Effects of Acute Insulin Excess and Deficiency on Gluconeogenesis and Glycogenolysis in Type 1 Diabetes

Guenther Boden, Peter Cheung, Carol Homko

To determine whether insulin induces acute changes in endogenous glucose production (EGP) via changes in gluconeogenesis (GNG), glycogenolysis (GL), or both, we measured GNG (with ^2H_2O) and GL (EGP-GNG) in nine patients with type 1 diabetes during acute insulin excess produced by subcutaneous injection of insulin and during insulin deficiency which developed between 5 and 8 h after insulin injection. During insulin excess, free insulin concentration rose fivefold (from 36 to 180 pmol/l). Plasma glucose was maintained between 6.2 and 6.7 mmol/l for ~4 h with IV glucose. EGP (with 6,6-^{2}H glucose) decreased from 17.1 to 9.8 μmol · kg^{-1} · min^{-1} after 1 h. This decrease was almost completely accounted for by a decrease in GL (from 10.7 to 4.6 μmol · kg^{-1} · min^{-1}). During insulin deficiency, plasma glucose rose from 6.2 to 10.5 mmol/l and EGP from 9.5 to 14.3 μmol/kg min. The increase in EGP again was accounted for by an increase in GL.

We conclude that in type 1 diabetes acute regulation of EGP by insulin is mainly via changes in GL while GNG changes little during the early hours of acute insulin excess or deficiency. *Diabetes* 52:133–137, 2003

Many patients with type 1 diabetes experience almost daily episodes of hypoglycemia caused by insulin excess or hyperglycemia caused by insulin deficiency (1). These problems are due in large part to excessive or insufficient insulin-induced suppression of endogenous glucose production (EGP). It is not known, however, whether these hypo- or hyperglycemic episodes in patients with type 1 diabetes are the consequence of excessive or insufficient suppression of gluconeogenesis (GNG) or glycogenolysis (GL), the two components of EGP, or a combination of both. The main reason for this uncertainty is that until recently, in vivo rates of GNG and GL could not be measured reliably in human subjects, primarily because hepatic GNG precursor–specific activities were unknown due to unpredictable dilution of the labeled precursors in the oxalacetic acid pool, which is shared by GNG and the tricarboxylic acid cycle (2). Recently, several methods have become available that allow accurate and noninvasive measurement of GNG (3–5). In the present study, we have used the ^2H_2O method, developed and validated by Landau et al. (4), to determine effects of acute insulin excess and deficiency on rates of GNG and GL in patients with type 1 diabetes.

From the Division of Endocrinology/Diabetes/Metabolism and the General Clinical Research Center, Temple University School of Medicine, Philadelphia, Pennsylvania.

Address correspondence and reprint requests to Guenther Boden, Temple University Hospital, 3401 N. Broad St., Philadelphia, PA 19140. E-mail: bodengh@tuhs.temple.edu.

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EGP, endogenous glucose production; FFA, free fatty acid; GL, glycogenolysis; GNG, gluconeogenesis; G_{Ra}, rate of glucose appearance; G_{Rd}, rate of glucose disappearance.

RESEARCH DESIGN AND METHODS

Subjects. Nine patients with type 1 diabetes were studied; some of their characteristics are shown in Table 1. Body weights were stable for ≥2 months, and diets contained a minimum of 250 g/day of carbohydrate for ≥2 days before the studies. The women were studied in the follicular phase of their menstrual cycle. Informed written consent was obtained from all the subjects after explanation of the nature, purpose, and potential risks of the study. The study protocol was approved by the Institutional Review Board of Temple University Hospital.

Study design. All patients were admitted to the General Clinical Research Center at Temple University Hospital the day before the studies. Their evening dose of insulin was withheld. At 6:00 P.M., they were fed a standard meal consisting of 53% carbohydrate, 15% protein, and 32% fat. After that, they fasted for the duration of the study but were allowed water ad libidum. At 11:00 P.M. and again at 3:00 A.M., they drank 2.5 g/kg body water of ^2H_2O (99% hydrogen 2; Isotec, Miamisburg, OH). Body water was assumed to be 50% of body weight in women and 60% of body weight in men. Additional water ingested during the fast was enriched to 0.5% with ^2H_2O to prevent dilution of the isotopic steady state. During the night, blood glucose concentration was maintained between 5.5 and 6.6 mmol/l with an intravenous infusion of regular insulin. The insulin infusion was discontinued at ~7:00 A.M. the following day. At ~6:00 A.M., an infusion of 6,6$^2H_2$ glucose was started and continued until the end of the study. At 8:00 A.M., patients received a subcutaneous injection of a rapid-acting insulin analog (four received insulin Asparte and five received insulin Lis-pro) at a dose equal to one-half their normal daytime insulin dose. Using the same experimental design, we have recently compared these two insulins in eight patients with type 1 diabetes. Both insulins were given in random order to all patients. No differences were found with respect to onset and duration of insulin actions on plasma glucose, free fatty acids (FFAs), and ketone body levels and on rates of glucose disappearance (G_{Rd}) and EGP (C.H., A. Deluzio, C. Jimenez, J. Kolaczynski, G.B., unpublished observations). Then, plasma glucose levels were kept between 6.2 and 6.7 mmol/l with a variable infusion of 20% dextrose in water. The glucose infusion was discontinued when plasma glucose rose above 7.2 mmol/l. Eight hours after insulin injection the study was terminated; the patients were restarted on their normal insulin regimen, fed a meal, and discharged from the hospital.

Overnight intravenous infusion of regular insulin. Regular human insulin was infused intravenously at rates ranging from 1.5 to 6.0 pmol · kg^{-1} · min^{-1} between 8:00 P.M. and 7:00 A.M. the following day. This maintained plasma glucose concentrations between 5.5 and 6.6 mmol/l.

Euglycemic clamp. At 8:00 A.M., all patients received a subcutaneous injection of 8 ± 1.7 units (range 2–14 units) of a rapid-acting insulin analog. Four patients received insulin Asparte (Novo Nordisk), and the other five received insulin Lis-pro (Elli Lilly). (These two insulin analogs have very similar pharmacokinetic properties and blood glucose, FFA, and ketone body–lowering activities (C.H., A. Deluzio, C. Jimenez, J. Kolaczynski, G.B., unpublished data). Plasma glucose levels were maintained between 6.2 and 6.7 mmol/l with a variable rate infusion of 20% dextrose in water. The glucose infusions were discontinued when plasma glucose levels rose above the target range (7.2 mmol/l). This occurred in most patients between 4 and 5 h after insulin injection.

Glucose turnover. Glucose turnover was determined by isotope dilution analysis with the nonradioactive isotope 6,6$^2H_2$ glucose. At ~6:00 A.M., 6,6$^2H_2$

TABLE 1
Patients

Sex (M/F)	1/8
Age (years)	24 ± 3
Weight (kg)	68.8 ± 3.1
Height (cm)	169 ± 4
BMI (kg/m^2)	24.2 ± 1.2
Duration of type 1 diabetes (years)	7.5 ± 1.5
HbA_{1c} (%)	7.6 ± 0.7
Insulin dose (units/24 h)	33.3 ± 5.1

Data are means ± SE.

glucose was infused intravenously starting with a bolus of 30 μmol/kg followed by a continuous infusion of 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ until the end of the study. Glucose was isolated from blood for determination of isotope enrichment by gas chromatography-mass spectrometry (6). Rates of glucose appearance (G_{Ra}) (Table 2) and disappearance (G_{Rd}) were calculated from the isotope enrichments before (−30 through 0 min) and during the 8-h study using Steele's equation for non–steady-state conditions (7). Underestimation of G_{Ra} during hyperinsulinemia was avoided by adding 6,6-$^2H_2$-glucose (6.9 mmol/100 ml) to the unlabeled glucose infused to maintain euglycemia (8). Because the patients were slightly hyperglycemic at the beginning of the study, the 2-h isotope infusion might not have been sufficient to produce isotopic steady state at that time, which could have resulted in a minor underestimation of G_{Ra}.

GNG and GL. Rates of GNG were determined with the ^2H_2O method of Landau et al. (4). We have used the C5-to-^2H_2O ratio that gives the same results as those obtained with the C5-to-C2 ratio (9). ^2H enrichment of C5 (Table 2) was determined by gas chromatograph–mass spectrometry (5973MSD, HP5890GC; Hewlett Packard) as previously described (9). Enrichment of ^2H in plasma water was determined with an isotope ratio mass spectrometer (PDZ Europa, London) by use of an ABCA-G module and a standard curve with known enrichments ranging from 0.25 to 1.0%. ^2H_2O enrichment was stable throughout the study (Table 2). To correct for the dilution of the ^2H on C5 of glucose, which occurred as result of the infusion of unlabeled glucose during the euglycemic-clamp, the C5-to-^2H_2O ratio was multiplied with G_{Ra}, i.e., the sum of exogenous and endogenous glucose entering the glucose space (10).

GNG (measured in micromoles per kilogram per minute) = ^2H C5/$^2H_2O \times G_{Ra}$. Because non–steady-state conditions existed in the current study, GNG values are approximations of mean values between two timepoints. GL is calculated as the difference between EGP and GNG.

GL (measured in micromoles per kilogram per minute) = EGP − GNG.

This method determines the amount of glucose derived from all GNG precursors that enter the glucose space. It does not detect GNG-derived glucose-6-phosphate, which is deposited in glycogen. Therefore, it underestimates the pyruvate/glycerol to glucose-6-phosphate flux to the extent that there is glycogen cycling.

Indirect calorimetry. Respiratory gas exchange rates were determined before and at hourly intervals until the end of the 8-h study, with a metabolic measurement cart as previously described (11). Rates of protein oxidation were estimated from urinary nitrogen excretion after correction for changes in urea nitrogen pool size (12). Rates of protein oxidation were used to determine npRQ. Rates of carbohydrate oxidation were determined with the npRQ tables of Lusk, which are based on an npRQ of 0.707 for 100% fat and 1.000 for 100% carbohydrate oxidation.

Body composition. Body composition was determined by bioelectrical impedance analysis (13).

Substrate and hormone analyses. Plasma glucose was measured with a glucose analyzer (YSI, Yellow Springs, OH). Free insulin was determined by radioimmunoassay using an antiserum with minimal (0.2%) cross-reactivity with proinsulin (Linco, St. Charles, MO) after deproteinization of serum. Glucagon was determined by radioimmunoassay with a kit from Linco. Plasma FFA, β-hydroxybutyrate, acetoacetate, glycerol, lactate, alanine, glutamine, and glutamate concentrations were determined enzymatically.

Statistical analysis. All data are expressed as the mean ± SE. Statistical analysis was performed using the SAS program (SAS Institute, Cary, NC). ANOVA with repeated measures was used to determine the differences in flux rates, substrates, or hormones across time points. Pairwise comparison for each time point was then performed if the overall comparison was statistically significant.

RESULTS

Insulin, glucose, GIR, and G_{Rd}. Free insulin concentrations increased from 36 ± 6 before to 180 ± 60 pmol/l ($P < 0.01$) 30 min after subcutaneous injection of insulin. After that, insulin levels declined, decreasing to 24 ± 2.4 pmol/l at 8 h (Fig. 1, *upper panel*).

Plasma glucose was 7.6 ± 0.4 mmol/l at 0 min (preinsulin injection). Between 1 and 4 h, plasma glucose was maintained between 6.2 ± 0.3 and 6.7 ± 0.6 mmol/l by intravenous infusion of glucose. Between 4 and 8 h, glucose increased from 6.6 ± 0.4 to 10.5 ± 1.9 mmol/l ($P < 0.02$) (Fig. 1, *middle panel*).

GIR, the rate of glucose infusion necessary to prevent glucose levels from falling below the target range, increased from 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ (the rate of 6,6^{2}H glucose infusion) at 0 min to 10.7 ± 4.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 2 h and thereafter declined to rates not significantly different from 0.3 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 5 h.

G_{Rd} increased from to 16.6 ± 1.2 to 21.4 ± 3.4 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.05$) 2 h after insulin injection and then declined to 13.1 ± 1.2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 8 h (Fig. 1, *bottom panel*).

GNG, GL, and EGP. GNG was 6.5 ± 1.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ before insulin injection and remained at about this level for the first 3 h after insulin injection. Between 3 and 4 h, GNG decreased to 4.2 ± 1.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ ($P < 0.03$). However, the decrease became statistically significant only at this one time point; therefore, its physiological relevance is uncertain. After that, GNG increased to 5.5 ± 1.0 $\mu mol \cdot kg^{-1} \cdot min^{-1}$ at 8 h. However, this increase did not become statistically significant (Fig. 2, *top panel*).

GL decreased from 10.7 ± 0.5 before to 1.0 ± 1.3 μmol

TABLE 2

Hours	$^2H \cdot C5$ (% enrichment)	^2H_2O (% enrichment)	$^2H \cdot C5/^2H_2O$	G_{Ra} ($\mu mol \cdot kg^{-1} \cdot min^{-1}$)
0	0.152 ± 0.017	0.383 ± 0.010	0.357 ± 0.396	17.4 ± 1.16
1	0.179 ± 0.017	0.373 ± 0.010	0.432 ± 0.034	11.7 ± 2.1
2	0.144 ± 0.021	0.368 ± 0.011	0.346 ± 0.047	20.9 ± 3.9
3	0.118 ± 0.021	0.367 ± 0.011	0.285 ± 0.050	19.9 ± 2.1
4	0.103 ± 0.016	0.367 ± 0.012	0.251 ± 0.040	16.0 ± 1.9
5	0.116 ± 0.020	0.369 ± 0.011	0.282 ± 0.048	15.5 ± 1.8
6	0.134 ± 0.015	0.368 ± 0.009	0.325 ± 0.032	16.0 ± 1.7
7	0.138 ± 0.022	0.351 ± 0.012	0.350 ± 0.055	14.9 ± 1.4
8	0.157 ± 0.023	0.355 ± 0.008	0.389 ± 0.053	13.9 ± 1.4

Data are means ± SE.

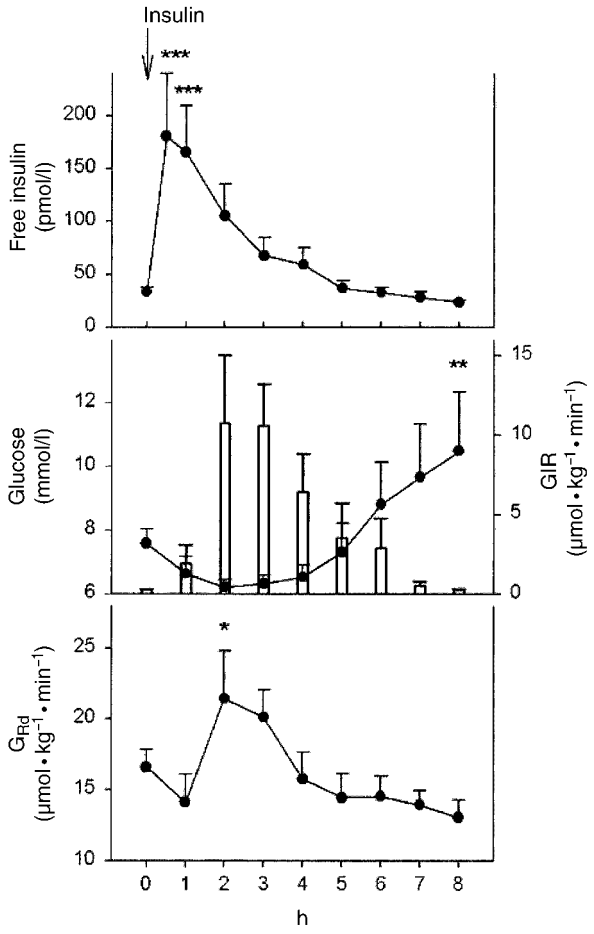

FIG. 1. Effects of subcutaneous insulin injection on serum free insulin and plasma glucose levels and on GIRs (□) and G_{Rd} in nine patients with type 1 diabetes. Shown are means ± SE. Statistical analysis: *P < 0.04 vs. 1 h; **P < 0.02 vs. 4 h; ***P < 0.01 vs. 0 h.

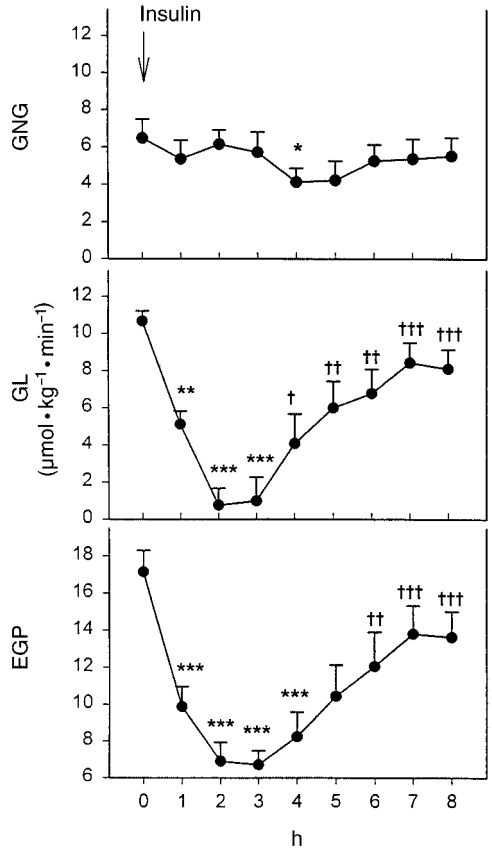

FIG. 2. Effects of subcutaneous insulin on rates of GNG (*A*), GL (*B*), and EGP (*C*) in nine patients with type 1 diabetes. Statistical analysis: *P < 0.03 vs. 0 h; **P < 0.02 vs. 0 h; ***P < 0.001 vs. 0 h; †P < 0.04 vs. 3 h; ††P < 0.01 vs. 3 h; †††P < 0.001 vs. 3 h.

· kg^{-1} · min^{-1}) 3 h after insulin injection ($P < 0.001$). After that, GL increased again reaching 8.1 ± 1.0 μmol · kg^{-1} · min^{-1} ($P < 0.001$) after 8 h (Fig. 2, *middle panel*). EGP mirrored the changes in GL, declining from 17.1 ± 1.2 to 6.7 ± 0.8 μmol · kg^{-1} · min^{-1} ($P < 0.01$) at 3 h and then increasing to 13.6 ± 1.4 μmol · kg^{-1} · min^{-1} ($P < 0.001$) at 8 h (Fig. 2, *bottom panel*).

FFA, ketone bodies, fat, and carbohydrate oxidation. Plasma FFA levels decreased from 585 ± 93 before to 220 ± 63 μmol/l 2 h after insulin injection and then increased to 1,157 ± 150 μmol/l at 8 h (Fig. 3, *bottom panel*). Total ketone body levels (sum of β-hydroxybutyrate and acetoacetate levels) decreased from 373 ± 100 before to 125 ± 24 μmol/l 2 h after insulin injection and then increased to 1,145 ± 214 μmol/l after 8 h (Fig. 3, *top panel*). Fat oxidation rates (calculated with 860 mol fat) declined from 1.1 ± 0.1 (at 0 h) to 0.8 ± 0.1 μmol · kg^{-1} · min^{-1} (at 3 h) ($P < 0.04$) and then increased to 1.3 ± 0.1 μmol · kg^{-1} · min^{-1} ($P < 0.002$) at the end of the studies (Fig. 3, *middle panel*). CHO oxidation rates increased from 4.6 ± 1.4 (at 0 h) to 9.4 ± 1.6 μmol · kg^{-1} · min^{-1} (at 3 h) ($P < 0.05$) and then declined, reaching 5.6 ± 1.2 μmol · kg^{-1} · min^{-1} ($P < 0.04$) at the end of the study.

Plasma concentrations of three (lactate, glycerol, and glutamate) of the five measured GNG precursors declined during the initial 3 h. Glycerol decreased from 64 ± 10 to 19 ± 3 μmol/l ($P < 0.001$), glutamine decreased from 411 ± 35 to 368 ± 31 μmol/l ($P < 0.03$), and lactate decreased from 787 ± 28 to 659 ± 54 μmol/l ($P < 0.04$). Total GNG precursor concentration, however, did not change significantly (1.69 ± 0.05 vs. 1.53 ± 0.08 mmol/l, NS).

Glucagon, epinephrine, norepinephrine, and cortisol. Plasma glucagon decreased from 46 ± 4 to 36 ± 3 pg/ml ($P < 0.01$) between 0 and 2 h and then returned to 49 ± 8 pg/ml ($P < 0.03$) between 2 and 8 h. Plasma epinephrine and norepinephrine levels were 55 ± 20 and 225 ± 75 pg/ml at 0 min and did not change significantly during the 8 h studies. Plasma cortisol decreased from 376 ± 38 to 203 ± 21 nmol/l ($P < 0.03$) between 0 and 4 h and after that tended to increase (to 347 ± 90 nmol/l (NS).

DISCUSSION

Insulin excess. Subcutaneous injection of rapidly acting insulin increased plasma free insulin fivefold within 30 min (from 36 to 180 pmol/l) and resulted in a 29% increase (from 16.6 to 21.4 μmol · kg^{-1} · min^{-1}) in G_{Rd} and a 46% decrease (from 17.1 to 9.5 μmol · kg^{-1} · min^{-1}) in EGP. This would have resulted in a sharp decrease in blood glucose concentration and release of several glucose counterregulatory hormones. These events, however, were prevented by intravenous infusion of glucose, which maintained near euglycemia and enabled us to study the effects of selective insulin excess on GNG and GL. The results showed that

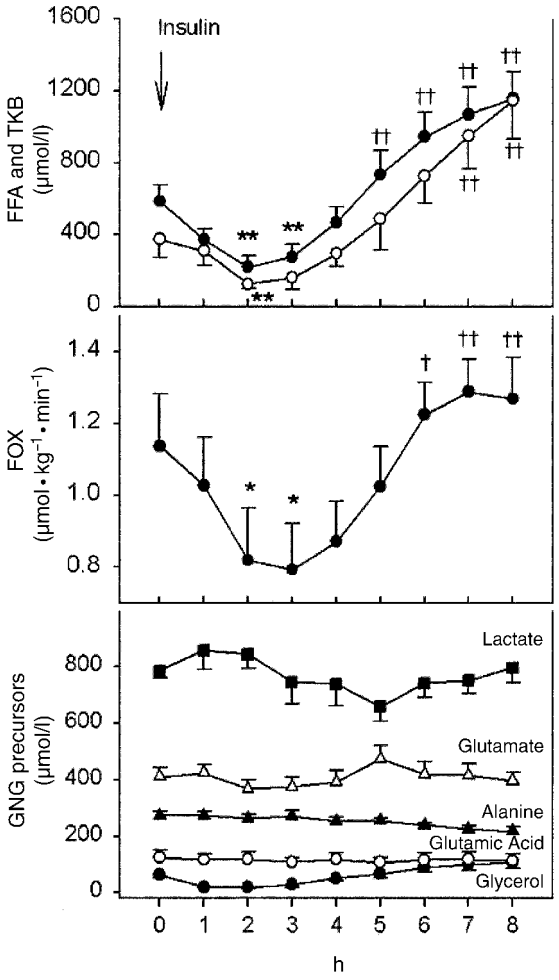

FIG. 3. Effects of subcutaneous insulin on plasma FFA (●) and total ketone body (TKB) levels (○) (*A*), on rates on fat oxidation (FOX) (*B*), and on plasma levels of five GNG precursors. Statistical analysis: **P* < 0.05 vs. 0 h; ***P* < 0.03 vs. 0 h; †*P* < 0.05 vs. 4 h; ††*P* < 0.01 vs. 4 h.

the rapid (within 1 h) 43% insulin-induced decrease in EGP was the result of a 54% decrease in GL. Similar results have recently been reported in normal subjects (10,14) and in dogs (15). Gastadelli et al. (10) and Boden et al. (14), using the ^2H_2O method to determine GNG, reported that acute increases in peripheral serum insulin to ~600 pmol/l decreased GNG modestly but totally abolished GL within 4 h. Edgerton et al. (15) examined acute effects of much smaller increases (from 60 to 114 pmol/l) in peripheral serum insulin in dogs using three different methods to measure GNG (^2H_2O, arteriovenous difference of GNG precursors, and ^{14}C phosphoenolpyruvate). These investigators also came to the conclusion that insulin decreases EGP acutely by lowering GL.

Insulin deficiency. The period of insulin excess had waned ~3 h after insulin injection and was followed by a period of slowly increasing insulin deficiency. This period was characterized by an approximate 30% decrease in free insulin below basal levels, a 35% decrease of G_{Rd} (from 20.1 to 13.1 μmol · kg^{-1} · min^{-1}), and an approximate 103% increase in EGP (from 6.7 to 13.6 μmol · kg^{-1} · min^{-1}), which together resulted in an increase in plasma glucose (from 6.6 to 10.5 mmol/l) and an increase in FFA and ketone body levels (from ~200 to >1,110 μmol/l). The increase in EGP was almost entirely due to an increase in GL (from 1.0 to 8.1 μmol · kg^{-1} · min^{-1}) with only a very small and statistically nonsignificant contribution from GNG. These findings indicated that in patients with type 1 diabetes, increases in EGP and plasma glucose concentrations during the early stages of insulin deficiency were essentially due to increased GL. Taken together with results of effects of insulin excess in patients with type 1 diabetes (this study) and in nondiabetic individuals (10,14), the data suggest that acute regulation of EGP by insulin in diabetic and in nondiabetic subjects is mainly via changes in GL and that GNG changes little or not at all during the early phases of either insulin excess or deficiency.

This represents a significant change from the previously held belief that insulin acutely regulates GNG (16). This belief was based to a large extent on in vitro data that showed rapid and profound suppression by insulin of the expression of several key GNG enzymes, including PEPCK, fructose-1–6-phosphatase, and glucose-6-phosphatase) (17, 18). The apparent discrepancy between the in vitro and in vivo data may have several reasons but can best be explained by the long half-life of these enzymes; for instance, the half-life of PEPCK is ~6 h (19). Thus, even if insulin had suppressed enzyme expression completely within 1–2 h, there would still be sufficient enzyme protein available to maintain GNG activity for several hours albeit at a continuously decreasing rate. The decline in GNG (from 6.5 to 4.3 μmol · kg^{-1} · min^{-1}) at 4 h was compatible with this concept. On the other hand, the decrease in plasma FFA and perhaps also the decrease in glycerol, glutamate, and lactate levels, which occurred at that time, may have played contributory roles (9).

The mechanism by which insulin acutely suppresses GL was not investigated. Since insulin acutely lowered plasma glucagon, known to be a potent regulator of GL, one might suspect that the decrease in glucagon could have been responsible for the decrease in GL. We believe, however, that this was very unlikely, as we have recently shown that under very similar experimental conditions acute hyperinsulinemia suppressed GL to the same extent, regardless of whether glucagon levels decreased (as in the present study) or were prevented from decreasing (with pancreatic clamping and glucagon infusion) (14).

The finding that insulin reduces EGP primarily by suppressing GL may explain, at least in part, why insulin action is decreased in conditions associated with reduced liver glycogen content, for instance during starvation or in patients with in chronic cirrhosis of the liver (20) and in conditions where the breakdown of liver glycogen is impaired, such as in glycogen storage diseases.

Lastly, it is likely that chronic effects of insulin deficiency on GNG/GL, which have not yet been investigated with newer methods, may be different from the effects of acute insulin deficiency described here. This is suggested by the finding that elevated rates of GNG in patients with type 2 diabetes correlated best with more advanced degrees of insulin deficiency (21).

In summary, we have determined rates of GNG and GL in nine patients with type 1 diabetes during short periods of acute insulin excess and deficiency. The decreases in EGP and blood glucose that occurred during insulin

excess were virtually completely accounted for by suppression of GL, while the increases in EGP and blood glucose during insulin deficiency were caused by a rise in GL. These results suggest that acute regulation of EGP in type 1 diabetes is via regulation of GL with little or no participation of GNG.

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