30}$ diet (bottom). Note that the IGFBP-1 concentration increases when the insulin concentration is between approximately 20 and 30 μU/mL.

diabetes mellitus, nor has a potential difference induced by a change in diet composition.

4. Discussion

4.1. *Glucose, insulin, and %tGHb*

The glucose and insulin data obtained in the current study are similar to those we have obtained previously [3]. That is, the LoBAG$_{30}$ diet resulted in a decrease in the fasting glucose concentration and in the 24-hour integrated glucose area response without a change in the fasting and 24-hour integrated insulin area responses. These were associated with a statistically significant decrease in %tGHb from 8.7% to 7.5%.

4.2. *Ghrelin*

Ghrelin is a hormone produced predominately by cells in the fundus of the stomach [13]. It has been shown to bind to the GH secretagogue receptor and to potently stimulate secretion of GH [14,15]. Ghrelin is increased with fasting and decreases following ingestion of meals. Because high-protein meals have been suggested to increase satiety, we wanted to determine whether ghrelin would remain low for a longer period following ingestion of the LoBAG meals. It did not. We also wanted to determine whether the ghrelin response was related to the GH response; again, it was not.

4.3. *Growth hormone*

In our previous studies in which a LoBAG diet was compared with a standard diet, GH was measured only in the fasted state at 8:00 AM [9,16,17]. It was not significantly increased when

the protein content of the diets was increased from 15% in the control arm to 30% of total food energy in the LoBAG arm. Because the majority of GH is secreted during stage 3 and 4 sleep, as was demonstrated using a 30-second sampling technique [18], we were interested in measuring GH during a 24-hour period in subjects with type 2 diabetes mellitus following ingestion of high-protein mixed meals.

In the present study, blood samples were obtained for GH determination hourly during the day, but every 20 minutes from 11:00 PM to 3:00 AM to increase the likelihood of capturing some of the spikes in GH release during sleep. We realize that it would have been desirable to obtain samples more frequently during the night, but the volume of blood required and other technical issues made this not feasible. At the time points measured, episodic increases in concentration occurred during the nighttime hours, as expected (Fig. 4). However, some occurred during the day. We did not prohibit daytime naps [19], which may or may not explain some of the peaks observed during the daytime hours. The GH response was not affected by the change in dietary composition (Fig. 4).

To our knowledge, 24-hour profiles of circulating GH concentration have not been determined in subjects with type 2 diabetes mellitus, nor have they been determined following ingestion of high-protein mixed meals.

As discussed below, ingestion of protein alone has been reported to increase circulating GH concentration and ingestion of carbohydrates, to suppress it. In the present study, it is clear that doubling the protein content of the diet at the expense of carbohydrate, from 15% in the control to 30% of total food energy in the $LoBAG_{30}$ diet, did not affect the 24-hour integrated GH concentration.

The effect on GH concentration of altering the diet composition for several days was studied many years ago [20]. In 5 to 8 young men, an 80% carbohydrate diet ingested over 8 to 10 days markedly reduced the 24-hour integrated GH concentration compared with the control diet of 40% carbohydrate, 40% fat, and 20% protein. A 75% fat or a 70% protein diet did not, and also did not increase it.

In the same study, 7 women also were studied while ingesting the high-carbohydrate and the control diet. The GH values were not suppressed by high carbohydrate intake, but primary data were not provided.

Although these diets were extreme in their composition, the data suggest that dietary carbohydrate lowers GH concentration in healthy men, but not significantly in women. A high-protein diet did not increase the GH concentration. In the same study, 72 hours of fasting resulted in an approximately 290% increase in GH in men but only a 125% increase in women. To our knowledge, these are the only data in the literature reporting 24-hour GH responses in which subjects ingested diets of varying composition for an extended period.

In acute studies, intravenous amino acids given in large amounts [21], as well as single meal ingestion of amino acids [22-24] or dietary protein [25-27], have been reported to rapidly increase the circulating GH. In several of these studies, when carbohydrate was ingested with protein, the GH response was attenuated. In contrast, Baker et al [28] reported that GH was not affected by a high-protein breakfast.

More recently, a group from the Netherlands has determined the effect on circulating GH concentrations of ingestion of drinks composed of complete soy protein, hydrolyzed soy protein, or a mixture of amino acids resembling the composition of soy protein in healthy young women over 5 hours [29]. All 3 drinks resulted in a similar increase in GH concentration. The increase was statistically significantly greater than the response to a placebo drink (presumably water, but not stated). When soy protein was ingested with carbohydrate or fat, an increase in GH was observed, but not when both carbohydrate and fat were present [30,31]. Thus, both carbohydrate and fat were required to negate a dietary protein-induced increase in GH.

4.4. *Insulin-like growth factor–I*

Insulin-like growth factor–I is a 70–amino acid hormone, similar in structure to insulin. It is produced by several organs in the body. However, the majority (~75%) is produced in the liver under the control of GH [32]. In liver, GH also stimulates the production of IGFBP-3 and another protein referred to as the *acid-labile subunit*. These 3 protein peptides circulate bound together in a ternary complex. This complex represents the major form in which IGF-I is present in serum [33]. Interestingly, a total of 6 well-characterized mammalian IGFBPs have been identified that have a high affinity for IGF-I (K_d ~10^{-10} mol/L) [34]. In addition, 9 other proteins have been identified that have a lower affinity for IGF-I. These have been referred to as *IGFBP-related proteins* (IGFPB-rP1-9) [34]. However, the metabolic role of these IGF-I binding proteins remains to be clarified.

Insulin-like growth factor–I was measured because it is also an anabolic hormone. An increase in IGF-1 has been reported to stimulate β-cell proliferation [35,36] and inhibit apoptosis in vitro [37]. Furthermore, IGF-I has insulin-like effects and improves insulin sensitivity [38]. However, there is concern that it may stimulate cancer cell proliferation [39].

Determination of IGF-I concentration by immunoassay is affected by the dissociation of IGF-I from its bound form(s), the specificity of the antibody, the standard used to calibrate the assay, as well as the collection and storage of the sample [40]. Thus, as elucidated by Bidlingmaier [41] and Frystyk et al [40], determination of total IGF-I concentration can be difficult. In the present study, all samples were assayed in batches using the same method (DSL) in our laboratory. Thus, analytical variability should be minimal and should not influence our interpretation of the present data. Of note, we determined IGF-I concentrations using 3 different methods: DSL, ImmunoDiagnostic Systems (Fountain Hills, AZ), and Siemens Immulite. Although the absolute value of IGF-I was different with the various methods, the pattern of response was similar (unpublished data).

In people ingesting typical mixed diets, circulating IGF-I is known to be quite stable throughout a 24-hour period (reviewed in Rajpathak et al [42]). Thus, the overnight fasting values should approximate the average 24-hour concentration. However, to our knowledge, it was not known whether IGF-I was stable at all time points in subjects with untreated type 2 diabetes mellitus ingesting a LoBAG diet. Therefore, we considered it necessary to document that the 24-hour integrated value was elevated for 24 hours. Indeed, that was the case in the present study (Fig. 5).

After 5 weeks on a $LoBAG_{30}$ diet, which contained double the protein of the baseline control diet, the fasting IGF-I concentration was increased by 31%. This is strikingly similar to data obtained in our previous studies of LoBAG diets, all of which contained 30% protein, although the carbohydrate content varied from 20% to 40%. The fasting IGF-I concentration was increased by 31% in the $LoBAG_{40}$ diet [16], 34% in the $LoBAG_{30}$ diet [9], and 40% in the $LoBAG_{20}$ diet [17]. Thus, it is likely that the increase in IGF-I was due to the increase in protein. Insulin-like growth factor–I was reported to be increased by a very high protein (65%) hypocaloric (6.25 kcal/kg body weight) diet in obese female subjects many years ago [43]. In subjects with type 1 diabetes mellitus, increasing the protein content from 10% to 20% of total food energy did not result in an increase in IGF-I [44].

In our study, because the increase in IGF-I was highest with the $LoBAG_{20}$ diet, which had the lowest carbohydrate, this may represent partial inhibition of IGF-I synthesis by carbohydrate. Nevertheless, this needs to be verified in a larger study.

To our knowledge, an effect on the circulating IGF-I concentration of diets varying in composition, but not in food energy, has not been reported in people either with or without type 2 diabetes mellitus.

Insulin-like growth factor–I inhibits GH secretion by direct feedback, but also indirectly by stimulating the release of somatostatin [45]. Thus, a decrease in GH with an increase in IGF-I due to the increased dietary protein would have been expected. If this is the case, the IGF-I is extremely sensitive to GH in this population. This we consider to be unlikely. Growth hormone is known to be low and less responsive to stimuli such as sleep, exercise, GH releasing hormone, etc, in older men with an increased fat mass [33]. In addition, it has been reported that infused IGF-I has less of an inhibitory effect on GH release in the elderly [46]. We consider that the best explanation for the current data is that dietary protein is stimulating IGF-I, independent of GH.

Another example of IGF-I being regulated independently of GH is with starvation. The GH concentration is increased, whereas the IGF-I concentration is decreased [47-50].

In reviewing the literature, we found only 1 report in which the total IGF-I in age-and weight-matched subjects with and without type 2 diabetes mellitus was compared [51]. The authors reported that the fasting total IGF-I was not different.

Theoretically, because both GH and IGF-I are anabolic hormones, an increase in IGF-I with no change in GH should result in an increase in lean body mass. Protein balance, amino acid concentrations, and body composition data will be reported separately.

4.5. *Insulin-like growth factor–I binding protein 3*

Insulin-like growth factor–I binding protein 3 is the most abundant of the IGF-I binding proteins [52]. It consists of 264 amino acids, with a molecular weight of 28 700 d [34]. It is produced in the liver, and its production is stimulated by GH. It is reported to have distinct biological effects independent of IGF-I binding (for review, please see Jogie-Brahim et al [53]).

In the present study, IGFBP-3 concentrations were similar following either the control diet or the $LoBAG_{30}$ diet. This reinforces the concept that dietary protein stimulates IGF-I independent of GH because IGFBP-3 is GH dependent, which did not change.

The morning IGFBP-3 concentration in subjects with type 2 diabetes mellitus has been reported to be approximately 40% lower than in similar-aged control subjects [54]. The values in our subjects with type 2 diabetes mellitus are similar to those with type 2 diabetes mellitus in the latter report [54]. We do not have data in healthy and age- and weight-matched control subjects. In men without diabetes, Benbassat et al [55] reported that fasting IGFBP-3 concentrations were approximately 15% lower in older (63-81 years) compared with younger subjects (20-39 years). From these limited data, it appears that type 2 diabetes mellitus has a greater lowering effect on IGFBP-3 than does aging. However, to our knowledge, the definitive comparative study has not been done.

4.6. *Insulin-like growth factor–I binding protein 1*

Insulin-like growth factor–I binding protein 1 is the only one of the IGF binding proteins that shows dynamic circadian changes. The principal regulator of IGFBP-1 concentration is insulin. Insulin inhibits hepatic synthesis and secretion of IGFBP-1 [51]. Indeed, an inverse relationship between IGFBP-1 and insulin in the fasting state has been reported in young as well as in aged subjects [52,55]. However, to our knowledge, the present study provides the first evidence of a 24-hour relationship in older subjects with type 2 diabetes mellitus. In the present study, when the insulin concentration decreased to less than approximately 30 to 40 μU/mL, the IGFBP-1 concentration was increased, suggesting a threshold effect in these subjects with type 2 diabetes mellitus. Interestingly, several years ago, it was reported that IGFBP-1 levels rose when insulin fell to less than 30 μU/mL, a relationship determined using samples from fasting obese female subjects without diabetes [43]. Because of the relationship between IGFBP-1 and insulin, determination of IGFBP-1 concentration has been recommended as a measurement of insulin resistance [56]. Our data indicate that although subjects with type 2 diabetes mellitus are considered to be insulin resistant, the IGFBP-1 results are similar to those in nondiabetic subjects.

In summary, to our knowledge, these are the first studies to report the 24-hour profiles of ghrelin, GH, IGF-I, IGFBP-1, and IGFBP-3 in older subjects with type 2 diabetes mellitus and the effect of a change in dietary composition. The data indicate that ingestion of a $LoBAG_{30}$ diet, that is, a higher-protein–and lower-carbohydrate–content diet, results in an increase in total IGF-I. The increased IGF-I occurred without an increase in GH or IGFBP-3. These and our previous data suggest that dietary protein regulates IGF-I concentration independently. Regulation of IGFBP-1 by insulin also was unchanged by the difference in diet composition.

The metabolic effects of the increase in IGF-I over the long term remain to be determined. Dysregulation of the IGF system has been associated with multiple aspects of cancer progression [39], particularly IGF-I receptor expression and activity. Thus, the argument could be made that an increase in IGF-I may be deleterious. However, a counter argument is most compelling. Theoretically, an increase in IGF-I could reduce the expected increase in fat mass associated with a decrease in lean body mass in aging [57]. Thus, a diet-

induced increase in IGF-I could result in an improvement in, or better maintenance of, lean body mass relative to fatness. Added benefits include possible stimulation of β-cell proliferation and inhibition of apoptosis, as well as improvement in insulin sensitivity and a decrease in %tGHb. These effects would be of considerable benefit for the person with type 2 diabetes mellitus.

5. Funding

Supported in part by Merit Review Funds from the Department of Veterans Affairs and a grant from The National Pork Board.

Acknowledgment

The authors thank the Staff of the SDTU and the Staff of the Clinical Chemistry Laboratory for their assistance and Dr Iranmanesh, Salem, VA, for GH assays. We are particularly grateful for the continuing assistance of our research team, Heidi Hoover, MS, RD, research dietitian; Jan Thurgood, BS, study coordinator; and Linda Hartich, MT, laboratory technologist, as well as our volunteer patients with type 2 diabetes mellitus, without whom these studies would not have been possible.

REFERENCES

[1] Gannon MC, Nuttall FQ, Saeed A, Jordan K, Hoover H. An increase in dietary protein improves the blood glucose response in persons with type 2 diabetes. Am J Clin Nutr 2003;78:734-41.
[2] Gannon MC, Nuttall FQ. Effect of a high-protein, low-carbohydrate diet on blood glucose control in people with type 2 diabetes. Diabetes 2004;53:2375-82.
[3] Nuttall FQ, Schweim K, Hoover H, Gannon MC. Effect of the $LoBAG_{30}$ diet on blood glucose control in people with type 2 diabetes. Br J Nutr 2008;99:511-9.
[4] Gannon MC, Hoover H, Nuttall FQ. Further decrease in glycated hemoglobin following ingestion of a $LoBAG_{30}$ diet for 10 weeks compared to 5 weeks in people with untreated type 2 diabetes. Nutr Metab (Lond) 2010;7:64.
[5] American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010;33(Suppl 1):S62-9.
[6] Dietary guidelines for healthy American adults. A statement for physicians and health professionals by the Nutrition Committee, American Heart Association. Circulation 1986;74: 1465A-8A.
[7] USDA, US Department of Health and Human Services. Nutrition and your health: dietary guidelines for Americans. Washington (DC): US Government Printing Office; 1995.
[8] Kushi LH, Byers T, Doyle C, Bandera EV, McCullough M, Gansler T, et al. American Cancer Society guidelines on nutrition and physical activity for cancer prevention: reducing the risk of cancer with healthy food choices and physical activity. Cancer 2006;56:254-81.
[9] Nuttall FQ, Schweim K, Hoover H, Gannon MC. Metabolic effect of a $LoBAG_{30}$ diet in men with type 2 diabetes. Am J Physiol Endocrinol Metab 2006;291:E786-91.
[10] Nuttall FQ. Comparison of percent total GHb with percent HbA1c in people with and without known diabetes. Diabetes Care 1998;21:1475-80.
[11] Rech ME. Observations on the decay of glycated hemoglobin HbA1c in diabetic patients. Exp Clin Endocrinol Diabetes 1996;104:102-5.
[12] Gannon MC. A computer program to calculate the rate of appearance of glucose in the peripheral circulation following infusion of labeled glucose. Copyright. Minneapolis (Minn): University of Minnesota; 1991.
[13] Kojima M, Hosoda H, Date Y, Nakazato M, Matsuo H, Kangawa K. Ghrelin is a growth-hormone–releasing acylated peptide from stomach. Nature 1999;402:656-60.
[14] Takaya K, Ariyasu H, Kanamoto N, Iwakura H, Yoshimoto A, Harada M, et al. Ghrelin strongly stimulates growth hormone release in humans. J Clin Endocrinol Metab 2000;85:4908-11.
[15] Hataya Y, Akamizu T, Takaya K, Kanamoto N, Ariyasu H, Saijo M, et al. A low dose of ghrelin stimulates growth hormone (GH) release synergistically with GH-releasing hormone in humans. J Clin Endocrinol Metab 2001;86:4552.
[16] Nuttall FQ, Gannon MC, Saeed A, Jordan K, Hoover H. The metabolic response of subjects with type 2 diabetes to a high-protein, weight-maintenance diet. J Clin Endocrinol Metab 2003;88:3577-83.
[17] Nuttall FQ, Gannon MC. The metabolic response to a high-protein, low-carbohydrate diet in men with type 2 diabetes mellitus. Metabolism 2006;55:243-51.
[18] Holl RW, Hartman ML, Veldhuis JD, Taylor WM, Thorner MO. Thirty-second sampling of plasma growth hormone in man: correlation with sleep stages. J Clin Endocrinol Metab 1991;72: 854-61.
[19] Ho KY, Evans WS, Blizzard RM, Veldhuis JD, Merriam GR, Samojlik E, et al. Effects of sex and age on the 24-hour profile of growth hormone secretion in man: importance of endogenous estradiol concentrations. J Clin Endocrinol Metab 1987;64:51-8.
[20] Merimee TJ, Pulkkinen AJ, Burton CE. Diet-induced alterations of hGH secretion in man. J Clin Endocrinol Metab 1976;42: 931-7.
[21] Knopf RF, Conn JW, Fajans SS, Floyd JC, Guntsche EM, Rull JA. Plasma growth hormone response to intravenous administration of amino acids. J Clin Endocrinol Metab 1965;25:1140-4.
[22] Welbourne TC. Increased plasma bicarbonate and growth hormone after an oral glutamine load. Am J Clin Nutr 1995;61:1058-61.
[23] Groschl M, Knerr I, Topf HG, Schmid P, Rascher W, Rauh M. Endocrine responses to the oral ingestion of a physiological dose of essential amino acids in humans. J Endocrinol 2003;179:237-44.
[24] Collier SR, Casey DP, Kanaley JA. Growth hormone responses to varying doses of oral arginine. Growth Horm IGF Res 2005;15:136-9.
[25] Knopf RF, Conn JW, Floyd Jr JC, Fajans SS, Rull JA, Guntsche EM, et al. The normal endocrine response to ingestion of protein and infusions of amino acids. Sequential secretion of insulin and growth hormone. Trans Assoc Am Physicians 1966;79:312-21.
[26] Rabinowitz D, Merimee TJ, Maffezzoli R, Burgess JA. Patterns of hormonal release after glucose, protein, and glucose plus protein. Lancet 1966;2:454-6.
[27] Pallotta JA, Kennedy PJ. Response of plasma insulin and growth hormone to carbohydrate and protein feeding. Metabolism 1968;17:901-8.
[28] Baker HW, Best JB, Burger HG, Cameron DP. Plasma human growth hormone levels in response to meals: a reappraisal. Aust J Exp Biol Med Sci 1972;50:715-24.
[29] van Vught AJ, Nieuwenhuizen AG, Brummer RJ, Westerterp-Plantenga MS. Effects of oral ingestion of amino acids and proteins on the somatotropic axis. J Clin Endocrinol Metab 2008;93:584-90.

[30] van Vught AJ, Nieuwenhuizen AG, Brummer RJ, Westerterp-Plantenga MS. Somatotropic responses to soy protein alone and as part of a meal. Europ J Endocrinol 2008;159:15-8.
[31] van Vught AJ, Nieuwenhuizen AG, Veldhorst MAB, Brummer RJ, Westerterp-Plantenga MS. Growth hormone responses to ingestion of soy protein with or without fat and/or carbohydrate in humans. e-SPEN. Eur J Clin Nutr Metab 2009;4: e239-44.
[32] Yakar S, Wu Y, LeRoith D. Systemic versus local IGF-1 production in normal development and disease. Central and peripheral mechanisms in pituitary disease. J Clin Invest 2002;110:771-81.
[33] Giustina A, Veldhuis JD. Pathophysiology of the neuroregulation of growth hormone secretion in experimental animals and the human. Endocr Rev 1998;19:717-97.
[34] Hwa V, Oh Y, Rosenfeld RG. The insulin-like growth factor–binding protein (IGFBP) superfamily. Endocr Rev 1999;20: 761-87.
[35] Hugl SR, White MF, Rhodes CJ. Insulin-like growth factor I (IGF-I)–stimulated pancreatic beta-cell growth is glucose-dependent. Synergistic activation of insulin receptor substrate-mediated signal transduction pathways by glucose and IGF-I in INS-1 cells. J Biol Chem 1998;273:17771-9.
[36] Lingohr MK, Dickson LM, McCuaig JF, Hugl SR, Twardzik DR, Rhodes CJ. Activation of IRS-2-mediated signal transduction by IGF-1, but not TGF-alpha or EGF, augments pancreatic beta-cell proliferation. Diabetes 2002;51:966-76.
[37] van Haeften TW, Twickler TB. Insulin-like growth factors and pancreas beta cells. Eur J Clin Invest 2004;34:249-55.
[38] Zenobi PD, Graf S, Ursprung H, Froesch ER. Effects of insulin-like growth factor–I on glucose tolerance, insulin levels, and insulin secretion. J Clin Invest 1992;89:1908-13.
[39] Rosenzweig SA, Atreya HS. Defining the pathway to insulin-like growth factor system targeting in cancer. Biochem Pharmacol 2010;80:1115-24.
[40] Frystyk J, Freda P, Clemmons DR. The current status of IGF-I assays—a 2009 update. Growth Horm IGF Res 2010;20:8-18.
[41] Bidlingmaier M. Pitfalls of insulin-like growth factor I assays. Horm Res 2009;71(Suppl 1):30-3.
[42] Rajpathak SN, Gunter MJ, Wylie-Rosett J, Ho GY, Kaplan RC, Muzumdar R, et al. The role of insulin-like growth factor–I and its binding proteins in glucose homeostasis and type 2 diabetes. Diabetes Metab Res Rev 2009;25:3-12.
[43] Musey VC, Goldstein S, Farmer PK, Moore PB, Phillips LS. Differential regulation of IGF-1 and IGF-binding protein–1 by dietary composition in humans. Am J Med Sci 1993;305:131-8.
[44] Hedman CA, Frystyk J, Fridell K, Jonsson A, Flyvbjerg A, Lindstrom T, et al. The IGF-system is not affected by a twofold change in protein intake in patients with type 1 diabetes. Growth Horm IGF Res 2005;15:304-10.
[45] Giustina A, Mazziotti G, Canalis E. Growth hormone, insulin-like growth factors, and the skeleton. Endocr Rev 2008;29: 535-59.
[46] Chapman IM, Hartman ML, Pezzoli SS, Harrell Jr FE, Hintz RL, Alberti KG, et al. Effect of aging on the sensitivity of growth hormone secretion to insulin-like growth factor–I negative feedback. J Clin Endocrinol Metab 1997;82:2996-3004.
[47] Isley WL, Underwood LE, Clemmons DR. Dietary components that regulate serum somatomedin-C concentrations in humans. J Clin Invest 1983;71:175-82.
[48] Thissen JP, Underwood LE, Ketelslegers JM. Regulation of insulin-like growth factor–I in starvation and injury. Nutr Rev 1999;57:167-76.
[49] Norrelund H, Frystyk J, Jorgensen JO, Moller N, Christiansen JS, Orskov H, et al. The effect of growth hormone on the insulin-like growth factor system during fasting. J Clin Endocrinol Metab 2003;88:3292-8.
[50] Chen JW, Hojlund K, Beck-Nielsen H, Sandahl Christiansen J, Orskov H, Frystyk J. Free rather than total circulating insulin-like growth factor–I determines the feedback on growth hormone release in normal subjects. J Clin Endocrinol Metab 2005;90:366-71.
[51] Frystyk J, Skjaerbaek C, Vestbo E, Fisker S, Orskov H. Circulating levels of free insulin-like growth factors in obese subjects: the impact of type 2 diabetes. Diabetes Metab Res Rev 1999;15:314-22.
[52] Frystyk J. Aging somatotropic axis: mechanisms and implications of insulin-like growth factor-related binding protein adaptation. Endocrinol Metab Clin North Am 2005 34:865-76, viii.
[53] Jogie-Brahim S, Feldman D, Oh Y. Unraveling insulin-like growth factor binding protein-3 actions in human disease. Endocr Rev 2009;30:417-37.
[54] Jehle PM, Jehle DR, Mohan S, Bohm BO. Serum levels of insulin-like growth factor system components and relationship to bone metabolism in type 1 and type 2 diabetes mellitus patients. J Endocrinol 1998;159:297-306.
[55] Benbassat CA, Maki KC, Unterman TG. Circulating levels of insulin-like growth factor (IGF) binding protein–1 and –3 in aging men: relationships to insulin, glucose, IGF, and dehydroepiandrosterone sulfate levels and anthropometric measures. J Clin Endocrinol Metab 1997;82:1484-91.
[56] Mohamed-Ali V, Pinkney JH, Panahloo A, Cwyfan-Hughes S, Holly JM, Yudkin JS. Insulin-like growth factor binding protein–1 in NIDDM: relationship with the insulin resistance syndrome. Clin Endocrinol (Oxf) 1999;50:221-8.
[57] van Beek AP, Wolffenbuttel BH, Runge E, Trainer PJ, Jonsson PJ, Koltowska-Haggstrom M. The pituitary gland and age-dependent regulation of body composition. J Clin Endocrinol Metab 2010;95:3664-74.