Effect of Dietary Energy Restriction on Glucose Production and Substrate Utilization in Type 2 Diabetes

Mark P. Christiansen, Peter A. Linfoot, Richard A. Neese, and Marc K. Hellerstein

A total of 8 obese subjects with type 2 diabetes were studied while on a eucaloric diet and after reduced energy intake (25 and then 75% of requirements for 10 days each). Weight loss was 2, 3, and 3 kg after 5, 10, and 20 days, respectively; all of the weight lost was body fat. Fasting blood glucose (FBG) levels fell from 11.9 ± 1.4 at baseline to 8.9 ± 1.6, 7.9 ± 1.4, and 8.8 ± 1.3 mmol/l at days 5, 10, and 20, respectively (P < 0.05, baseline vs. 5, 10, and 20 days). Endogenous glucose production (EGP) was 22 ± 2, 18 ± 2, 17 ± 2, and 22 ± 2 μmol · kg⁻¹ body mass (LB M) · min⁻¹ (P < 0.05, days 5 and 10 vs. baseline). Gluconeogenesis measured by mass isotopomer distribution analysis (MIDA) provided 31 ± 4, 41 ± 5, 40 ± 4, and 33 ± 4%, respectively, of the EGP (NS); absolute glycolytic glyconeogenic contribution to the EGP was 15 ± 2, 11 ± 2, 11 ± 2, and 15 ± 2 μmol · kg⁻¹ LBM · min⁻¹, respectively (P < 0.001, baseline vs. days 5 and 10 and day 10 vs. day 20). The blood glucose clearance rate increased significantly at day 20 (P < 0.05). Neither lipolysis nor flux of plasma nonesterified fatty acids were altered compared with baseline. In conclusion, severe energy restriction per se independent of major changes in body composition reduces both FBG concentration and EGP in type 2 diabetes, the reduction in EGP results entirely from a reduction of glycogenolytic input into blood glucose, and the duration of reduced glucose production is short-lived after relaxation of energy restriction even without weight gain, but effects on plasma glucose clearance persist and partially maintain the improvement in fasting glycemia. Diabetes 49:1691–1699, 2000

Dietary restriction of total energy intake has been shown to influence fasting blood glucose (FBG) and endogenous glucose production (EGP) before significant changes in body weight or composition occur (1–3). The changes in FBG and EGP are correlated, and most of the effects are seen within 7–10 days of starting caloric restriction (3,4).

The glucose-producing pathways affected by energy restriction remain uncertain. Glycogenolysis and gluconeogenesis both contribute to EGP in the postabsorptive state. In non diabetic humans and rodents, starvation or carbohydrate-restricted diets reduce EGP (5,6); in rats, the change in EGP results entirely from reduced glycogenolytic flux to glucose, not reduced gluconeogenesis input. Some previous studies have suggested that predominantly gluconeogenesis is increased in type 2 diabetes, whereas other results suggest that the primary contribution to EGP is from hepatic glycogenolysis (7–9). FBG could also be reduced by energy restriction if the metabolic clearance of glucose were increased. This could be reflected in either increased oxidative or nonoxidative disposal of glucose under fasting conditions.

Our laboratory has previously shown that fractional and absolute gluconeogenesis can be measured in rats and in humans by infusing [2-¹³C]glycerol and [U-¹³C]glucose using mass isotopomer distribution analysis (MIDA) to measure the fractional gluconeogenesis contribution to EGP (5,10). We investigated the metabolic mechanisms by which dietary energy restriction reduces EGP and FBG in obese type 2 diabetes subjects and whether metabolic changes during energy restriction relate to body composition changes or energy balance per se. Some of these results have previously been presented in abstract form (11,12).

RESEARCH DESIGN AND METHODS

Subjects. A total of 8 subjects (4 men and 4 women, means ±SD 51 ± 4 years of age) with type 2 diabetes (duration of 5 ± 3 years) were recruited from the Diabetes Clinic at San Francisco General Hospital (SFGH). Their average weight was 107 ± 14 kg, the average BMI was 36 ± 3 kg/m², and the average glycosylated hemoglobin level was 8.1 ± 0.5% (normal range 4–6.5%). After giving written informed consent to participate in the protocol, subjects were admitted to the General Clinical Research Center (GCRC) at SFGH for 25 days. If they had been taking oral hypoglycemic agents or insulin, these medications were discontinued for at least 2 weeks before admission. Studies received prior approval from the University of California at San Francisco Committee on Human Research.

Study design. The study consisted of a 25-day inpatient admission to the GCRC during which time 3 dietary phases were imposed (Fig. 1A). The first

A1-gluconeogenesis, B1A, bioelectrical impedance analysis; EGP, endogenous glucose production; EM-gluconeogenesis, measured molar excess of the M₁ iso tomer of glucose; FBG, fasting blood glucose; f_M₁ glucose, fraction of glucose synthesized by the gluconeogenesis pathway; GC/MS, gas chromatogra phy/mass spectrometry; GCRC, General Clinical Research Center; I, rate of infusion; LBM, lean body mass; M₀, the lowest mass isotopomer in the envelope monitored (typically the parent or all ¹³C isotopomer), with other mass isotopomers (e.g., M₂, M₃, ...Mₗ) distinguished by their mass difference from M₀; ME, molar excess; MIDA, mass isotopomer distribution analysis; NEFA, nonesterified fatty acid; NMR, nuclear magnetic resonance; p, hepatic triose-phosphate precursor pool enrichment; Rₚ, rate of appearance; SFGH, San Francisco General Hospital; SIR, selected ion recording.

From the Department of Medicine (M.P.C., P.A.L., M.K.H.), University of California, Berkeley, California. Address correspondence to Marc K. Hellerstein, MD, PhD, or Mark P. Christianson, MD, Department of Nutritional Sciences, 309 Morgan Hall, University of California, Berkeley, CA 94720-3104. E-mail: march@nature.berkeley.edu.

Received for publication 1 October 1998 and accepted in revised form 18 September 2000.
phase was a 5-day eucaloric baseline period. An estimated weight-maintaining (eucaloric) diet was determined for each subject based on body weight and diet records taken by the dietitians in the GCRC. The average composition of this diet was 18% protein, 35% fat, and 47% carbohydrate. The total energy content of the diet was adjusted if the subject's daily weight was not stable during the 5-day baseline period in the GCRC. The second phase consisted of 10 days of dietary energy restriction to 25% of eucaloric needs (30% protein, 9% fat, 61% carbohydrate). This was followed by the third and final phase in which 75% of eucaloric needs (20% protein, 30% fat, and 50% carbohydrate) were provided for the final 10 days. Isotope infusion studies with indirect calorimetry were performed 4 times: at baseline (after documented weight stabilization, day 5 of the GCRC admission) and at 5, 10, and 20 days after beginning the dietary intervention.

Subjects ate dinner at 1800 the night before each infusion study and remained fasting until the infusion studies were completed at 0900 the next day. Intravenous lines (both antecubital spaces) were started at 2200 and kept open with 0.45% saline. At 0200, a primed/continuous low-dose infusion of [U-13C6]glucose (6.7 µmol/kg prime, 0.11 µmol · kg–1 · min–1) was started (Fig. 1B). At 0500, [1,2,3,4-13C4]palmitate (27 nmol · kg–1 · min–1) mixed with human serum albumin and [2-13C]glycerol (2.7 µmol · kg–1 lean body mass consumed. The rate of appearance of NEFA (µmol · kg–1 · LBM · min–1) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)

where \( I_{\text{glycerol}} \) is the rate of infusion of labeled glycerol and \( M_1 \)-glycerol (ME) is the molar fraction of \([2-13C]_1 \) glycerol calculated by comparison to true standards. The rate of appearance of NEFA \( (R_a \text{NEFA}) \) was calculated by dilution:

\[ R_a \text{NEFA} = \frac{[\text{NEFA}] - [\text{palmitate}]/[\text{M4-palmitate}] \times [\text{ME}]}{[\text{ME}]} \times [\text{NEFA}] \times [\text{ME}] \] (7)
RESULTS

Body composition. Weight by phase is shown in Table 1. Average weight loss during the first 5 days of phase 1 (25% of eucaloric requirements) was 1.84 ± 0.29 kg, and, after another 5 days, total weight loss was 3.04 ± 0.52 kg. Despite energy restriction to 75% of eucaloric needs between days 10 and 20, no further weight loss was documented. The average weight at day 5 was significantly different from that at baseline, and day 10 was statistically different from day 5 but not different from day 20. All of the weight loss involved a loss of body fat based on BIA measurements; fat-free mass did not change (Table 1).

Carbohydrate metabolism. As seen in Table 2, FBG fell by day 5 and remained significantly lower than baseline throughout the study. The decrease in FBG was associated with a significant decrease in EGP at days 5 and 10 but a return to the baseline EGP values at day 20. The absolute flux of gluconeogenesis into blood glucose did not change; all of the changes in EGP were because of altered contributions from glycerol to blood glucose. The proportion of gluconeogenesis derived from labeled plasma glycerol carbon did not change by phase (32 ± 3, 34 ± 3, 33 ± 2, and 33 ± 4% from baseline and days 5, 10, and 20, respectively). For each subject, by phase, the values for \( \text{EM}_1 \)-glucose and \( \text{EM}_2 \)-glucose, the calculated hepatic triose-phosphate precursor pool enrichments \((p)\), and \( f_{\text{glucose}} \) are shown in Table 3. Plasma glucose clearance was \( 2.0 \pm 0.2, 2.1 \pm 0.2, 2.3 \pm 0.3, \) and \( 2.7 \pm 0.3 \) \( \mu \text{mol} \cdot \text{kg}^{-1} \text{LBM} \cdot \text{min}^{-1} \) at baseline and at days 5, 10, and 20, respectively (\( P < 0.05, \text{day 20 vs. baseline and day 20 vs. day 5} \)).

DISCUSSION

Previous studies (1–3,14) have shown that the reduction in FBG during energy restriction in type 2 diabetes often occurs within the first week, before major alterations in body weight; that the reduction in FBG during energy restriction correlates closely with reduced EGP; and that relaxation of caloric restriction without weight gain is associated with a return to elevated FBG and EGP levels. The new observations in our study relate to the underlying metabolic changes, including the sources of EGP and the effects on fatty acid metabolism. Our major findings were that the rapid reduction in EGP resulted entirely from a decreased contribution from glycogenolysis; that the effects on glycogenolysis and EGP were reversed rapidly by relaxation of the degree of energy restriction, even though the diet remained hypocaloric; that FBG remained reduced from baseline after relaxation of the energy restriction because of increased plasma glucose clearance; and that high rates of lipolysis and fat oxidation present at baseline did not increase further with energy restriction.

EGP has 2 sources of metabolic input in the postabsorptive state: glycogenolysis and gluconeogenesis. Our fractional

<table>
<thead>
<tr>
<th>TABLE 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Body weight and composition by phase</strong></td>
</tr>
<tr>
<td><strong>Day</strong></td>
</tr>
<tr>
<td><strong>Weight</strong></td>
</tr>
<tr>
<td><strong>Fat free mass</strong></td>
</tr>
<tr>
<td><strong>Fat</strong></td>
</tr>
</tbody>
</table>

Data are means ± SD. Values are expressed in kilograms. Values with different superscripts in the same row are significantly different at \( P < 0.05 \).

<table>
<thead>
<tr>
<th>TABLE 2</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Metabolic parameters by phase</strong></td>
</tr>
<tr>
<td><strong>Baseline</strong></td>
</tr>
<tr>
<td><strong>FBG (mmol/l)</strong></td>
</tr>
<tr>
<td><strong>EGP</strong></td>
</tr>
<tr>
<td><strong>( f_{\text{glucose}} ) (%)</strong></td>
</tr>
<tr>
<td><strong>Absolute glycogenolysis</strong></td>
</tr>
<tr>
<td><strong>Glycogen contribution</strong></td>
</tr>
<tr>
<td><strong>Whole-body carbohydrate oxidation</strong></td>
</tr>
<tr>
<td><strong>Plasma glucose clearance (ml · kg(^{-1}) LBM · min(^{-1}))</strong></td>
</tr>
<tr>
<td><strong>R(_1) NEFA</strong></td>
</tr>
<tr>
<td><strong>R(_1) glycerol</strong></td>
</tr>
<tr>
<td><strong>Whole-body lipid oxidation</strong></td>
</tr>
</tbody>
</table>

Data are means ± SD. *Units are micromoles per kilogram\(^{-1}\) LBM per minute\(^{-1}\) unless otherwise noted. Values with different superscripts in the same row are significantly different at \( P < 0.05 \).
gluconeogenesis results in 8 individuals with type 2 diabetes were lower at the baseline measurement than those published by Magnusson et al. (7) using nuclear magnetic resonance (NMR) spectroscopy, although our findings were similar to older estimates based on splanchnic arteriovenous differences in type 2 diabetes (15). Potential difficulties in using [13C1]glycerol for MIDA calculations have been suggested (16,17). This and several factors relating to the isotope infusion protocol and analytical approach may affect these measurements. Reviewing the potential effect of these factors in detail is therefore worthwhile. We have recently published a more general review of MIDA, including potential limitations, based on our experience during the past 8 years (18).

The first issue relating to experimental design is the optimal duration for labeled glucose and glycerol infusions. Other groups have previously reported (19,20) that up to 7-10 h of labeled glucose administration may be necessary to reach plateau-specific activities in plasma glucose in severely hyperglycemic subjects (glucose >250 mg/dl). The time required for equilibration in subjects with normoglycemia or modest hyperglycemia (<200 mg/dl), as was present in our subjects, was considerably shorter (i.e., <6 h) (19). We thus provided a 7-h infusion of labeled glucose. In contrast, our infusion time for [2-13C2]glycerol was designed to be only 4 h for several reasons. First, we anticipated that plasma glucose enrichments would have reached nearly plateau values at 7 h based on studies in modestly hyperglycemic diabetic subjects (19). The enrichments of plasma glucose at 4 h were measured at each phase of the study, and the values were 86 ± 3, 86 ± 2, 89 ± 3, and 89 ± 2% of the 7-h values at days 0, 5, 10, and 20, respectively. This suggests that significant differences in cycling between phases do not occur. Moreover, the gluconeogenesis precursor infusion was intentionally kept shorter than the labeled glucose infusion to minimize the effect of delayed glycogen cycling on fractional gluconeogenesis estimates. The potential effect of differences in glycogen cycling between phases on gluconeogenesis estimates is modeled in Table 4.

A second issue relating to isotopic experimental design is whether the gluconeogenesis measurements could be contaminated by the other isotopic labels given ([1,2,3,4-13C4]palmitate or [U-13C6]glucose). Note that any entry of 13C as a single labeled species into the triose-phosphate pool from sources other than [13C1]glycerol does not pose a theoretical problem for MIDA (i.e., as long as these enter as M1-triose-phosphate). The combinatorial technique will simply include these in the calculated value of p. An important advantage of MIDA is that the ultimate labeling source or route into the true precursor pool makes no difference (6,18). The only potential problem would be if the 13C-label enters the triose-p pool as higher masses (M2 and M3). Occurrence of M2-triose-p would result in overestimation of p and underestimation of f葡萄糖 (6). [1,2,3,4-13C4]palmitate is unlikely to pose an important problem in this regard. For this isotopomer of palmitate to interfere with MIDA, [1,2,3,4-13C4]palmitate would need to be generated. The maximum enrichment of this molecule can be estimated from the plasma enrichment of [1,2,3,4-13C4]palmitate (~1%). Of the 8 acetates in palmitate, only one-fourth could be doubly labeled as acetates. In addition, palmitate accounts for ~25% of the NEFAs in the plasma. This would be diluted further by other sources of acetyl-CoA (glycolysis and protein metabolism, which is likely a small contribution under fasting conditions, perhaps 20%) (26) and by exchange and dilution of carbon within oxaloacetate (by anaplerotic sources, including amino acids). The latter has generally been estimated to...
be in the range of 50% (27). Also, even if oxaloacetate were to leave the tricarboxylic acid cycle before the completion of 1 turn of the cycle, decarboxylation of oxaloacetate to pyruvate results in the loss of 1 of the labeled carbons 50% of the time (27). Therefore, the maximum enrichment of a glycolate results in the loss of 1 of the labeled carbons 50% of 1 turn of the cycle, decarboxylation of oxaloacetate to alanine may exist across the liver when labeled glycolate is administered (16,17). This conclusion was based on avid uptake of glycolate by the liver and on dilution of labeled glycolate leaving the liver. Depletion of labeled glycolate across the hepatic lobule may limit the delivery of the label to hepatocytes distal along the lobule; a labeling gradient was postulated to explain lower-than-expected values of fractional gluconeogenesis contribution in fasted rats or perfused livers (16,17). However, several investigators have reported fractional gluconeogenesis values that were not low (i.e., in the range of 90% or appropriately high fractional contribution) in fasted rats (5,6,28), perfused livers (28), and humans (10,29,30). When labeled glycolate is administered at the rates used in our study (e.g., to achieve hepatic precursor pool enrichments >0.10 ME), gluconeogenesis appears to not be underestimated (5,6,10,28–30), perhaps because the increased glycolate delivery overcomes any potential gradient.

Distinguishing between uptake of a metabolite by a tissue and a metabolic gradient within the tissue is also important. The liver could take up glycolate avidly in many ways without resulting in isotopic inhomogeneity within the tissue. In many cases, metabolite mixing in the space of Disse, which is not subject to size exclusion, may result in a metabolic gradient within the hepatocyte. This should result in an isotopic inhomogeneity ranging from ~0.5% to ~10% within the liver with the potential for a labeled substrate to become enriched in a hepatic precursor pool. Enrichments for labeled substrates in liver can be large enough to be detected by stable isotope enrichment measurements by mass spectrometric methods. A third issue is the effect of various metabolic or analytical complicating factors on estimates of p and f_{glucose} for gluconeogenesis were simulated by computer modeling as described previously (18). Basis of lower-bound, upper-bound, and anticipated (reasonable) estimates are described below. *Contamination of M_2-triose-p was calculated by adding 0.0005–0.0010 ME to M_2 glucose and back-calculating p and f_{glucose}. Values used for upper and lower estimates and for anticipated estimate are from human subjects given [U^{13}C_6]glucose in the fasted state. †Simple glycolate labeling gradient was calculated as described previously (18). The range was based on maximal likely [13C]-glyceraldehyde enrichment and measured gluconeogenesis values previously reported for these 13C-glycolate infusion rates (16). The gradient was simulated as the sum of 100 steps with the gradient of plasma glycolate contribution to p from 50 to 10% across the lobule (5-fold gradient) or 40 to 20% across the lobule (2-fold gradient) with plasma glycolate enrichment of 0.45 ME (and thus average p = 0.30 × 0.45 ME = 0.135 ME for both simulations). ‡Label exchange into lactate was previously measured to be 25% of the value of p during 13C-glycolate infusion (6). Simulation was performed by adding 25% value of p for nonglycolate gluconeogenesis substrates (lactate, alanine, etc.) across the lobule to 13C-glycolate input as calculated in footnote †. §The biosynthetic gradient was calculated by imposing a gradient of relative contribution to total gluconeogenesis falling from 100:1 across the liver (in steps of 1) paralleling a gradient of label input from plasma 13C-glyceraldehyde (enrichment of 0.45 ME). ||Stimulation of gluconeogenesis was assumed to be identical to an increase in the triose-phosphate pool (i.e., ~10% increase in p attributable to 13C-glycolate, with 100% of triose-phosphates proceeding to gluconeogenesis), either with no hepatic autoregulation (for upper-bound estimate) or with complete autoregulation (for minimum estimate) and 50% autoregulation (for reasonable estimate). ¶This calculation assumes that a 4-h plateau value represents 95% of true blood equilibrium value and that the increase during hours 4–7 represents mostly isotope cycling from labeled glycogen (21–25). A range of 4- vs. 7-h values measured in these subjects is used. #Analytical error estimates were calculated as described previously (18). The ±2% value represents the actual accuracy criteria that were used for inclusion of GC/MS data in the present study.
to bulk flow of blood; gap junction communications between hepatocytes along the lobule; or exchange and release of labeled metabolites among cells). Regarding metabolite exchange, we have observed that plasma 13C-lactate enrichments reach ~25% of values of p during 13C-glycerol infusions (6). This labeled lactate/alanine will then tend to dampen any gradients of substrate glycerol uptake across the hepatic lobule. This is another way that complex label behavior works to the benefit of combinatorial (MIDA) methods; the net effect being that all of the 13C that enters hepatic triose-p during a labeled glycerol infusion need not have entered in the form of 13C-glycerol, so that extreme labeling gradients are mitigated even if glycerol itself follows a lobular uptake pattern.

Simulating the quantitative effect on gluconeogenesis of various labeling gradients across the hepatic lobule is possible by computer modeling (18). If a simple gradient of labeled glycerol uptake exists across the lobule (i.e., p begins at 0.18 and ends at 0.09 for an average value of 0.135), then \( f_{\text{glucose}} \) is only underestimated by 3.8% of the true value (Table 4). Even if p varies dramatically (by 5-fold from 0.225 to 0.045 with the average value still being 0.135) across the lobule, then \( f_{\text{glucose}} \) is reduced by 13.3% (Table 4). Such a severe gradient is unlikely to occur, particularly at the 13C-glycerol infusion rates herein, for the reasons discussed above, including the mixing of 13C-label into lactate/alanine pools. If label exchange into these other pools is included in a computer simulation, then even a 5-fold glycerol gradient reduces \( f_{\text{glucose}} \) only by 8.2% (i.e., to 91.8% of the correct value) (Table 4).

Finally, one cannot ignore the possibility that a true biosynthetic gradient may also exist that parallels any gradient for label uptake. If most gluconeogenesis as well as label uptake occurs in the first portion of the lobule, then MIDA becomes the measurement methodology of choice, even compared with direct tissue sampling of triose-p enrichment (if the latter were possible). This is because the presence of a biosynthetic gradient paralleling a labeling gradient makes the mixed-tissue precursor pool enrichment misleading (i.e., the bulk tissue average will not represent the precursor pool actually used for synthesis). In contrast, combinatorials are based on measurements of the synthesized product molecules themselves. MIDA thereby provides the most effective strategy in principle for measuring biosynthesis when unpredictable or inhomogenous biosynthetic pools are present because only a combinatorial technique can correct for unequal synthetic contributions across the tissue. The simulated effect of a biosynthetic gradient using MIDA versus direct tissue sampling is shown in Table 4. Even a massive, physiologically unrealistic gradient (e.g., 100-fold from 0.45 to 0.0045 in p) results in almost no underestimation of gluconeogenesis (\( f_{\text{glucose}} = 95.7\% \) of the true value).

The effect of these and other potential complicating factors on MIDA estimates of gluconeogenesis are summarized in Table 4. These factors aside, in our view, the most important source of potential error in estimating gluconeogenesis by MIDA is analytical. Attention to analytical constraints and avoiding potential problems are critical for accurate use of MIDA in general and particularly for measurement of gluconeogenesis. To calculate p and \( f_{\text{glucose}} \) one must be able to measure the M0, M1, and M2 isotopomers accurately. This requires sufficient enrichment of each isotopomer to differentiate from natural abundance background and proper performance of the mass spectrometer for each ion. The least abundant mass isotopomer in the case of gluconeogenesis is the M2 glucose. Our experience and calculations of error sensitivity (6,18) indicate that the least abundant mass isotopomer must contain at least 0.0050 (0.50%) ME for reliable results. M2 enrichment is determined by f (not under the investigator's control) and p (under the investigator's control through the infusion rate of 13C-glycerol). The capacity of the mass spectrometer to measure M2 and M1 abundances accurately in the unlabeled standards is also critical because calculation of enrichment requires subtraction of natural abundance values. To avoid systematic errors, the instrument should generate values within ±2% of theoretical values for all mass isotopomers; higher degrees of inaccuracy have extremely large effects on calculated values of gluconeogenesis (e.g., ±5% accuracy results in \( f_{\text{glucose}} \) values ranging from 68 to 126% of the true value) (Table 4). We use ±2% baseline accuracy as a requirement for inclusion of a data run.

Another analytical point is that the amount of sample material in the mass spectrometer source (the total ion abundance) affects the relative abundances measured for different masses (the isotope ratios). This effect, termed "abundance sensitivity" (31,32), may be because of factors such as detector nonlinearity and gas-phase bimolecular interactions (e.g., H-extraction). Matching the abundance of the major ion (M0) in all samples (labeled and natural abundance) is therefore essential analytically. Furthermore, this total ion abundance must be set at a value at which all mass isotopomers measured in unlabeled samples fall within the ±2% accuracy range compared with theoretical values. To meet these analytical requirements, preliminary runs of standards and samples are performed, followed by adjustment of injection volumes before making final measurements. An example of acceptable gluconeogenesis data is shown in Table 5. Note that the total ion abundances are similar for samples and standards; the values for M1 and M2 fractional abundances for the standard are close to theoretical values (0.1348 and 0.0256, respectively), and M2-glucose is >0.0050 ME. If any of these features were absent, then the calculation of p and \( f_{\text{glucose}} \) would be uncertain, and the measurement would be discarded.

To attain adequate M2 enrichments at \( f_{\text{glucose}} = 20-40\% \) perturbing the triose-phosphate pool by at least 8-10% is necessary (6,18). This can be achieved in human subjects by administration of [2-13C]glycerol at 0.1 mg · kg\(^{-1} \) · LBM · min\(^{-1} \). Although this nontracer dose of label could perturb hepatic physiology, infusions of much greater quantities of glycerol (e.g., 1.5 mg · kg\(^{-1} \) · min\(^{-1} \)) have been shown not to alter EGP (33). Infusions of labeled glycerol at this rate result in plasma glycerol enrichments of 40-50%, thereby doubling plasma glycerol concentrations (which may help to overcome potential hepatic labeling gradients). The consequence of doubling glycerol delivery to the liver may be to increase the contribution from glycerol to the gluconeogenesis precursor pool and perhaps increase gluconeogenesis modestly (see Table 4 for a simulated effect).

Consistent with the notion that flux from glycolysis provides most of the EGP in type 2 diabetes are our results during energy restriction. The reduction in EGP occurred early after energy restriction and resulted entirely from the change in the contribution from glycolysis, not gluconeogenesis. This is compatible with previously documented effects of acute starvation to reduce liver glycogen content.
and glycogenolytic contributions to EGP (6). That reduction in EGP by 21% could occur by a starvation-induced glycogen depletion mechanism is unlikely a priori if glycogen provided only 12% of EGP at baseline in type 2 diabetes, as was concluded using the NMR method (7). A primary role for glycogenolysis as a modulating pathway over EGP in type 2 diabetes is consistent with the failure of either reducing gluconeogenesis substrate availability (with ethanol) (34) or increasing gluconeogenesis substrate availability (with fructose) (35) to alter EGP in diabetic subjects, as in healthy control subjects (33,36).

Regardless of the metabolic source of EGP, the observation that short-term energy restriction per se reduces fasting hyperglycemia by reducing EGP before changes in body composition occur raises several interesting questions. Perhaps the most obvious from a clinical perspective is how long this energy restriction effect lasts. When the degree of energy restriction was relaxed during the last 10 days of the protocol, no significant changes were evident in weight or FBG. However, EGP increased back to baseline levels entirely because of restoration of the basal contribution from glycogenolysis. The absence of a concomitant rise in FBG at day 20, despite the change in EGP, means that the metabolic clearance of glucose in the postabsorptive state had improved compared with baseline. This may reflect secondary effects such as improved peripheral insulin sensitivity resulting from the prior period of energy restriction; improved peripheral insulin secretion, as has been reported during energy restriction in type 2 diabetes (1,37); or an effect on insulin sensitivity of the reduction in hyperglycemia per se. The increased clearance of glucose was not associated with any significant changes in either glucosuria or whole-body carbohydrate oxidation. Thus, the improved glucose disposal was not associated with any significant changes in either the concentration of whole-body glucose availability or the rate of glucose disposal under the postabsorptive conditions studied. This effect is likely to reside in the muscle, although changes in hepatic uptake of plasma glucose could also contribute. Improvements in monounsaturated fatty acid oxidation, muscle lipid oxidation, and ketone body production have been reported in response to caloric restriction (48–50), but these changes are likely to be more pronounced in situations involving a greater reduction in energy intake (51–53). In any event, our results suggest that persistent glycemic benefits of energy-restricted diets after reequilibration at a new lower body weight may involve additional effects beyond those associated with weight loss (54–56)."
reported a similar response to energy restriction in type 2 diabetes. Individuals with type 2 diabetes require higher insulin levels to suppress lipolysis (41). Rates of lipolysis were supranormal in our diabetic subjects at baseline compared with nondiabetic subjects studied previously in our laboratory using identical methods (39,42). One possible interpretation of our results is that rates of lipolysis were high enough at baseline that whole-body lipid oxidation was already near maximal and therefore did not increase further in response to caloric deprivation. The very low nonprotein respiratory quotients values present at baseline in these diabetic subjects (0.74 ± 0.01) are consistent with this interpretation. This explanation for altered lipid metabolic response to energy restriction in type 2 diabetes is speculative, however, and requires further study.

In conclusion, short-term energy restriction is associated with significant changes in EGP before the occurrence of substantial changes in body weight or composition. The reduction in EGP resulted entirely from a decrease in the contribution from glycogenolysis, whereas absolute rates of gluconeogenesis remained constant. The short-term effects of energy restriction on glucose metabolism in type 2 diabetes therefore appear to represent a starvation or hepatic glycogen-depleting mechanism of action. The duration of this effect on EGP is fairly short in that relaxation of the energy restriction without weight gain was associated with a return to baseline values of EGP and glycogenolysis within 10 days. Persistent effects on metabolic clearance of plasma glucose partially preserved the improvements in glycemia despite the return of EGP to baseline. High rates of lipolysis and fat oxidation were present at baseline in these subjects and did not increase further with energy restriction in contrast with the previously reported response of subjects without diabetes (39,40).

ACKNOWLEDGMENTS

This work was supported by an American Diabetes Association Clinical Research Grant (M.K.H.) and grant 5-M01-RR-00826-3 from the National Center for Research Resources, National Institutes of Health. M.P.C. is the recipient of a National Institutes of Health Clinical Associate Award.

REFERENCES

1. Wing RR, Blair EH, Bononi P, Marcus MD, Watanabe R, Bergman RN: Caloric restriction per se is a significant factor in improvements in glycemic control and insulin sensitivity during weight loss in obese NIDDM patients. Diabetes Care 17:30-36, 1994
23. Hellerstein MK, Kaempfer S, Reid IS, Wu K, Shackleton CH: Rate of glucose entry into hepatic uridine diphosphoglucone by the direct pathway in fasted and fed states in normal humans. Metabolism 44:172-182, 1995
27. Weinman EO, Strissower EH, Chaikoff IL: Conversion of fatty acids to carbohydrolyte: applications of isotopes to this problem and the role of the Krebs cycle as a synthetic pathway. Physiol Rev 37:252-272, 1957