RESEARCH REPORT
HUMAN NUTRITION

Title: Effect of a High Protein Diet on 24-hr Profile of Ghrelin, GH and IGF-1, #08-017

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Date Submitted : July 1, 2009

Industry Summary:

Background: It is well known that type 2 diabetes is a major health problem in the United States, and indeed in the world. With the aging population, it is likely to increase. Therefore, there are a significant number of people who are, or should be, concerned about the effect of diet on their blood glucose (sugar) concentration.

Our data indicate that a diet that is high in protein, including pork, and low in starch will improve the blood glucose concentration in people with type 2 diabetes. In addition, our preliminary data indicated that this diet also resulted in an increase in blood Insulin-like Growth Factor 1 (IGF) and growth hormone (GH). GH and IGF-1 are hormones which could ameliorate, or reduce the rate at which the muscle and bone loss associated with aging develops. Proteins are made up of 20 different amino acids. Certain of these amino acids help the body to make new muscle proteins. These amino acids are increased in the blood after consuming a high protein diet. An increase in these amino acids from the diet also could help to maintain muscle mass and function. With an aging population, both diabetic and non-diabetic, loss of bone and muscle mass, resulting in increased frailty, falls, hip fractures, etc. is a major problem for the individual. Thus, data obtained in these studies could have a major impact on the dietary recommendations for this population.

Explanation of objectives: Our objectives were to measure the hormones and metabolites mentioned above that are associated with maintaining muscle mass. These determinations were done following ingestion of a test diet for 5 weeks by subjects with untreated type 2 diabetes. The test diet contains approximately 1.5 to 2 times the amount of protein in the normal American diet. Pork is included in the menu on several days. The results were compared with the concentrations of those hormones and metabolites measured in the same subjects following ingestion of a control diet.

Narrative of how research was conducted:

Eight male subjects, 52 – 70 years old, with type 2 diabetes were studied in a "Special Diagnostic and Treatment Unit" (SDTU), (similar to a Clinical Research Center). None of the subjects was being treated with oral hypoglycemic agents or insulin at the time they were enrolled in the study. A 5-week randomized, crossover study design was used with a 5-week washout period between diets.

The control (15% protein) diet was designed according to the recommendations of the American Heart Association, and the United States Department of Agriculture. The diet consisted of 55% carbohydrate, with an emphasis on starch-containing foods, 15% protein, 30% fat (10% monounsaturated, 10% polyunsaturated, 10% saturated fatty acid content). A second diet was designed to consist of 30% carbohydrate, 30% protein, and 40% fat. The saturated fatty acid content of the test diet was ~10% of total food energy, thus the majority of the fat was mono- and polyunsaturated. We refer to this diet as a "Low Biologically Available Glucose" (LoBAG) diet. A subscript notation is included to indicate the amount of carbohydrate in the diet, thus this is a $LoBAG_{30}$ diet.

Subjects were randomized to begin the study with either the $LoBAG_{30}$ or the control diet by a flip of a coin. Subjects were admitted to the SDTU on the evening prior to the study. The following day, standardized meals containing 55% carbohydrate, 30% fat, and 15% protein were given for breakfast, lunch and dinner, at 0800, 1200, and 1800 h.

Blood was obtained fasting at 0730, 0745 and 0800, and then every 15 minutes for the first hour after meals, every 1/2 hour for the next two hours, and then hourly until the next meal. Blood was drawn at a total of 46 time points for determination of most hormones and metabolites. GH is secreted episodically, and mainly during the night. Samples for GH were obtained hourly until 11 P.M., and then at 20-minute intervals until 3 A.M. Samples for ghrelin were obtained at frequent time points after meals, and at 20-minute intervals during the night, for a total of 52 time points.

Following this 24-hour data accumulation period, the subjects were sent home with all the necessary food for the next 2-3 days as appropriate for the diet to which they were randomized.

Subjects returned to the SDTU every 2-3 days to pick up food and meet with the study dietitian. At that time they provided a urine specimen for analysis of creatinine and urea to determine dietary compliance. They also were weighed, and had blood pressure, total glycohemoglobin and blood glucose measured. If their body weight decreased, or increased on 2 successive occasions the total food energy of the meals was increased or decreased as appropriate to attempt to maintain weight stability throughout the study. In addition, subjects were interviewed regarding dietary compliance, questions or concerns about the study, etc. At the end of the 5-week period, the subjects again were admitted to the SDTU and blood was drawn as described above. At this time the control or LoBAG meals (breakfast, lunch, dinner, and a snack) were given, as appropriate.

Following a washout period, the same procedure outlined above was repeated with the other diet. Thus each subject served as his own control.

Discussion of research findings (explanation of results)

All assays have been completed on all 8 study subjects.

1. The fasting IGF-1 concentrations were identical before and after the control diet. After the test diet the concentration increased significantly ($P = 0.049$), and confirmed one of our original hypotheses.

The 24 hour integrated insulin-like growth factor-1 (IGF-1) area response was greatest following 5 weeks on the $LoBAG_{30}$ diet. However, the increase did not reach statistical significance by the most stringent analysis, but the difference between $LoBAG_{30}$ pre and post was significantly different. Thus, while not significant for all measurements, there is the suggestion that IGF-1 is increased by the $LoBAG_{30}$ diet. This should have positive effects on stimulating protein synthesis, and perhaps muscle accumulation.

2. The growth hormone concentration did increase during the night, as expected. However, the total GH area responses were not statistically significantly increased. We had expected a greater increase following ingestion of the $LoBAG_{30}$ diet.

3. The ghrelin concentration decreased after meals, as expected. There was little difference in the responses between the control diet and the $LoBAG_{30}$ diet. The role of ghrelin, if any, in the physiological regulation of GH secretion in vivo is unclear. It also has been implicated in the control of appetite. Our results did not provide further insight into the physiological regulation of GH secretion.

4. We have determined individual amino acid concentrations on samples from all 8 of the subjects before and after the control diet, and before and after the $LoBAG_{30}$ diet. Initially we concentrated on analyzing data from leucine and isoleucine because the branched chain amino acids, especially leucine, have been shown to stimulate protein synthesis through a signaling mechanism.

The amino acid concentrations were indeed elevated to a greater extent and for a longer period of time when subjects ingested the $LoBAG_{30}$ diet. There was a statistically significant increase in leucine, isoleucine, valine and lysine.

5. We have determined the body composition using 4 different methods: bioelectric impedance, tritiated water, DEXA and CT on all 8 subjects.

Changes in lean body mass were modest and not significantly increased following ingestion of the $LoBAG_{30}$ diet for 5 weeks. This was not totally unexpected given the relatively short time frame of the experiment. However, protein balance was positive in this study, as we had reported previously. We had hoped that a significant increase in lean body mass would have been determined. The trend is in the right direction, but the statistics are not definitive.

Also of importance, though not the focus of this study, the fasting glucose was significantly decreased, as was the net glucose area (reflecting the glucose concentration after meals) and the total glucose area response following 5 weeks on a $LoBAG_{30}$ diet. The fasting insulin concentrations were not different. The net insulin area was modestly, but not significantly decreased. There was no difference in total insulin area response. Following the $LoBAG_{30}$ diet, the glycated hemoglobin was significantly decreased. There was no change in the plasma lipid profile, nor in kidney function. These results confirm our previous studies regarding the beneficial effects of a $LoBAG_{30}$ diet for people with type 2 diabetes.

6. We have determined the 24-hour integrated Insulin Like Growth Factor Binding Protein 3 (IGFBP-3) concentration on samples from all 8 subjects obtained hourly before and after the control diet, and before and after the high protein-$LoBAG_{30}$ diet. IGFBP-3 is part of a ternary complex which includes an "acid-labile subunit" and represents the major form in which IGF-1 is present in the plasma. As stated above, IGFBP-3 is GH dependent. However, we did not observe an increase in growth hormone in the present study, and the IGFBP-3 responses were not greatly increased following the $LoBAG_{30}$ diet.

Explain what these findings mean to industry

The results of our study confirmed our previous data indicating that glucose control is improved in subjects with type 2 diabetes following 5 weeks of ingestion of a $LoBAG_{30}$ diet. There also is a positive protein balance, as we have reported previously. The present study indicates that the amino acids known to stimulate protein synthesis also were increased following 5 weeks on a $LoBAG_{30}$ diet. One of the hormones involved in maintaining lean muscle mass also was increased. Although not all hormones involved in maintaining muscle and bone mass were statistically significantly increased, nor was lean muscle mass increased significantly, the trend was in a positive direction. We think these are very positive indicators for the recommendation for increasing the protein content of the diet for people with type 2 diabetes. Given the positive nitrogen (presumably protein) balance, the recommendation is likely to be true for people without type 2 diabetes, as well, although that hypothesis was not tested directly in the present study.

Our results indicate that an increase in the protein content of the diet could be beneficial for the aging population of the United States. Since pork is a popular component of the dietary protein, increased consumption of pork should follow.

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Scientific Abstract:

Background: We previously reported that a weight-maintenance, non-ketogenic diet containing 30% carbohydrate (CHO), 30% protein, 40% fat, (30:30:40) ($LoBAG_{30}$), ingested for 5 weeks, resulted in an increase in the A.M. fasting insulin-like growth factor-1 (IGF-1) and a positive nitrogen balance, in addition to improving glucose control, in subjects with untreated type 2 diabetes.

Objective: The objective of the present study was to determine whether a $LoBAG_{30}$ diet ingested by an elderly population would ameliorate the sarcopenia of aging by resulting in: 1) an increase in the 24 hour integrated IGF-1: 2) an increase in the 24 hour integrated growth hormone: 3) an increased and prolonged elevation in essential amino acids, particularly the branched chain amino acids, and 4) and increase in lean body mass.

Design: Eight men, age 52-70, with untreated type 2 diabetes were studied using a randomized crossover diet design with a washout period in between. Blood was drawn and urine was collected over a 24 hour period before and after 5 weeks following ingestion a standard diet of 55% CHO, 15% Pro, 30% fat, and before and after 5 weeks of ingesting a $LoBAG_{30}$ diet.

Results: Fasting IGF-1 was significantly increased following 5 weeks on a $LoBAG_{30}$ diet, however, the 24 hour integrated area response was not. Growth hormone concentrations were not increased. The leucine, isoleucine, and valine concentrations were elevated to a greater extent and for a longer period of time when subjects ingested the $LoBAG_{30}$ diet. Lean body mass was little changed.

Conclusions: A $LoBAG_{30}$ diet may be beneficial in ameliorating the sarcopenia of aging based on a prolonged elevation in branched chain amino acids, and an increase in IGF-1. However, it is likely that longer-term studies are necessary to demonstrate a change in lean body mass, and thus prove this hypothesis. In the present study, there were no deleterious changes in lipid profile or kidney function after 5 weeks on a high protein diet.

Introduction: An overview of the researchable question and its importance to producers.

We have completed three 5 week randomized, cross-over designed studies in which subjects with untreated type 2 diabetes ingested a diet in which the protein content was increased from 15% to 30% of total food energy.

The major objective of those studies was to determine if an increase in protein content, and a decrease in the carbohydrate content of the diet, would decrease the 24-hr integrated glucose concentration and thus the % glycohemoglobin. This indeed was the case. The diets resulted in a highly significant decrease in glycated hemoglobin without an increase in insulin and without weight change.

At the end of the 5-week period we observed an increase in the overnight fasting mean IGF-1 (insulin-like-growth factor-1) concentration with the high protein diets. The mean growth hormone (GH) concentration was approximately double that when the same subjects ingested the 15% protein diet, although this did not reach statistical significance. The increase in dietary protein resulted in a modest increase, or no change in 24 hour integrated insulin concentration, depending on the carbohydrate content of the diet. Insulin is known to decrease protein catabolism, particularly in skeletal muscle. The total amino acids were elevated for a more prolonged period of time with all 30% protein diets.

In addition, the 30% protein diets, but not the 15% protein diet, resulted in a positive nitrogen balance without a significant difference in body weight. The latter is compatible with an increased lean body mass and loss of fat mass as reported when adults with growth hormone (GH) deficiency are treated with exogenous GH. Insulin-like growth factor-1 (IGF-1) and GH are anabolic hormones. IGF-1 is reported to be relatively stable. However, GH is secreted predominantly in large bursts during deep sleep. Insulin has an anticatabolic effect on protein metabolism in muscle. Essential amino acids, particularly the branched chain amino acids, not only are substrates for new protein synthesis, but also are signaling molecules for synthesis of protein in skeletal muscle.

IGF-1 and GH were measured because in single meal studies it has been reported that protein resulted in a late increase in growth hormone, but this has not been a consistent finding. Short-term starvation results in a decrease in IGF-1 but an increase in GH. This is corrected when the subjects are refed adequate calories, but complete correction requires not only adequate calories but addition of dietary protein as well, suggesting that dietary protein itself may have a regulatory role in GH and IGF-1 metabolism. To our knowledge however, data regarding an increased dietary protein content on the circulating concentration of IGF-1 and GH have not been reported.

Specific amino acids were not measured in our previous studies. Both an increase in insulin and a prolonged increase in essential amino acids during each 24-hour period may occur with a high protein diet. Thus, the anti-catabolic effect of insulin and direct anabolic effect of essential amino acids also could contribute to a positive protein balance, i.e. an increase in lean body mass in these subjects.

IGF-1 also has been reported to stimulate proliferation of beta cell mass in vitro and to inhibit apoptosis.

Aging results in a gradual decrease in GH secretion and circulating IGF-1 concentration. The IGF-1 concentration also has been reported to be further reduced in people with type 2 diabetes. Aging also results in a decrease in muscle mass and strength, bone mass and skin mass (or thickness).

The factors contributing to the loss of mass in these organs are not understood. However, a decrease in GH, IGF-1, decreased ingestion of protein, as well as developing insulin resistance likely are factors involved. Also, a decrease in IGF-1 could contribute to the decrease in insulin secretory capacity with both aging and particularly in people with type 2 diabetes.

In the present study we have determined whether a 30% protein diet results in an increase in 24 hr integrated GH, IGF-1, ghrelin and essential amino acids. GH stimulates synthesis of IGF-1 in the liver. GH is regulated by GHRH/somatomedin. Ghrelin binds to a receptor separate from that of GHRH, and at least in animals stimulates GH secretion. For this reason, we also measured ghrelin concentrations.

If a high protein diet can be shown to ameliorate the effects of aging, this will result in an increase in protein consumption by the aging American population. Pork is a popular protein component of the American diet, thus pork consumption likely will increase.

Objectives:

1. To determine the 24-hr integrated insulin-like growth factor-1 (IGF-1) concentration following 5 weeks on a 30% protein diet, compared to 5 weeks on a 15% protein diet.

2. To determine if the anticipated increased 24-hr integrated IGF-1 due to a 30% protein diet is mediated by an increase in 24-hr integrated growth hormone (GH) concentration.

3. To determine the 24-hr integrated ghrelin concentration and the dynamic changes with meals in a 15% and 30% protein diet.

4. To determine if indispensable amino acids are elevated to a greater extent and for a longer period of time during a 24-hr period when subjects ingest a 30% protein diet (particularly leucine).

5. To determine if there is a change in lean body mass (and possibly fat mass) in the absence of a change in total body weight when the subjects ingest a 30% protein diet.

6. To determine the 24-hr integrated IGFBP-3 concentration because its production and secretion are GH dependent.

Materials & Methods:

Subjects and Meals

Male subjects, 52-70 years old, with mild, untreated type 2 diabetes were studied in a "Special Diagnostic and Treatment Unit" (SDTU), (similar to a Clinical Research Center). All subjects met the National Diabetes Data Group criteria for the diagnosis of type 2 diabetes mellitus. The study was approved by the Department of Veterans Affairs Medical Center, and the University of Minnesota Committees on Human Subjects and written informed consent was obtained from all subjects. The subjects did not have hematologic abnormalities, kidney disease, liver disease, macroalbuminuria (>300mg/24 hours), congestive heart failure, or untreated thyroid disease. Prior to the study all subjects were interviewed to determine their physical activity profile, food aversions, and to explain the study process and commitment in detail. Subjects confirmed they had been weight stable for at least 3 months. They were instructed to maintain their current activity level throughout the study. Two weeks prior to beginning the study the subjects completed a 3-day food frequency questionnaire with one of the days being a Saturday or Sunday. This information was used to calculate the total food energy necessary to maintain body weight. None of the subjects was being treated with oral hypoglycemic agents or insulin at the time they were enrolled in the study. A 5-week randomized, crossover study design was used with a washout period between diets.

The control (15% protein) diet was designed according to the recommendations of the American Heart Association, and the United States Department of Agriculture. The diet consisted of 55% carbohydrate, with an emphasis on starch-containing foods, 15% protein, 30% fat (10% monounsaturated, 10% polyunsaturated, 10% saturated fatty acid content). A second diet was designed to consist of 30% carbohydrate, 30% protein, and 40% fat. The saturated fatty acid content of the test diet was ~10% of total food energy, thus the majority of the fat was mono- and polyunsaturated. We refer to this diet as a "Low Biologically Available Glucose" (LoBAG) diet. A subscript notation is included to indicate the amount of carbohydrate, thus this is a $LoBAG_{30}$ diet.

Subjects were randomized to begin the study with either the $LoBAG_{30}$ or the control diet by a flip of a coin. Subjects were admitted to the SDTU on the evening prior to the study. The following day, standardized meals containing 55% carbohydrate, 30% fat, and 15% protein were given for breakfast, lunch and dinner, at 0800, 1200, and 1800 h. Subjects were asked to remain in the SDTU during the study period with minimal activity.

Blood was obtained fasting at 0730, 0745 and 0800, every 15 minutes for the first hour after meals, every 1/2 hour for the next two hours, and then hourly until the next meal. Blood was drawn at a total of 46 time points. GH is secreted episodically, and mainly during the night. Samples for GH were obtained hourly until 11 P.M., and then at 20-minute intervals until 3 A.M. Samples for ghrelin were obtained at frequent time points after meals, and at 20-minute intervals during the night, for a total of 52 time points. Following this 24-hour data accumulation period, the subjects were sent home with all the necessary food for the next 2-3 days as appropriate for the diet to which they were randomized.

Subjects returned to the SDTU every 2-3 days to pick up food and meet with the study dietitian. At that time they provided a urine specimen for analysis of creatinine and urea to determine dietary compliance. They also were weighed, and had blood pressure, total glycohemoglobin and blood glucose measured. If their body weight decreased, or increased on 2 successive occasions the total food energy of the meals was increased or decreased as appropriate to attempt to maintain weight stability throughout the study. In addition, subjects were interviewed regarding dietary compliance, questions or concerns about the study, etc. At the end of the 5-week period, the subjects again were admitted to the SDTU and blood was drawn as described above. At this time the control or LoBAG meals (breakfast, lunch, dinner, and a snack) were given, as appropriate.

Following a washout period, the same procedure outlined above was repeated with the other diet. Thus each subject served as his own control.

Assay Methods

Serum glucose, urea nitrogen, and creatinine concentrations were determined using an Abbott Architect analyzer (Abbott Laboratories, Abbott Park, Illinois). Serum immunoreactive insulin was measured using an automated chemiluminescent assay on DPC's IMMULITE machine (Diagnostic Products Corp., Los Angeles, California). Glucagon and ghrelin were measured by radioimmunoassay (RIA) using kits from Linco Research (a subsidiary of Millipore, Inc, Billerica, MA). Growth hormone was measured using the Immulite 2000, in the laboratory of Dr. Ali Iranmanesh (Salem, VA). IGF-1 was measured using an Enzyme-Labeled Chemiluminescent Immunometric Assay (ELISA) kit from Quest (Quest, New Brighton, MN). IGF-1 also was measured using ELISA kits from ImmunoDiagnostic Systems (IDS, Fountain Hills, AZ) and Diagnostic Laboratory Systems, Inc (DSL, Webster, TX). IGFBP-1 and IGFPB-3 were measured using ELISA kits from DSL. Fecal nitrogen was measured by the Dumas combustion method at the Mayo Laboratories (Rochester, MN). Alpha amino nitrogen was measured with an o-phthaldialdehyde dye-binding method (Gusmer Enterprises, Inc, Waupaca, WI). Individual amino acids were measured by high performance liquid

chromatography (HPLC) using precolumn online derivatization with o-phthalaldehyde and 3-mercaptopropionic acid and 9-fluorenylmethylchloroformate and ultraviolet detection.

Weight was determined in street clothes without shoes on a digital scale (Scalitronix, White Plains, NY). Blood pressure was measured using a Dinemap instrument (Critikon/Mediq, Pennsauken, NJ). Lean body mass was determined 1) using a portable body impedance analyzer (RJL Systems, Clinton Township, MI), 2) by tritiated water, 3) dual energy x-ray absorptiometry (DEXA) and 4) computed tomography (CT).

The total amount of protein deaminated was determined by quantifying the urine urea nitrogen and fecal nitrogen excreted over the 24 hours of the study, adjusted for a change in the amount of urea nitrogen retained endogenously. The latter was calculated by determining the change in plasma urea nitrogen concentration between the fasting baseline and at the end of the 24-hour study period and by correcting for plasma water by dividing by 0.94. In this calculation, it is assumed that there is a relatively rapid and complete equilibration of urea in total body water. Total body water as a percentage of body weight was calculated using the equation of Watson et al. The overall assumption is that a change in plasma urea concentration is indicative of a corresponding change in total body water urea concentration. In this study the beginning and ending urea nitrogen concentrations were essentially identical indicating no retention of urea.

The net 24-hour incremental area responses were calculated using the overnight fasting value as baseline. Total 24-hour area responses were calculated using zero as the baseline. Both area calculations were done using a computer program based on the trapezoid rule. Statistics were determined using ANOVA or Student's t test for paired variates, as appropriate, with the Statview 512+ program (Brain Power, Calabasas, California) for the Macintosh computer (Apple Computer, Cupertino, California). A P value of <0.05 is the criterion for significance. Data are presented as the mean ± SEM.

Results:

1. The 24 hour integrated insulin-like growth factor-1 (IGF-1) area response was 6.16 and 5.86 before and after 5 weeks on the control diet. It was 5.29 and 6.48 before and after 5 weeks on the test diet on samples from the 8 subjects. Although the area response was greatest following 5 weeks on the $LoBAG_{30}$ diet, the increase did not reach statistical significance by ANOVA ($P = 0.91$) but the difference between $LoBAG_{30}$ pre and post was significantly different ($P = 0.05$). IGF-1 was being measured because it is an anabolic hormone. An increase in IGF-1 results in an increase in lean body mass and a decrease in fat mass. In addition, it has been reported to stimulate beta cell proliferation and inhibit apoptosis in vitro. Also, IGF-1 has insulin-like effects and improves insulin sensitivity.

The fasting IGF-1 concentrations were identical before and after the control diet (149 ± 26 ng/ml). After the test diet the concentration increased from 144 ± 25 to 159 ± 28 ng/ml. The increase was statistically significant ($P = 0.049$), and confirmed one of our original hypotheses.

2. We have determined the 24 hour integrated growth hormone (GH) area responses on samples from all 8 subjects. GH is secreted episodically, and mainly during the night. Samples were obtained hourly until 11 P.M., and then at 20-minute intervals until 3 A.M. before and after the control diet, and before and after the high protein-$LoBAG_{30}$ diet. The growth hormone concentration did increase during the night, as expected. The total GH area responses were 9.2 ± 2.1 and 11.4 ± 2.7 ng hr/ml before and after the control diet and 9.8 ± 1.6 and 12.0 ± 3.5 ng hr/ml before and after the test diet. These differences were not statistically significant ($p = 0.74$ pre vs. post $LoBAG_{30}$).

GH was measured because it also is an anabolic hormone. An increase in GH results in an increase in nitrogen balance, and increase in lean body mass, an increase in fat oxidation and a decrease in fat mass. IGF-1 and IGFBP-3 (see below) are both GH dependent.

3. We have determined the 24 hour integrated ghrelin concentration on samples from all 8 subjects. Samples were obtained at frequent time points after meals, and at 20-minute intervals during the night, for a total of 52 time points before and after the control diet, and before and after the high protein-$LoBAG_{30}$ diet. The ghrelin concentration decreased after meals. The total area response was 21.9 ± 3.4 and 19.5 ± 3.5 pg.hr/ml before and after the control diet. It was 20.4 ± 3.4 and 19.6 ± 2.4 pg.hr/ml before and after the $LoBAG_{30}$ diet. These differences were not statistically significantly different from one another. Ghrelin is a hormone produced predominantly by the neuroendocrine X/A-like cells in the oxyntic mucosa of the stomach. It has been shown to bind to a receptor called the GH secretagogue receptor, and to strongly stimulate secretion of GH. The role of ghrelin, if any, in the physiological regulation GH secretion in vivo is unclear.

4. We have determined individual amino acid concentrations on samples from all 8 of the subjects before and after the control diet, and before and after the $LoBAG_{30}$ diet. Initially we concentrated on analyzing data from leucine and isoleucine because the branched chain amino acids, especially leucine, have been shown to stimulate protein synthesis through a signaling mechanism. The total integrated area response data are presented below.

Area response data in μmol.hr/L

Amino Acid	Control Pre	Control Post	LoBAG30 Pre	LoBAG30 Post
Leucine	2341 ± 315	2278 ± 287	2462 ± 344	3217 ± 262**
Isoleucine	1569 ± 73	1581 ± 71	1673 ± 82	2104 ± 88**
Valine	4454 ± 235	4233 ± 128	4379 ± 228	6000 ± 336**
Glycine	3311 ± 175	3365 ± 179	3216 ± 141	3413 ± 170
Lysine	3364 ± 203	3157 ± 142	3386 ± 100	3633 ± 193*
Phenylalanine	1268 ± 29	1264 ± 37	1413 ± 55	1505 ± 52
Proline	3343 ± 268	3901 ± 514	3344 ± 19	3358 ± 228
Serine	1938 ± 133	1836 ± 68	1979 ± 117	2175 ± 80*
Alanine	7367 ± 443	7181 ± 274	7558 ± 366	7177 ± 248

**P≤ 0.05 by ANOVA
*P ≤ 0.05 compared to Control Post by Student's t test

The amino acid concentrations were indeed elevated to a greater extent and for a longer period of time when subjects ingested the $LoBAG_{30}$ diet.

5. We have determined the body composition using 4 different methods: bioelectric impedance, tritiated water, DEXA and CT on all 8 subjects.

The data are presented below

Kg Lean Body Mass

Test	Control Pre	Control Post	LoBAG30 Pre	LoBAG30 Post
B Impedance	74 ± 1.7	72 ±2.1	73 ± 1.8	74 ± 2.0
Tritiated Water	63 ± 2.0	63 ± 2.8	66 ± 2.8	68 ± 2.8
DEXA	63 ± 1.9	62 ± 1.6	64 ± 1.7	64 ± 1.8
CT -	Pending*			

*Our colleague at the Mayo Clinic, Dr. Michael Jensen, has the computer program to analyze the CT scans. We had a miscommunication regarding data identification, and are in the process of solving the problem. Thus, the CT body composition data are not available today.

% Lean Body Mass

Test	Control Pre	Control Post	LoBAG30 Pre	LoBAG30 Post
B Impedance	76 ± 1.0	75 ± 1.0	76 ±1.2	77 ± 0.8
Tritiated Water	64 ± 1.7	66 ± 2.7	69 ± 1.8	71 ± 2.7
DEXA	65 ± 1.6	65 ± 1.3	66 ± 1.3	67 ± 1.3
CT	Pending*			

Changes in lean body mass were modest and not significantly increased following ingestion of the $LoBAG_{30}$ diet for 5 weeks. This was not totally unexpected given the relatively short time frame of the experiment. However, protein balance was positive in this study, as we had reported previously. We had hoped that a significant increase in lean body mass would have been determined. The trend is in the right direction, but the statistics are not definitive.

Also of importance, though not the focus of this study, the fasting glucose was significantly decreased, as was the net glucose area (reflecting the postprandial glucose concentration) and the total glucose area response following 5 weeks on a $LoBAG_{30}$ diet. The fasting insulin concentrations were not different. The net insulin area was modestly, but not significantly decreased. There was no difference in total insulin area response. Following the $LoBAG_{30}$ diet, the glycated hemoglobin was significantly decreased. There was no change in the plasma lipid profile, nor in kidney function. These results confirm our previous studies regarding the beneficial effects of a $LoBAG_{30}$ diet for people with type 2 diabetes.

6. We have determined the 24-hour integrated Insulin Like Growth Factor Binding Protein 3 (IGFBP-3) concentration on samples from all 8 subjects obtained hourly before and after the control diet, and before and after the high protein-$LoBAG_{30}$ diet. The total area responses were 651 ± 811 and 618 ± 81 ng.hr/ml before and after the control diet; 685 ± 83 and 730 ± 78 ng.hr/ml before and after the $LoBAG_{30}$ diet. The increase following 5 weeks on the $LoBAG_{30}$ diet did not reach statistical significance (P = 0.14). IGFBP-3 is part of a ternary complex which includes an "acid-labile subunit" and represents the major form in which IGF-1 is present in the plasma. As stated above, IGFBP-3 is GH dependent. However, we did not observe an increase in growth hormone in the present study.

Discussion:

The purpose of this study was to obtain evidence to support the hypothesis that an increase in the protein content of the diet for aging subjects with type 2 diabetes would result in an improvement in the sarcopenia that occurs with aging. The protein content of the test diet was 30% of total food energy. This is 1.5 – 2 times that of the normal American diet. We have previously demonstrated that a diet containing 30% protein, and either 20%,

30%, or 40% carbohydrate would result in an improvement in blood glucose control without deleterious effects on plasma lipids or kidney function in people with untreated type 2 diabetes.

The hormones and metabolites measured to provide evidence, and perhaps a mechanism for our hypothesis were IGF-1, GH, ghrelin, and individual amino acids, particularly branched chain amino acids.

IGF-1 circulates in the plasma as a ternary complex (IGF-1, IGF-BP-3, acid labile subunit). This complex has been reported to have metabolic effects not found for IGF-1 independently. IGF-1 was measured because it is likely to have metabolic effects that are independent of both GH and the ternary IGF complex. It also is an important anabolic hormone with direct effects on bone and other organs. IGFBP-3 was measured because its production also is GH dependent. GH was measured because it is an important anabolic hormone. Its secretion is stimulated by ghrelin as well as by GH releasing hormone (GHRH). It directly stimulates IGF-1 and IGFBP-3 production in the liver. Ghrelin was measured because it stimulates GH release. Individual amino acids were measured because they are substrate for protein synthesis. In addition, some, particularly leucine, are also signaling molecules for protein synthesis.

It is generally accepted that the IGF-1 concentration is quite stable. However, we were interested in documenting whether the 24 hr integrated IGF-1 concentration would increase significantly. This did not occur. However, we did obtain information on the dynamics of the IGF-1 concentration throughout a 24-hour period.

The circulating IGF-1 is largely synthesized in the liver and the synthesis rate is regulated by GH. However, short-term starvation or a low food energy diet in association with a decreased protein intake results in a decreased circulating IGF-1 concentration but an increase in GH and an apparent GH-resistant state. Therefore, it was important to determine if the overnight fasted elevated IGF-1 concentration resulting from an increased dietary protein intake was mediated by an increased GH secretion or is GH independent. In a nutritionally adequate state, the great majority of GH enters the circulation as secretory bursts during deep sleep, which we were able to demonstrate. During the day, the concentration GH is reported to be very low or unmeasurable. However, we did measure modest increases as secretory bursts throughout the day. Our data indicate that the overnight fasted elevated IGF-1 concentration was GH independent.

Our original hypothesis was that the increase in GH was not secondary to increased ghrelin. However, we did not observe an increase in GH.

The major focus on ghrelin has been its possible role as an initiator and/or terminator of feeding. It is high in a fasted state, but ingestion of mixed meals results in a rapid decrease in concentration. Thus, it is likely to play a role in regulating food intake. We did observe the decrease in ghrelin following meals. However, there was no difference in response whether the meals were high protein or not. This is intriguing because high protein diets have been reported to increase satiety and are popular for weight reduction. Our data would suggest that the satiating effect of high protein diets is not mediated by ghrelin.

Essential amino acids, especially the branched chain amino acids and even more specifically leucine, directly stimulate protein synthesis in muscle. We now have demonstrated that the branched chain amino concentrations are higher and remain elevated longer when the subjects ingest a 30% protein diet.

The results of this study confirmed our previous data indicating that glucose control is improved in subjects with type 2 diabetes following 5 weeks of ingestion of a $LoBAG_{30}$ diet.

There also is a positive protein balance, as we have reported previously. The present study indicates that branched chain amino acids, particularly leucine which is known to stimulate protein synthesis, were increased following 5 weeks on a $LoBAG_{30}$ diet. IGF-1, which is involved in maintaining lean muscle mass also was increased. Although not all hormones involved in maintaining muscle and bone mass were statistically significantly increased, nor was lean muscle mass increased significantly, the trend was in a positive direction. These are very positive indicators for the recommendation for increasing the protein content of the diet for people with type 2 diabetes. Given the positive nitrogen (presumably protein) balance, the recommendation is likely to be true for people without type 2 diabetes, as well, although that hypothesis was not tested directly in the present study.

Our results indicate that an increase in the protein content of the diet could be beneficial for the aging population of the United States. Since pork is a popular component of the dietary protein, increased consumption of pork should follow.