Effects of diet composition and ketosis on glycemia during very-low-energy-diet therapy in obese patients with non-insulin-dependent diabetes mellitus[1-3]

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ABSTRACT   To determine whether high-ketogenic very-low-energy diets (VLEDs) can reduce hepatic glucose output (HGO) and hyperglycemia more effectively than can low-ketogenic VLEDs in obese patients with non-insulin-dependent diabetes mellitus (NIDDM), seven patients were treated with a high-ketogenic VLED for 3 wk and were compared with six patients treated with a low-ketogenic VLED. All patients were then crossed over and treated with the alternate diet for another 3 wk. Basal HGO, fasting ketone bodies, and glycemia, insulin, and C-peptide after fasting and an oral-glucose-tolerance test (OGTT) were measured. Before treatment, prediet weight and fasting, OGTT, and HGO measurements were not different between groups. After dieting, weight loss was not different between the groups. However, fasting and OGTT glycemia were lower during treatment with the high-ketogenic VLED than with the low-ketogenic VLED (treatment effect: $P < 0.05$, by analysis of variance). Moreover, there was a strong correlation between basal HGO and fasting plasma ketone bodies ($r = -0.71$ at 3 wk, $r = -0.67$ at 6 wk; both $P < 0.05$). In contrast, fasting and OGTT plasma insulin and C-peptide concentrations were not different between treatment groups. These data indicate that in obese patients with NIDDM, high-ketogenic VLEDs have a more clinically favorable effect on glycemia than do low-ketogenic VLEDs.   *Am J Clin Nutr* 1996;63:110–5.

KEY WORDS   Very-low-energy diet, VLED, non-insulin-dependent diabetes mellitus, NIDDM, weight loss, diet composition, ketosis

INTRODUCTION

Previous studies demonstrated that hepatic glucose output (HGO) is responsive to circulating ketone body concentrations. In healthy, nondiabetic subjects, ketoacid infusions lower glycemia by decreasing HGO either by a direct effect on the liver (1) or secondarily through increased insulin secretion (2). In obese patients with non-insulin-dependent diabetes mellitus (NIDDM), ketoacid infusions have no effect on insulin secretion but glycemia declines and HGO is reduced, particularly when plasma insulin and glucagon concentrations are suppressed (3).

Very-low-energy-diet (VLED) therapy is the physiologic correlate of the infusion experiments described above—ketone bodies increase and plasma insulin and glucagon concentrations decrease significantly (4). It is not known, however,

whether ketosis associated with dieting can account for the decrease in HGO and glycemia, which occurs during weight loss in obese patients with NIDDM. To test the hypothesis that ketosis modulates the reduction in glycemia during dieting in obese patients with NIDDM, the carbohydrate content of VLED formulas was varied to induce high or low ketone body formation (5) and the glycemic responses of obese patients with NIDDM to these VLEDs were compared.

SUBJECTS AND METHODS

Subjects

Thirteen obese patients with a history of NIDDM and who had not been dieting for the 2 mo preceding enrollment participated in the study. Two weeks before beginning the protocol, those patients receiving oral agents or insulin had their medications discontinued. Medications for other medical disorders that could interfere with the metabolic indexes being measured were also discontinued before the protocol. The metabolic and clinical characteristics of the patients are summarized in **Table 1**.

All inpatient and outpatient studies were performed at the University of Rochester General Clinical Research Center (GCRC). The protocol was approved by the University of Rochester Research Subjects Review Board and informed, written consent was obtained from all participants before enrollment in the protocol.

Protocol

Patients were counseled on consuming a high-carbohydrate meal plan recommended by the American Diabetes Association (ADA) for 2 wk before the studies began (6). Intensive diet stabilization and monitoring were then performed after admission to the GCRC for 4 d. Patients consumed a standardized

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TABLE 1
Baseline clinical and metabolic characteristics[1]

	VLED sequence	
	High ketogenic/ low ketogenic (n = 5 F, 2 M)	Low ketogenic/ high ketogenic (n = 5 F, 1 M)
Age (y)	48 ± 4	55 ± 5
Weight (kg)	111 ± 7	94 ± 4
Height (m)	1.66 ± 0.03	1.66 ± 0.02
FBS (mmol/L)	10.5 ± 1.2	12.7 ± 1.8
HbA$_{1c}$ (g/L)	110 ± 7	122 ± 15
HGO (mg·kg^{-1}·min^{-1})	2.28 ± 0.18	2.57 ± 0.30

[1] $\bar{x}$ ± SEM. VLED, very-low-energy diet; FBS, fasting blood sugar; HbA$_{1c}$, hemoglobin A$_{1c}$; HGO, hepatic glucose output.

isoenergetic ADA meal plan ($\approx$125 kJ/kg, or $\approx$30 kcal/kg) composed of 55% carbohydrate, 30% fat, and 15% protein. Fat-free mass (FFM) was determined before the studies by using the ^{40}K method (7). All measurements during the protocol were made after a 14-h overnight fast.

Prediet studies

Before the diet began, basal HGO was measured with the isotope-dilution method (8). After placement of an intravenous catheter, an intravenous bolus of 6,6-^{2}H$_2$-glucose (3 mg/kg) was given followed by a continuous infusion at 3 mg·kg^{-1}·h^{-1}. A retrograde intravenous line was then placed in a dorsal hand vein for blood sampling and the hand was warmed to 70 °C to arterialize the venous samples (9). The infusion continued for 4 h to ensure that steady state was achieved (10, 11). After 4 h, four samples were obtained at 10-min intervals for measurements of fasting whole-blood glucose and plasma 6,6-^{2}H$_2$-glucose. In addition, duplicate samples were obtained for measurements of fasting plasma insulin, β-hydroxybutyrate (BOHB), acetoacetate, and glucagon. One hour after the basal HGO measurement was completed, a standard 75-g oral-glucose-tolerance test (OGTT) was performed with measurements of venous blood glucose and plasma insulin and C-peptide at 30-min intervals for 3 h (12).

Diets

VLED specifications

After the baseline studies, all patients began a VLED (13). The diets consisted of powdered formulas (Ross Laboratories, Columbus, OH) plus safflower oil reconstituted in water. The diets were designed such that the energy deficit in all patients was the same. This was accomplished by providing a total energy intake of 12.5 kJ/kg FFM (10 kcal/kg FFM) divided into four servings. All diets contained 1.2 g protein/kg ideal body wt, and carbohydrate and fat contents were manipulated to either induce (low carbohydrate) or suppress (high carbohydrate) ketosis.

High-ketogenic VLED

To generate ketosis, a protein-fortified, low-carbohydrate formula [New Directions formula; Ross Laboratories: 55% of energy as protein (D Whey, calcium caseinate), 20% of energy as carbohydrate (hydrolyzed corn starch and sucrose), and 25% of energy as fat (corn oil)] was supplemented with safflower

oil. The powdered formula was measured to achieve the required protein intake (1.2 g protein/kg ideal body wt; $\bar{x}$: 65.1 ± 10.1 g, range: 32–104 g); the carbohydrate content was restricted to ≤ 40 g ($\bar{x}$: 24.1 ± 3.9 g; range: 12–40 g). Safflower oil was then added in the amount necessary to meet the individual's energy intake requirement (mean total fat 29.3 ± 5.0 g, range: 13–47 g). To achieve similar energy deficits in all patients (12.5 kJ/kg FFM, or 10 kcal/kg FFM), the average energy content of the high-ketogenic diet was 2.60 ± 0.15 MJ (624 ± 39 kcal; range: 1.90–3.35 MJ, or 455–800 kcal).

Low-ketogenic VLED

To suppress ketosis, a high-carbohydrate, fat-restricted formula [Surgical Liquid Diet; Ross Laboratories: 78% of energy as carbohydrate (sucrose and hydrolyzed corn starch)] was supplemented with a highly concentrated protein formula [ProMod; Ross Laboratories: 71% of energy as protein (soy lecithin, D Whey] to meet daily protein needs (1.2 g protein/kg ideal body wt; $\bar{x}$: 57.3 ± 9.0 g, range: 32–97 g). This formulation ensured that the carbohydrate content was such that it would maximize suppression of ketosis ($\bar{x}$: 94.4 ± 14.4 g, range: 65–156 g). The addition of safflower oil to each diet was minimal (4 g). These combined formulas met the energy requirements of the study design (12.5 kJ/kg FFM, or 10 kcal/kg FFM) by providing an average energy intake of 2.68 ± 0.21 MJ (644 ± 51 kcal; range: 2.09–3.35 MJ, or 500–800 kcal).

Implementation of VLED therapy

To ensure that the groups were closely matched, patients were assigned to either the low- or high-ketogenic diet on the basis of their fasting blood glucose concentrations, weight, age, and sex. For 3 wk, seven patients were treated with the high-ketogenic formula and six patients were treated with the low-ketogenic formula. Patients were then crossed over to the alternate diet until completion of the study at 6 wk. After crossover, the energy and protein contents of the diets remained the same for each patient but the carbohydrate and fat contents were adjusted appropriately to induce or suppress ketosis. In addition, all patients consumed ≥ 2 L water/d as well as an energy-free fiber supplement (Hydrocil; Solvay Pharmaceuticals, Marietta, GA) to prevent constipation. No other source of energy was consumed during the diet period.

Monitoring and follow-up studies

Patients were monitored daily as outpatients for fasting glucose and weight. The morning formula was consumed in the GCRC Outpatient Clinic. The remainder of the formula for that day was consumed outside the GCRC in conjunction with the patient's normal daily routine. Patients were instructed to not alter their routine from prediet activities, particularly with respect to exercise.

Patients were readmitted weekly to the GCRC for metabolic measurements. After a 14-h overnight fast, blood glucose and plasma insulin, C-peptide, glucagon, BOHB, and acetoacetate were measured. After 3 and 6 wk of dieting, the basal HGO and OGTT procedures were repeated. After completion of the studies, patients were refed mixed meals as outpatients under the supervision of the GCRC's research dietitian until their energy intake met the individual's new weight-maintenance requirements.

Analytical methods

Hormone and substrate concentrations

Blood glucose was measured with the automated glucose oxidase method (YSI 23A; Yellow Springs Instruments, Yellow Springs, OH). Plasma insulin was measured with the Linco radioimmunoassay kit (Sigma Diagnostics, St Louis). Plasma glucagon and C-peptide were measured with a standard radioimmunoassay kit method (Diagnostic Products Corp, Los Angeles). BOHB and acetoacetate were measured by using standard enzymatic methods (14).

Plasma concentrations of $6,6\text{-}^2H_2$ glucose were determined by the Biomedical Mass Spectrometry Facility of the University of Rochester and Monroe Community Hospital according to standard methods (15). The boroacetylated derivative of glucose was separated on a capillary gas chromatography column (HP-1; Hewlett Packard, Palo Alto, CA) and analyzed by selected ion monitoring with a 5988A gas chromatography mass spectrometer (Hewlett Packard).

Calculations

Basal hepatic glucose output

The rate of glucose appearance (R_a) in the basal state was calculated from the isotope enrichment of $6,6\text{-}^2H_2$ glucose by using Steele's equations as modified for stable isotope methodology (8). Because virtually all endogenous glucose originates from the liver after an overnight fast, basal HGO is equivalent to the R_a.

Enrichment of $6,6\text{-}^2H_2$ glucose was determined by the increase (above a pretracer sample) in the area ratio of m/z 299 to m/z 297 (m/z is the mass-to-charge ratio), according to the following formula:

$$MPE = 100 \times (R_S - R_B) / [1 + (R_S - R_B)] \qquad (1)$$

where MPE is the molar percentage excess, R_S is the area ratio of the sample of m/z 299 to m/z 297, and R_B is the area ratio of the baseline (pretracer) sample of m/z 299 to m/z 297.

Ketone bodies

BOHB and acetoacetate concentrations were summed and the data analyzed as total ketone body concentrations.

Statistical analyses

Data are expressed as mean ± SEM. This study is a two-period crossover design with repeated measures within each period for most outcome variables. Repeated-measures data were analyzed as described by Wallenstein and Fisher (16). These methods essentially involve performing standard repeated-measures analysis of variance (ANOVA) on within-subject differences and totals. The periods analyzed were the first 3 wk of treatment and the second 3 wk of treatment. The responses observed weekly during treatment were analyzed. F tests, adjusted by using the Huynh-Feldt method when necessary, were performed for analysis of the direct treatment effect (high-compared with low-ketogenic VLEDs), period effect (first 3 wk compared with second 3 wk), and treatment $\times$ period interaction. F tests were also performed for analysis of the direct treatment effect, period effect, and treatment $\times$ period interaction on response variables for which there were no

repeated measures (17). Analyses were performed by using SAS statistical software on the University of Rochester Clinical Research Center Computer Data Management and Analysis System (CDMAS), Rochester, NY.

RESULTS

Weight loss

Prediet weights (Table 1) were not different between groups. Weight loss in patients initially treated with the high-ketogenic VLED and then crossed over to the low-ketogenic VLED was not significantly different from that of patients treated in the reverse order (−11.6 ± 1.0 kg and −10.3 ± 1.3 kg, respectively).

Fasting plasma ketone bodies

Patients manifested the expected changes in ketone bodies (**Figure 1**). Patients on the low-carbohydrate, high-ketogenic VLED had a marked increase in plasma ketone bodies during the first 3 wk of dieting. When these patients were crossed over to the high-carbohydrate, low-ketogenic VLED, plasma ketone bodies were suppressed. In contrast, plasma ketone body concentrations initially were suppressed in patients treated with a high-carbohydrate, low-ketogenic VLED and then increased when crossed over to the low-carbohydrate, high-ketogenic VLED.

Fasting blood glucose and plasma hormone concentrations

Prediet fasting glucose concentrations were not different between groups (Table 1, **Figure 2**). During each period (ie, 3-wk intervals of treatment), fasting glucose concentrations were significantly lower when patients were treated with the high-ketogenic VLED (treatment effect: $P < 0.001$), regardless of whether the high-ketogenic VLED was consumed during the first 3 wk or during the second 3 wk of the protocol (treatment $\times$ period interaction; NS). Consistent with previous reports (18), fasting plasma insulin, C-peptide, and glucagon concentrations decreased in conjunction with weight loss but there was no significant difference in the treatment effect in response to the high- compared with the low-ketogenic diet.
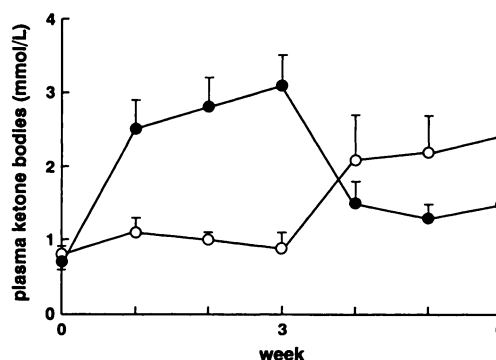

FIGURE 1. Fasting plasma ketone bodies in patients initially treated with the high-ketogenic diet followed by crossover to the low-ketogenic diet (●) compared with patients treated with the low-ketogenic diet followed by crossover to the high-ketogenic diet (○). The treatment effect of the high-ketogenic diet was significant ($P < 0.05$).
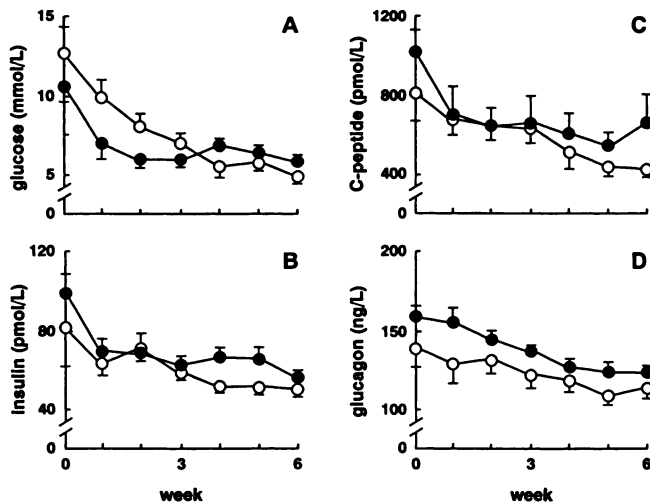
FIGURE 2. Fasting blood glucose, plasma insulin, C-peptide, and glucagon concentrations in patients initially treated with the high-ketogenic diet followed by crossover to the low-ketogenic diet (●) compared with patients treated with the low-ketogenic diet followed by crossover to the high-ketogenic diet (○). The treatment effect of the high-ketogenic diet on fasting glucose was significant ($P < 0.05$).

Basal hepatic glucose output

Prediet basal HGO between the groups did not differ (Table 1). Basal HGO in the patients treated with the high-ketogenic diet during the first 3 wk of the protocol was 1.27 ± 0.08 mg·kg^{-1}·min^{-1} and remained at a comparable level, 1.31 ± 0.08 mg·kg^{-1}·min^{-1}, when measured at 6 wk after crossing over to the low-ketogenic diet. In contrast, HGO in the patients treated with the low-ketogenic diet was 1.56 ± 0.14 mg·kg^{-1}·min^{-1} at 3 wk and continued to decline to 1.25 ± 0.11 mg·kg^{-1}·min^{-1} at 6 wk. The treatment effect of the high-ketogenic diet on HGO was highly significant ($P < 0.002$).

Fasting plasma ketone bodies compared with hepatic glucose output

Before dieting, there was no relation between fasting plasma ketone bodies and HGO (**Figure 3**). However, at both 3 and 6 wk, a significant correlation between the indexes was observed ($r = -0.71$ at 3 wk, $r = -0.67$ at 6 wk; both $P < 0.05$).
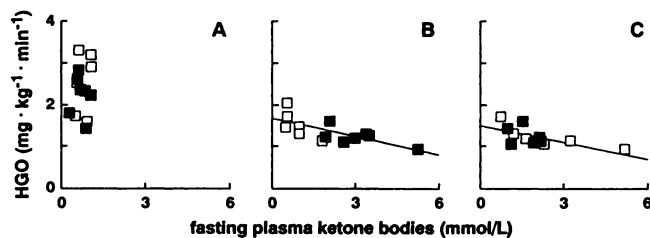

fasting plasma ketone bodies (mmol/L)

FIGURE 3. Regression analyses between hepatic glucose output (HGO) and fasting plasma ketone bodies in patients initially treated with the high-ketogenic diet followed by crossover to the low-ketogenic diet (■) compared with patients treated with the low-ketogenic diet followed by crossover to the high-ketogenic diet (□). $r = -0.71$ at 3 wk and $r = -0.67$ at 6 wk (both $P < 0.05$)

Oral-glucose-tolerance test

Results from prediet OGTTs were not different between groups (**Figure 4**). After the high-ketogenic diet was consumed, the decrease in OGTT glycemia was greater than after the low-ketogenic diet (Figure 4, A and D; treatment effect: $P < 0.05$). In contrast, plasma insulin concentrations did not change significantly under any of the study conditions (Figure 4, B and E). As demonstrated previously (19), weight loss resulted in increased plasma C-peptide concentrations (Figure 4, C and F), but there was no difference in this response after the high- or low-ketogenic diet.

DISCUSSION

Weight loss in obese patients with NIDDM results in a substantial decrease in fasting and postprandial glycemia (13, 18). The decrease is in part due to a reduction in the abnormally high HGO observed in patients with NIDDM with poor metabolic control. How weight loss modulates HGO and leads to improved glycemia is unknown. Previous studies demonstrated that during a ketoacid infusion, HGO and glycemia decreased in both nondiabetic individuals and in patients with NIDDM (1–3). On the basis of these observations, the current study tested the hypothesis that in obese patients with NIDDM, the ketosis associated with dieting modulates HGO and results in a
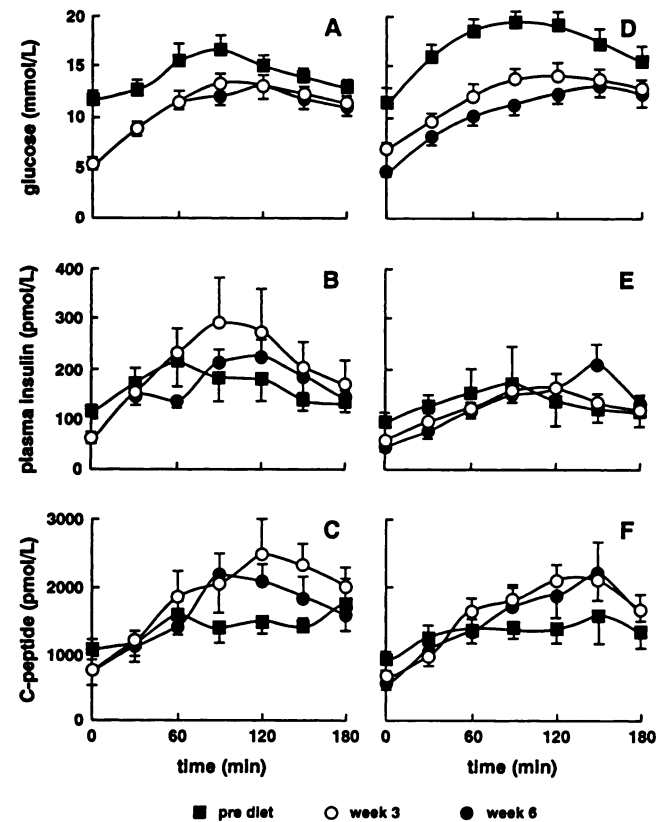

time (min)   time (min)

■ pre diet   ○ week 3   ● week 6

FIGURE 4. Results of an oral-glucose-tolerance test in patients treated with the high-ketogenic diet followed by crossover to the low-ketogenic diet (A, B, and C) and in patients treated with the low-ketogenic diet followed by crossover to the high-ketogenic diet (D, E, and F). Glycemia was significantly lower after the high-ketogenic diet (treatment effect: $P < 0.05$).

greater glucose-lowering effect than does a nonketogenic diet. The results provide evidence that changes in diet composition have a significant effect on glucose metabolism, but the degree of ketosis induced by dieting may not be the only modulating mechanism by which this occurs.

To address this hypothesis, differential ketotic responses to a constant energy deficit were achieved by manipulating the carbohydrate and fat contents of the VLEDs. The low-carbohydrate VLED induced a marked increase in plasma fasting ketone body concentrations to concentrations typically seen in persons with severe energy restriction. In contrast, the high-carbohydrate VLED suppressed ketosis to concentrations nearly equal to prediet concentrations, despite the significant energy deficit. This observation supports the findings of previous studies in nondiabetic patients, demonstrating the powerful effect of carbohydrate on ketosis (20), even with VLED therapy (21–23), and extends these observations to obese patients with NIDDM.

During treatment with the high-ketogenic diet, patients' fasting and OGTT glycemia were lower than during treatment with the low-ketogenic diets. This occurred whether the high-ketogenic diet was consumed during the initial 3 wk of the protocol or after crossover during the second 3 wk. In fact, after crossover from the high- to the low-ketogenic VLED, fasting glycemia increased slightly and OGTT glycemia plateaued despite continued weight loss. In contrast, the patients initially treated with the low-ketogenic diet manifested further substantial decreases in fasting and OGTT glycemia after crossover to the high-ketogenic diet.

Previous studies in which ketosis was generated by infusing ketoacids demonstrated that under those conditions, glycemia and HGO were acutely lowered in obese patients with NIDDM (3). On the basis of the strong correlation between HGO and ketosis, the current study suggests that ketosis also lowers glycemia and basal HGO under the physiologic conditions of dieting. However, the regression analyses also indicate that overlap in the ketotic responses to the high- compared with the low-ketogenic VLEDs occurred. Some patients on the low-ketogenic diet had a greater capacity for ketone body formation or clearance than did patients on the high-ketogenic diet. Consequently, no true threshold or cutoff was observed at which ketosis had an apparent effect on glycemia. This observation is further emphasized by the low covariance (r^2) between these indexes, indicating that < 50% of the glucose-lowering effect of dieting can be accounted for by ketosis. Therefore, it is difficult to attribute the differences in clinical response solely to ketosis. Rather, the differences in diet composition per se must also be considered.

Some of the mechanisms by which weight loss lowers HGO and glucose concentrations during dieting in obese patients with NIDDM have been elucidated. Both insulin secretion and insulin sensitivity improve (13, 18), and a recent study carefully documented the time-course changes in these indexes (24). Because the current study indicates that insulin did not change with the different diets (based on C-peptide concentrations), differences in diet composition may be having an impact on either substrate (ie, glucose) availability or insulin sensitivity. It is conceivable, for example, that liver glycogen stores differ under the two diet conditions. Recent studies indicate that in the short term, glycogen stores in patients with NIDDM may not be completely depleted (25, 26). During dieting, it is

possible that a high-carbohydrate VLED maintains or repletes stores, and/or a low-carbohydrate VLED may more effectively deplete glycogen. In addition, the greater decrease in OGTT glycemia associated with the high-ketogenic diet, even though there were no differences in C-peptide or insulin concentrations between the treatment conditions, may occur if insulin sensitivity increased more in response to the high-ketogenic VLED. Whether this is due to increased peripheral uptake of glucose or to increased suppression of HGO cannot be determined from this study.

In conclusion, the composition of VLEDs can have a significant effect on the ketone body formation during dieting in obese patients with NIDDM. Ketosis and manipulation of diet composition can have a favorable effect on glycemia during weight loss. However, before a recommendation that VLEDs be reformulated, further studies are needed to elucidate the underlying mechanisms causing the differences in metabolic response to high- compared with low-carbohydrate VLEDs, to determine the minimum carbohydrate intake that will maximize the clinical benefits, to identify the types of macronutrients that may further improve the response to dieting (eg, complex compared with simple carbohydrate, polyunsaturated compared with monounsaturated fatty acids), and to develop treatment strategies to maintain the short-term improvements in metabolic control so that long-term benefits result.   ▪

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