Effect of a High-Protein, Low-Carbohydrate Diet on Blood Glucose Control in People With Type 2 Diabetes

Mary C. Gannon1,2,3 and Frank Q. Nuttall1,3

There has been interest in the effect of various types and amounts of dietary carbohydrates and proteins on blood glucose. On the basis of our previous data, we designed a high-protein/low-carbohydrate, weight-maintaining, nonketogenic diet. Its effect on glucose control in people with untreated type 2 diabetes was determined. We refer to this as a low-biologically-available-glucose (LoBAG) diet. Eight men were studied using a randomized 5-week crossover design with a 5-week washout period. The carbohydrate:protein:fat ratio of the control diet was 55:15:30. The test diet ratio was 20:30:50. Plasma and urinary β-hydroxybutyrate were similar on both diets. The mean 24-h integrated serum glucose at the end of the control and LoBAG diets was 198 and 126 mg/dl, respectively. The percentage of glycohemoglobin was 9.8 ± 0.5 and 7.6 ± 0.3, respectively. It was still decreasing at the end of the LoBAG diet. Thus, the final calculated glycohemoglobin was estimated to be ~6.3–5.4%. Serum insulin was decreased, and plasma glucagon was increased. Serum cholesterol was unchanged. Thus, a LoBAG diet ingested for 5 weeks dramatically reduced the circulating glucose concentration in people with untreated type 2 diabetes. Potentially, this could be a patient-empowering way to ameliorate hyperglycemia without pharmacological intervention. The long-term effects of such a diet remain to be determined. Diabetes 53:2375–2382, 2004

RESEARCH DESIGN AND METHODS

Men with mild, untreated type 2 diabetes were studied in a special diagnostic and treatment unit (SDTU; similar to a clinical research center). All participants met the National Diabetes Data Group criteria for the diagnosis of type 2 diabetes (8). Participant characteristics are given in Table 1. 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 participants. The participants did not have hematologic abnormalities, kidney disease, liver disease, macroalbuminuria (>300 mg/24 h), congestive heart failure, or untreated thyroid disease. Before the study, all participants were interviewed to determine their physical activity profile and food aversions and to explain the study process and commitment in detail. Participants confirmed that they had been weight stable for at least 3 months. They were instructed to maintain their current activity level throughout the study. Two weeks before beginning the study, the participants completed a 3-day food frequency 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. None of the participants were being treated with oral hypoglycemic agents or insulin at the time of enrollment 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 (9) and the U.S. Department of Agriculture (10,11). The diet consisted of 55% carbohydrate, with an emphasis on starch-containing foods, 15% protein, and 30% fat (10% monounsaturated, 10% polyunsaturated, and 10% saturated fatty acid). A second diet was designed to consist of 20% carbohydrate, 30% protein, and 50% fat. The saturated fatty acid content of the test diet was ~10% of total food energy; thus, the majority of the fat was monounsaturated and polyunsaturated. This diet is referred to in the text as the LoBAG diet. The composition of the diets is given in Table 2.

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LoBAG, low biologically available glucose; NEFA, nonesterified fatty acid; SDTU, special diagnostic and treatment unit.

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Participants were randomized to begin the study with either the LoBAG or the control diet by a flip of a coin. Six participants started on the LoBAG diet, and five participants started on the control diet. Unfortunately, three of the participants who started on the control diet did not complete the study for personal reasons (death of spouse, move across country, chose not to finish). Therefore, the data are presented on eight participants who completed both arms of the study. Participants were admitted to the SDTU on the evening before the study. The next day, standardized meals that contained 55% carbohydrate, 30% fat, and 15% protein were given for breakfast, lunch, and dinner at 0800, 1200, and 1800. Participants were asked to remain in the SDTU during the study period with minimal activity.

On the second day in the SDTU, standardized meals again were given. This diet was similar for both baseline studies and is referred to as “control/pre” and “LoBAG/pre” diet in the figures, depending on which study diet followed the inpatient stay. In addition to the meals at 0800, 1200 and 1800, snacks were given at 1600 and 2100. Blood was obtained fasting at 0730, 0745, and 0800, every 15 min for the first hour after meals, every 30 min for the next 2 h, and then hourly until the next meal. Blood was drawn at a total of 46 time points. After this 24-h data accumulation period, the participants were sent home with all of the necessary food for the next 2–3 days as appropriate for the diet to which they were randomized.

Participants 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 (tGHb), and blood glucose measured. If their body weight decreased or increased on two successive occasions, then the total food energy of the meals was increased or decreased as appropriate to attempt to maintain weight stability throughout the study. In addition, participants were interviewed regarding dietary compliance, questions or concerns about the study, etc. At the end of the 5-week period, the participants 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 snacks) were given, as appropriate.

The plasma glucose concentration and β-hydroxybutyrate concentration

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Patient characteristics</th>
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</thead>
<tbody>
<tr>
<td>Patient</td>
<td>Age (years)</td>
</tr>
<tr>
<td>1</td>
<td>69</td>
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<tr>
<td>2</td>
<td>72</td>
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<tr>
<td>8</td>
<td>59</td>
</tr>
<tr>
<td>Mean</td>
<td>63.3</td>
</tr>
<tr>
<td>Range</td>
<td>51–82</td>
</tr>
</tbody>
</table>

ASA, acetylsalicylic acid.

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The plasma glucose concentration and β-hydroxybutyrate concentration

<table>
<thead>
<tr>
<th>TABLE 2</th>
<th>Composition of diets</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>LoBAG</td>
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<tr>
<td>Energy (kcal)</td>
<td>2,825</td>
</tr>
<tr>
<td>Protein (g)</td>
<td>106 (15%)</td>
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<tr>
<td>Carbohydrate (g)</td>
<td>388 (55%)</td>
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<td>Monosaccharides (g)</td>
<td>64</td>
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<tr>
<td>Disaccharides</td>
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<tr>
<td>Fat (g)</td>
<td>94 (30%)</td>
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<tr>
<td>Monounsaturated (g)</td>
<td>29</td>
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<tr>
<td>Polyunsaturated (g)</td>
<td>24</td>
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<tr>
<td>Saturated (g)</td>
<td>33</td>
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<tr>
<td>Cholesterol (mg)</td>
<td>375</td>
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<tr>
<td>Dietary fiber (g)</td>
<td>24</td>
</tr>
</tbody>
</table>

FIG. 1: A: Mean body weight while on the control (○) or LoBAG (●) diet. B: Plasma β-hydroxybutyrate concentration after 5 weeks on the control (○) or LoBAG (●) diet.
were determined by enzymic methods using an Analox analyzer with an O₂ electrode (Analox Instruments, London, U.K.). %tGHb was measured by boronate-affinity high-performance liquid chromatography (BioRad Variant; BioRad Labs, Hercules, CA). Serum immunoreactive insulin was measured using a standard double-antibody radioimmunoassay method using kits produced by Incstar (Stillwater, MN). Glucagon and C-peptide were measured by radioimmunoassay using kits from Linco Research (St. Louis, MO) and Diasorin (Stillwater, MN), respectively. NEFAs were measured enzymically using a kit manufactured by Wako Chemicals (Richmond, VA). 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).

The net 24-h incremental area responses were calculated using the overnight fasting value as baseline. Total 24-h 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 Student’s t test for paired variates, with the Statview 512+ program (Brain Power, Calabasas, CA) for the Macintosh computer (Apple Computer, Cupertino, CA). P < 0.05 is the criterion for significance. Data are presented as the mean ± SE.

RESULTS

The average body weight was 219 ± 10 lb (99 ± 4.5 kg) and 216 ± 10 lb (98 ± 4.5 kg) at the beginning of the control and LoBAG diets, respectively (Fig. 1A). At the end of the 5 weeks on the control diet, the average body weight was 215 ± 10 lb (98 ± 4.5 kg). After 5 weeks on the LoBAG diet, the average weight was 212 ± 9 lb (96 ± 4.1 kg). Thus, the average body weight decreased by 4 lb (1.8 kg) during the 5-week study period, regardless of diet.

Urine ketones were monitored twice weekly while participants were on the LoBAG diet. They were always zero to trace using nitroprusside impregnated Ketostix (Bayer, Elkhart, IN). Twenty-four-hour urine ketones were identical at the beginning and the end of the LoBAG diet (196 ± 8 and 196 ± 9 μmol/l, respectively). Before and after the

FIG. 2. A: Mean plasma glucose concentration before (Δ) and after (○) 5 weeks on the control diet. Side graph: Net and total 24-h integrated glucose area response. Area response was not significantly different. B: Mean plasma glucose concentration before (▲) and after (●) 5 weeks on the LoBAG diet. Side graph: Net and total 24-h integrated glucose area response. *Both the net and the total area responses were significantly lower after the LoBAG diet (P < 0.05).
They were 187 ± 7 and 203 ± 10 μmol/l, respectively.

The mean fasting β-hydroxybutyrate concentration was 225 ± 15 μmol/l after 5 weeks on the control diet (Fig. 1B). After 5 weeks on the LoBAG diet, the mean fasting concentration was 236 ± 27 μmol/l. The 24-h profiles were similar when the participants ingested either diet.

The mean fasting glucose concentration before starting the control diet was 180 ± 10 mg/dl (10 ± 0.6 mmol/l; Fig. 2A). After 5 weeks on the control diet, the fasting glucose concentration was decreased to 159 ± 11 mg/dl (8.8 ± 0.6 mmol/l), but this was not significant (P = 0.66). Before starting the LoBAG diet, the mean fasting glucose concentration was 167 ± 13 mg/dl (9.3 ± 0.7 mmol/l), similar to that before starting the control diet (P = 0.24). After 5 weeks on the LoBAG diet, the fasting glucose concentration was significantly decreased to 119 ± 7 mg/dl (66 ± 0.4 mmol/l; P < 0.003; Fig. 2B).

The mean 24-h integrated net glucose area responses were similar precontrol, pre-LoBAG, and postcontrol (681 ± 174, 731 ± 159, and 730 ± 236 mg · h · dl⁻¹ [38 ± 9.7, 41 ± 8.8, and 41 ± 13.1 mmol · h · 1⁻¹], respectively; Fig. 2 side graphs, left bars). After 5 weeks on the LoBAG diet, the net mean 24-h integrated glucose area response was decreased by 77% (165 ± 59 mg · h · dl⁻¹) (9.2 ± 3.3 mmol · h · 1⁻¹; P < 0.02).

Total 24-h integrated glucose area responses also were similar precontrol, pre-LoBAG, and postcontrol (4,998 ± 337, 4,746 ± 301, and 4,554 ± 347 mg · h · dl⁻¹ [278 ± 18.7, 264 ± 16.7, and 253 ± 19.3 mmol · h · 1⁻¹], respectively; Fig. 2 side graphs, right bars). The total area response after 5 weeks on the LoBAG diet was decreased significantly.
(3,023 ± 160 mg · h · dl⁻¹ [168 ± 8.9 mmol · h · l⁻¹]; P < 0.0004 vs. the 5-week postcontrol and P < 0.0001 vs. pre-LoBAG). On the basis of these integrated areas, the mean glucose concentration over the 24-h periods of study was reduced from 198 to 126 mg/dl (11–7 mmol/l) after 5 weeks on the LoBAG diet, a 36% decrease (P < 0.0001). The mean fasting insulin concentrations before and after 5 weeks on both the control and the LoBAG diets were identical (12 ± 2 μU/ml [72 ± 12 pmol/l]; Fig. 3). The mean 24-h integrated insulin area response above the fasting value was similar after the pre- and postcontrol diet and pre-LoBAG diet (534 ± 73 μU · h · ml⁻¹ · ml⁻¹ [554 ± 84 μU · h · ml⁻¹ · ml⁻¹]; and 530 ± 81 μU · h · ml⁻¹ · ml⁻¹ [3,024 ± 438, 3,324 ± 504, and 3,180 ± 486 pmol · h · l⁻¹], respectively; Fig. 3 side graphs, left bars). It was decreased at 5 weeks on the LoBAG diet (318 ± 39 μU · h · ml⁻¹ · ml⁻¹ [1908 ± 702 pmol · h · l⁻¹]). This was a decrease of 40% from the pre-LoBAG value (P < 0.01). The mean 24-h total integrated insulin area response decreased by 25%.

The mean fasting C-peptide concentration before and after the control diet was 0.86 ± 0.08 and 0.91 ± 0.08 pg/ml. It was 0.81 ± 0.09 and 0.92 ± 0.08 before and after the LoBAG diet (data not shown). The 24-h time course response was similar to the insulin response. The net C-peptide area response was decreased by 34% after 5 weeks on the LoBAG diet. This was statistically significant (P < 0.05).

The mean %tGHB was essentially unchanged during the 5 weeks on the control diet (Fig. 4). A decrease in tGHB was present 1 week after the institution of the LoBAG diet and became significant by 3 weeks on the diet. At the end of the 5-week period, the %tGHB had decreased 22%, from 9.8 ± 0.5 to 7.6 ± 0.3% (P < 0.0007).

The mean fasting glucagon concentrations were similar before and after both the control and the LoBAG diets (95 ± 11, 91 ± 8, 91 ± 7, and 94 ± 7 pg/ml, respectively; Fig. 5). After 5 weeks on the LoBAG diet, the glucagon response was similar to the control for the first hour after breakfast. Subsequently, the glucagon concentration was higher at every time point until 0700 the next morning, except for one time point after dinner. Both the net and the total glucagon area responses were significantly increased after the LoBAG diet (P < 0.05).

The mean fasting NEFA concentrations were 765 ± 67, 654 ± 59, 718 ± 70, and 593 ± 50 μEq/l before and after the control and LoBAG diets, respectively (data not shown). These differences were not statistically significant (P > 0.05). The 24-h excursions were similar on the control and LoBAG prediet days. When the LoBAG diet was ingested, the fasting NEFA was lower and the increase after the lunch meal was attenuated, as was the decrease before dinner. The rise after dinner was more rapid and reached a higher concentration.

The mean 24-h integrated net NEFA area responses were −5,323 ± 1,187, −2,468 ± 693, −4,525 ± 1,660, and 80 ± 1,809 μEq · h · l⁻¹ before and after the control and LoBAG diets, respectively. The small positive area response after the LoBAG diet was statistically significantly different compared with the response before the LoBAG diet (P < 0.05). Total areas were not statistically different from one another.

The mean fasting triacylglycerol concentrations were 264 ± 36, 226 ± 32, 246 ± 27, and 149 ± 23 mg/dl before and after the control and LoBAG diets, respectively (Fig. 6). The fasting triacylglycerol concentration was significantly lower after 5 weeks on the LoBAG diet (P < 0.05). After ingestion of either diet, the triacylglycerol concentration increased until ~1200–1400, decreased at 2000–2200, increased slightly at ~2400, and subsequently returned to the fasting value by 0800 the next morning.

The mean 24-h integrated net triacylglycerol area response was not significantly different between diets. However, the mean 24-h integrated total area response was significantly lower after 5 weeks on the LoBAG diet (P < 0.05).

The total cholesterol concentrations were 195 ± 7, 184 ± 17, 188 ± 10, and 177 ± 8 mg/dl before and after the control and the LoBAG diets, respectively. The LDL cholesterol concentrations were 105 ± 9, 102 ± 2, 105 ± 7, and 110 ± 6 mg/dl before and after the control and the LoBAG diets, respectively. The HDL cholesterol concentrations were 38 ± 1, 37 ± 2, 37 ± 2, and 36 ± 2 before and after the control and the LoBAG diets, respectively. These total, LDL, and HDL concentrations were not significantly different between diets or before and after each diet.

**DISCUSSION**

We previously reported that a diet in which the protein content was increased from 15 to 30% of total food energy, with a corresponding decrease in carbohydrate content, resulted in a moderate but highly statistically significant mean decrease in glycohemoglobin (8.1–7.3%) after 5 weeks on the diet. This was the consequence of smaller postmeal glucose increases. The fasting glucose concentration was unchanged (12).

In the present study, the diet contained the same 30% of food energy as protein. However, the carbohydrate content was further reduced from 40 to 20% of total food energy. The control diet in both studies is a diet that is
recommended for the general population as a means of reducing one’s risk for coronary heart disease (9).

In the present study, the lower carbohydrate diet not only reduced the postmeal glucose concentration but also considerably reduced the overnight fasting glucose concentration. It is interesting that the 29% decrease observed in the present study is similar to the 34% decrease that we observed previously after a 36-h fast in people with type 2 diabetes (5). The overall result was a striking decrease in the 24-h integrated glucose concentration (Fig. 2). In addition, the percentage of glycohemoglobin concentration at the end of the 5-week study period was decreased from a mean of 9.8 to 7.6 (Fig. 4).

The study was designed to be 5 weeks in duration because 33 days had been reported to be the half-time for glycohemoglobin to reach a new steady state (13). If this is the case, then the anticipated final percentage of glycohemoglobin would be ~5.4 (i.e., 2.2 × 2 = 4.4; 9.8 − 4.4 = 5.4%).

We previously determined that with the glycohemoglobin method that we use, each 1% glycohemoglobin represents ~20 mg/dl glucose integrated over a 24-h period (14). Using this information and the 24-h integrated glucose concentration observed at the end of the 5 weeks on the LoBAG diet, the estimated final percentage of glycohemoglobin would be 6.3%. Thus, the dietary modification that we refer to as the LoBAG diet has the potential for normalizing or nearly normalizing the blood glucose in people with mild to moderately severe type 2 diabetes. Nevertheless, these results should be considered to be merely a proof of concept. Only men were studied, and the diet was highly controlled and was of a relatively short
duration. Thus, the generalization of these results will depend on additional longer-term studies in which both men and women and different age and ethnic groups are included and a greater variety of foods are used. In addition, even though we attempted to keep the participants’ body weight stable, the participants lost a mean of ∼4 lb while ingesting both diets.

The present data also suggest that a diet modification in which the protein and the fat content is increased will facilitate an improvement in glycohemoglobin by the various pharmacological agents used to treat diabetes. However, this also remains to be determined.

The decrease in postmeal glucose concentrations observed can easily be explained by the smaller amount of carbohydrate in the diet and thus the smaller amount of glucose absorbed after ingestion of the meals. The reason for the greatly decreased fasting glucose concentration is uncertain but is likely to be the consequence of a reduced store of glycogen and thus a decrease in glycogenolysis rate (5,6,15). A priori, there is no reason to suspect that the LoBAG diet would result in a decreased rate of gluconeogenesis. Indeed, current evidence indicates that gluconeogenesis remains constant irrespective of the amount of carbohydrate in the diet (16) or of the gluconeogenic substrate supplied (15,17,18).

The LoBAG diet resulted in a decrease in 24-h integrated insulin concentration. In our previous study in which the protein content of the diet was increased from 15 to 30% of total food energy, the 24-h integrated insulin concentration was slightly increased when compared with the same control diet used in the present study (12). This was expected because dietary protein strongly stimulates insu-
lin secretion in people with type 2 diabetes (19). The decrease in integrated insulin secretion in the present study undoubtedly is due to the reduced food-derived glucose content of the diet. Dietary fat does not stimulate insulin secretion (20), or it facilitates a modest increase (4,21,22). Fructose (2,23) and galactose (24) ingestion also results in only a small increase in insulin concentration.

The serum total, LDL, and HDL cholesterol concentrations did not change significantly when the fat content of the diet was increased from 30 to 50% of total food energy. Most likely, this was because the saturated fatty acid content was kept at 10% of energy in both diets. The triglyceride concentration decreased as expected with a reduction in carbohydrate in the diet (25). A decrease in triglyceride might have been expected to increase HDL cholesterol (26); however, this has not been a consistent finding (27–29).

The glucagon area response increased 2.5-fold after the LoBAG diet. This increase is less than the fourfold increase that we observed in our previous study (12). However, the difference in fold increase is due, in part, to a difference in the response to the control diets. The net area response to the three 15% protein meals (control meals) was less in the previous study compared with the present study (139, 127, and 160 vs. 413, 293, and 349 pg·h·ml⁻¹, respectively). Nevertheless, the actual 24-h integrated glucagon response also was higher in the current study (893 vs. 525 pg·h·ml⁻¹).

In summary, a LoBAG diet can dramatically reduce the glucose concentration. The glucagon area response increased 2.5-fold after the LoBAG diet. This increase is less than the fourfold increase that we observed in our previous study (12). However, the difference in fold increase is due, in part, to a difference in the response to the control diets. The net area response to the three 15% protein meals (control meals) was less in the previous study compared with the present study (139, 127, and 160 vs. 413, 293, and 349 pg·h·ml⁻¹, respectively). Nevertheless, the actual 24-h integrated glucagon response also was higher in the current study (893 vs. 525 pg·h·ml⁻¹).

ACKNOWLEDGMENTS

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REFERENCES