Failure of Substrate-induced Gluconeogenesis to Increase Overall Glucose Appearance in Normal Humans

Demonstration of Hepatic Autoregulation without a Change in Plasma Glucose Concentration

Trond Jenssen, Nurjahan Nurjhan, Agostino Consoli, and John E. Gerich
Clinical Research Center, Departments of Medicine and Physiology, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania 15261

Abstract

It has been proposed that increased supply of gluconeogenic precursors may be largely responsible for the increased gluconeogenesis which contributes to fasting hyperglycemia in non-insulin-dependent diabetes mellitus (NIDDM). Therefore, to test the hypothesis that an increase in gluconeogenic substrate supply per se could increase hepatic glucose output sufficiently to cause fasting hyperglycemia, we infused normal volunteers with sodium lactate at a rate approximately double the rate of appearance observed in NIDDM while clamping plasma insulin, glucagon, and growth hormone at basal levels. In control experiments, sodium bicarbonate was infused instead of sodium lactate at equimolar rates. In both experiments, [6-^{3}H]-glucose was infused to measure glucose appearance and either [U-^{14}C]lactate or [U-^{14}C]alanine was infused to measure the rates of appearance and conversion of these substrates into plasma glucose. Plasma insulin, glucagon, growth hormone, C-peptide, and glycerol concentrations, and blood bicarbonate and pH in control and lactate infusion experiments were not significantly different. Infusion of lactate increased plasma lactate and alanine to 4.48±3 mM and 610±33 μM, respectively, from baseline values of 1.6±0.2 mM and 431±28 μM, both $P < 0.01$; lactate and alanine rates of appearance increased to 38±1.0 and 8.0±0.3 μmol/kg per min ($P < 0.01$ versus basal rates of 14.4±0.4 and 5.0±0.5 μmol/kg per min, respectively). With correction for Krebs cycle carbon exchange, lactate incorporation into plasma glucose increased nearly threefold to 10.4 μmol/kg per min and accounted for about 50% of overall glucose appearance. Alanine incorporation into plasma glucose increased more than twofold. Despite this marked increase in gluconeogenesis, neither overall hepatic glucose output nor plasma glucose increased and each was not significantly different from values observed in control experiments (10.8±0.5 vs. 10.8±0.5 μmol/kg per min and 5.4±0.4 vs. 5.3±0.3 mM, respectively). We, therefore, conclude that in normal humans there is an autoregulatory process independent of changes in plasma glucose and glucoregulatory hormone concentrations which prevents a substrate-induced increase in gluconeogenesis from increasing overall hepatic glucose output; since this process cannot be explained on the basis of inhibition of gluconeogenesis from other substrates, it probably involves diminution of glycogenolysis. A defect in this process could explain at least in part the increased hepatic glucose output found in NIDDM. (*J. Clin. Invest.* 1990. 86:489–497.) Key words: glucogenolysis • gluconeogenesis • hepatic glucose production • lactate

Introduction

Output of glucose from the liver is the result of two processes, glycogenolysis and gluconeogenesis. Although glycogenolysis normally accounts for more than 70% of hepatic glucose output in the postabsorptive state (1, 2), in other situations, such as postprandial hepatic glycogen repletion (3), recovery from hypoglycemia (4, 5) and non-insulin-dependent diabetes mellitus (NIDDM)[1] (6), gluconeogenesis may be the dominant process.

It remains unclear what critical factors determine the relative contributions of gluconeogenesis and glycogenolysis to overall hepatic glucose output in these situations.

Gluconeogenesis may be regulated at three sites: delivery of substrate from peripheral tissues, transport of substrate into hepatocytes, and intrahepatic conversion of substrate to glucose (7). Experiments using the perfused rat liver have demonstrated a linear relationship between gluconeogenesis and perfusate lactate and pyruvate concentrations over their physiologic range (8). In renal failure (9) and maple syrup urine disease (10) where gluconeogenic substrate availability is reduced, there is reduced gluconeogenesis and reduced overall hepatic glucose output. Conversely, in NIDDM, where circulating levels of gluconeogenic precursors have been noted to be increased (6, 11–13), there is increased gluconeogenesis (6, 14, 15) and increased overall hepatic glucose output (6, 11, 14–16).

These observations support the concept that substrate availability is a critical factor regulating gluconeogenesis and overall hepatic glucose output. Indeed it has been suggested that increased substrate delivery may be a major factor responsible for the increased gluconeogenesis and associated fasting hyperglycemia found in NIDDM (13, 16–18).

However, whether an increase in substrate availability, such as may occur under physiologic conditions, can augment gluconeogenesis enough to cause fasting hyperglycemia remains to be established (19–22). An autoregulatory effect of glucose on hepatic release of glucose formed via gluconeogenesis has been demonstrated in vitro (23, 24) and in the dog (25).

Dr. Jenssen's present address is Department of Medicine, University Hospital of Tromsoe, N-9012 Tromsoe, Norway.

Address reprint requests to Dr. Gerich, Clinical Research Center, 3488 Presbyterian-University Hospital, Pittsburgh, PA 15261.

Received for publication 8 September 1989 and in revised form 6 April 1990.

J. Clin. Invest.

0021-9738/90/08/0489/09 $2.00
Volume 86, August 1990, 489–497

1. *Abbreviations used in this paper:* NIDDM, non-insulin-dependent diabetes mellitus.

If this process were active in humans, it would limit the extent to which gluconeogenesis could increase hepatic glucose output and cause fasting hyperglycemia.

The present studies were therefore undertaken to test the hypothesis that an increase in substrate supply, independent of hormonal influences, could augment overall hepatic glucose output and cause fasting hyperglycemia in normal human volunteers. For this purpose, we infused lactate, the predominant gluconeogenic precursor (26) for 3 h at a rate approximately double the rate of appearance found in patients with NIDDM (18) while plasma insulin, glucagon, and growth hormone concentrations were clamped (27) at baseline levels. Under these conditions, gluconeogenesis increased more than twofold and was estimated to account for ~ 50% of glucose appearance; however, neither plasma glucose nor overall hepatic glucose output increased. We therefore conclude that in normal humans an increase in gluconeogenic substrate availability is insufficient in itself to produce fasting hyperglycemia and that there is an autoregulatory process which can prevent a substrate-induced increase in gluconeogenesis from increasing overall hepatic glucose output even in the absence of an increase in plasma glucose concentration.

Methods

Subjects. Informed written consent was obtained from 10 (four men and six women) healthy, nonobese (body mass index 24±1 kg/m^2) subjects aged 18–47 yr (mean±SEM 29±3 yr). None of the subjects were taking medication and none had a family history of diabetes mellitus; all were screened 2–4 wk before the experiments with a medical history, physical examination, and routine blood-work to exclude illness. Subjects consumed a weight-maintaining diet containing 200 g of carbohydrates for 3 d before the study.

Protocol. After obtaining approval from the University of Pittsburgh Institutional Review Board subjects were admitted to the University of Pittsburgh Clinical Research Center on two occasions between 5:00 and 7:00 p.m. the day before the experiments and were given a standard dinner (10 kcal/kg, 50% carbohydrate, 35% fat, 15% protein) between 6:00 and 8:00 p.m. At approximately 4:30 a.m. the next morning, the dorsal vein on the left hand was retrogradely cannulated and placed in a thermoregulated device maintained at 65°C for sampling of arterialized venous blood (28). In addition, a deep antecubital vein was cannulated on the opposite arm to obtain venous blood. At 5:00 a.m. primed (30 μCi), continuous infusions (0.3 μCi/min) of [6-^{3}H]glucose and [U-^{14}C]lactate (or [U^{14}C]alanine) (Amersham Corp., Arlington Heights, IL) were started in an antecubital vein of the left arm. To avoid potential confounding effects of changes in plasma glucoregulatory hormone levels, endogenous secretion of insulin, glucagon, and growth hormone was suppressed by infusion of somatostatin; exogenous insulin, glucagon, and growth hormone were infused as described by DeFeo et al. (27) in amounts designed to reproduce normal basal systemic levels. Briefly, at 6:00 a.m., a 250-μg/h somatostatin infusion was started along with constant infusions of glucagon (0.7 ng/kg per min) and growth hormone (3 ng/kg per min). The insulin infusion rate was initially adjusted to maintain plasma glucose concentrations between 6 and 6.5 mM; once plasma glucose concentrations were stable in this range for 30 min, the insulin infusion rate was fixed for the rest of the study. At approximately 9:00 a.m. either a 3-h continuous infusion of Na^+-L-lactate (30 μmol/kg per min) or an equimolar amount of Na-bicarbonate (control experiments) was started. The order of the infusions was random. Na-L-lactate was prepared by adjusting the acid form of L-lactate (Sigma Chemical Co., St. Louis, MO) with 10 N NaOH to pH 5.10, filtering the resultant solution through a filter (0.22 μM, Millipore Corp., Bedford, MA) and steam autoclaving it for 30 min. The Na^+-L-lactate was then kept at 4°C overnight before experiments. The lactate concentration of the infusate was measured in each experiment. Blood was drawn at −30, 0, 60, 120, 150, and 180 min for determination of plasma substrate and hormone concentrations, specific activities of glucose and lactate (or alanine), and blood pH and bicarbonate content. Whole body gas exchange was measured by indirect calorimetry at 0, 120, and 180 min, respectively, using the metabolic measurement cart (Sensormedics, Anaheim, CA), as previously described (29). Immediately before and after these measurements $^{14}CO_2$ in expiratory air was collected by directing the expiratory air through a 0.5 M hyamine hydroxide ethanol solution.

Analytical procedures. Plasma glucose was determined in duplicate on a glucose analyzer (Yellow Springs Instrument Co., Yellow Springs, OH). Plasma lactate, alanine, and glycerol were determined by standard fluorometric methods (30–32). The specific activities of plasma lactate and glucose were determined after isolating lactate and glucose using ion-exchange chromatography (33). The radioactivity of the isolated lactate and glucose were corrected for recovery using external standards. A co-oximeter (Instrumentation Laboratory, Inc., Lexington, MA) (29) was used to measure blood pH, P_{CO_2}, and bicarbonate concentrations. Plasma insulin (34), glucagon (35), growth hormone (36), and C-peptide (37) were measured by radioimmunoassay methods previously reported.

Calculations. The rates of appearance (*Ra*) and disappearance (*Rd*) of glucose, lactate, and alanine were determined with the nonsteady-state equation of DeBodo et al. (38) using a pool fraction of 0.65 and volumes of distribution of 200, 500, and 300 ml/kg, respectively, for glucose (38), lactate (39), and alanine (40).

The rate of lactate conversion to glucose was determined using the nonsteady-state equation of Chiasson et al. (41): [Glucose $Rd \times$ [^{14}C]-glucose SA + PV ([^{14}C]glucose$_2$ − [^{14}C]glucose$_1$)$(t_2 - t_1)^{-1}$]/[^{14}C]-Lactate SA, where glucose *Rd*, [^{14}C]glucose SA, and [^{14}C]lactate SA represent the mean values of plasma glucose *Rd*, [^{14}C]glucose SA, and [^{14}C]lactate SA between two consecutive time points. Δ[^{14}C]glucose radioactivity and Δt represent the Δ values for plasma glucose counts and time expressed in disintegrations per minute per milliliter and in minutes, respectively. P is the fractional constant and V the volume of distribution for glucose.

The percentage of glucose *Ra* derived from lactate was calculated according to the equation: [Lactate conversion to glucose (0.5)]/glucose *Ra* × 100.

The percent of lactate *Rd* converted to glucose was calculated according to the equation: Lactate conversion to glucose/Lactate *Rd* × 100.

Since the infusion of sodium lactate and sodium bicarbonate introduces a nonsteady-state situation in the bicarbonate pool, rates of lactate oxidation were determined by a revised version of the equation of Chiasson et al. (41) applied to the rate of $^{14}CO_2$ elimination from whole body. It is assumed that the specific activity of the $^{14}CO_2$ in the pulmonary artery is the same as the specific activity of $^{14}CO_2$ in expiratory air and that potential changes in the bicarbonate pool do not affect the recovery of label. The rate of $^{14}CO_2$ production in whole body is, therefore, reflected by the rate of disappearance of $^{14}CO_2$ measured in expiratory air, and the equation of Chiasson et al. can be applied as follows: Rate of oxidation of ^{14}C-labeled lactate in whole body = [(CO_2 expired × $SACO_2$) + PV ($SACO_{2_2}$ × venous $P_{CO_{2_2}}$ − $SACO_{2_1}$ × Venous $P_{CO_{2_1}}$)$(t_2 - t_1)^{-1}$]/([^{14}C]lactate SA × 3), where CO_2 expired is the mean rate of whole-body CO_2 production as measured in the expiratory air (micromoles per minute) and $SACO_2$ is mean specific activity of $^{14}CO_2$ (disintegrations per minute per micromole) in expiratory air between two time points. The expression $SACO_2$ × venous P_{CO_2} gives CO_2 radioactivity (disintegrations per minute per milliliter) at the corresponding time points t_2 and t_1, respectively. [^{14}C]Lactate SA is the mean corresponding specific activity of the ^{14}C-labeled lactate. Volume of distribution for CO_2(V) is set to total body water (600 ml/kg) and the fractional constant is set to 1, assuming total mixing in the CO_2 pool. In the experiments in which [^{14}C]alanine was infused, alanine oxidation was calculated in an analogous way.

Unless stated otherwise, data presented are means±SEM and were analyzed with analysis of variance; $P < 0.05$ was considered significant.

Results

Plasma insulin, C-peptide, glucagon, and growth hormone (Fig. 1). Baseline plasma insulin, C-peptide, glucagon, and growth hormone concentrations were comparable in lactate (77±10 pM, 0.113±0.012 nM, 214±31 ng/liter, and 1.73±0.07 μg/liter, respectively) and bicarbonate infusion experiments (78±11 pM, 0.096±0.015 nM, 231±15 ng/liter, and 1.86±0.07 μg/liter, respectively).

All hormone levels remained constant during both experiments and did not differ significantly in control and lactate infusion studies.

Blood pH and bicarbonate concentrations (Fig. 2). Baseline blood pH and bicarbonate concentrations were not significantly different in control and lactate infusion experiments (pH 7.39±0.001 and 7.38±0.01, respectively; bicarbonate 25.2±0.3 and 26.1±1.2 meq/liter, respectively). Blood bicarbonate increased significantly in both experiments, but the levels attained in both experiments were not significantly different from one another. During the final hour of the study, blood bicarbonate was 31.0±1.4 meq/liter in the control experiments vs. 32.4±0.6 meq/liter, in the lactate experiments P = NS. Blood pH also increased significantly in both experiments, but values attained in both experiments were not significantly different from one another. During the last hour, pH was 7.46±0.01 and 7.48±0.01 in control and lactate experiments, respectively.

Plasma lactate, alanine, and glycerol concentrations (Fig. 3). Baseline plasma lactate in the control and lactate infusion experiments (1.31±0.12 and 1.28±0.10 mM, respectively) were not significantly different. In the control experiment, plasma lactate did not change; in the lactate infusion experiment, plasma lactate increased to 4.48±0.38 mM during the last hour.

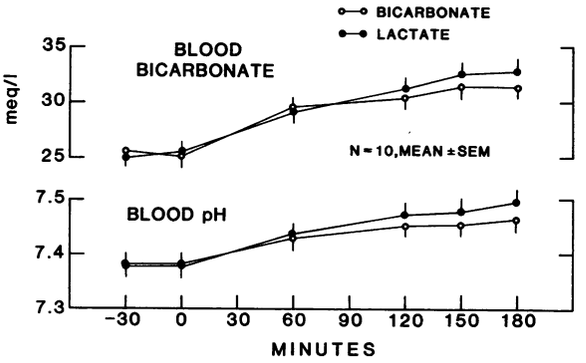

Figure 2. Blood pH and bicarbonate concentrations.

Baseline plasma alanine in control and lactate infusion experiments (417±34 and 431±28 μM, respectively) were not significantly different. A slight, but significant, decrease in plasma alanine occurred during bicarbonate infusions so that values during the last hour were 351±28 μM ($P < 0.01$). Conversely, during the lactate infusion experiments, plasma alanine increased significantly to 610±33 μM ($P < 0.01$) during the last hour.

Baseline plasma glycerol concentrations were comparable in both experiments and did not change significantly during bicarbonate and lactate infusion experiments.

Plasma lactate appearance, disappearance, and conversion to plasma glucose, and CO_2 (Fig. 4). Baseline plasma lactate rates of appearance were comparable in control and lactate infusion experiments (14.5±0.4 vs. 14.4±0.4 μmol/kg per min, respectively). With bicarbonate infusion (control experiments), plasma lactate appearance decreased slightly but significantly ($P < 0.01$) to 12.9±0.3 μmol/kg per min during the last hour. In the lactate infusion experiments, plasma lactate

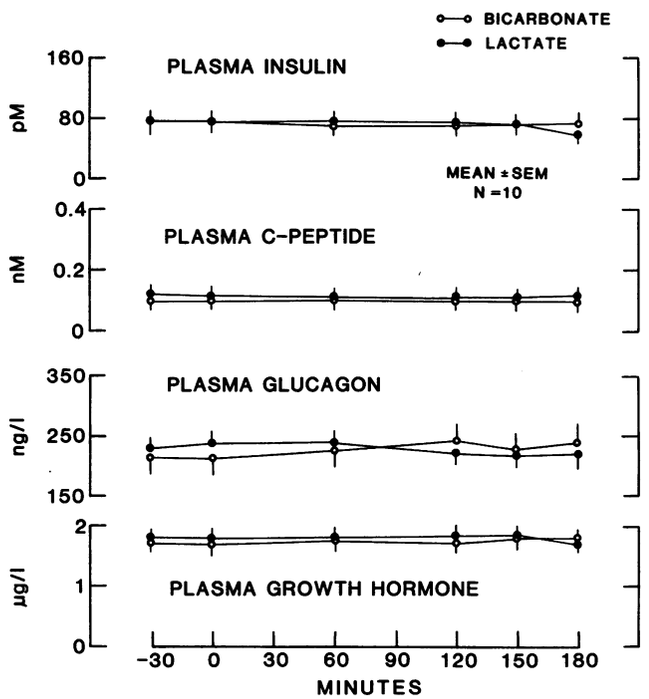

Figure 1. Plasma insulin, C-peptide, glucagon, and growth hormone concentrations.

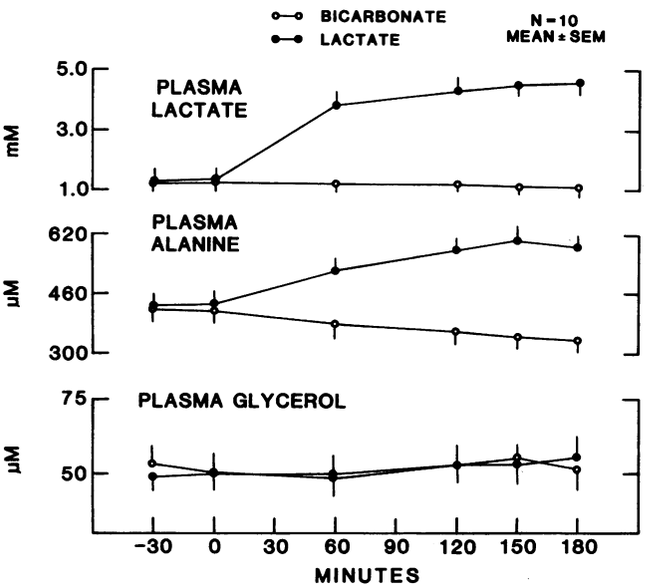

Figure 3. Plasma lactate, alanine, and glycerol concentrations.

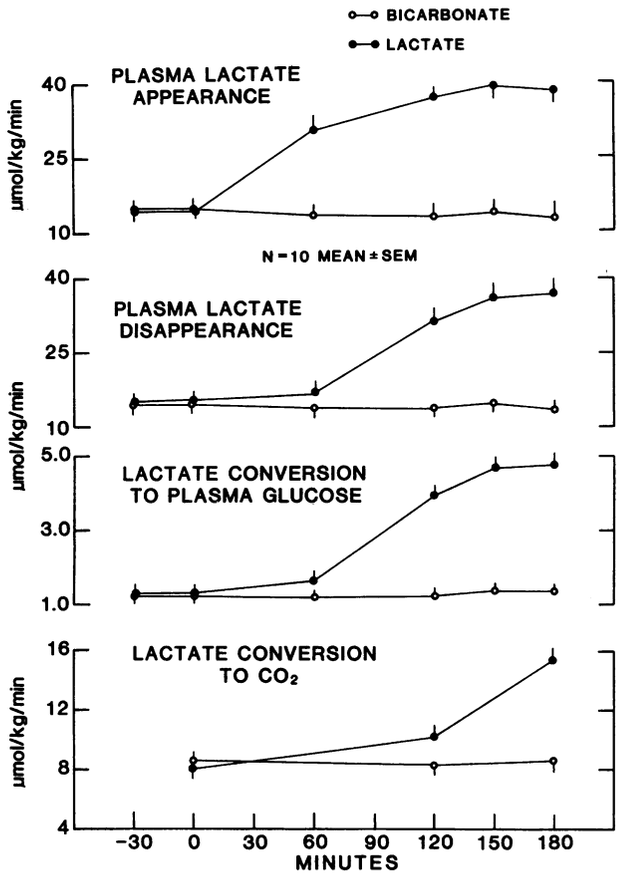

Figure 4. Rates of plasma lactate appearance (*Ra*), disappearance (*Rd*), and conversion to plasma glucose and CO_2.

appearance increased to 38.0±1.0 μmol/kg per min ($P < 0.001$) during the last hour.

Baseline plasma lactate rates of disappearance were comparable in control and lactate infusion experiments (14.5±0.5 vs. 14.8±0.5 μmol/kg per min, respectively, P = NS). In control experiments, plasma lactate disappearance did not change significantly and averaged 13.7±0.2 μmol/kg per min during the last hour. In lactate infusion experiments, plasma lactate disappearance increased significantly to 34.9±0.8 μmol/kg per min during the last hour.

Baseline rates of lactate conversion to glucose were comparable in control and lactate infusion experiments (1.4±0.2 and 1.6±0.2 μmol/kg per min, respectively, P = NS). The percentage of plasma lactate disappearance accounted for by conversion to glucose was also comparable in control (9.3±1.3%) and lactate (9.2±1.1%) infusion experiments. With bicarbonate infusion (control experiments), lactate conversion to glucose remained constant, averaging 1.5±0.2 μmol/kg per min during the last hour. In the lactate infusion experiments, lactate conversion to plasma glucose increased more than twofold to 3.5±0.4 μmol/kg per min during the last hour. The percent of lactate disappearance accounted for by conversion to plasma glucose was slightly but significantly greater in lactate infusion experiments than in control experiments (13.0±0.8 vs. 10.5±1.5, $P < 0.05$).

Baseline rates of plasma lactate oxidation were comparable in control and lactate infusion experiments (8.5±0.6 vs. 8.0±0.5 μmol/kg per min, respectively) and accounted for 57±4% and 54±3% of lactate disappearance in control and lactate infusion experiments, respectively. During bicarbonate infusion, lactate oxidation did not change significantly and averaged 7.9±0.6 μmol/kg per min during the last 30 min. During lactate infusions lactate oxidation increased significantly to 15.3±1.0 μmol/kg per min during the last 30 min. The percentage of lactate disappearance accounted for by oxidation did not change in control experiments (57±5%); however, in lactate infusion experiments, it decreased significantly to 43±1% during the last 30 min, $P < 0.05$.

Plasma [^{14}C] glucose radioactivity, percentage of plasma glucose derived from lactate, plasma glucose rate of appearance, and plasma glucose concentrations (Fig. 5, Table I). Plasma ^{14}C-glucose radioactivity did not change from baseline during the bicarbonate infusion while it increased consistently to approach a new steady state between times 150 and 180 min during lactate infusion (Table I). At baseline the percentage of plasma glucose derived from plasma lactate was comparable in control (5.8±0.7%) and lactate infusion experiments (5.6±0.5%). Plasma glucose rates of appearance (12.1±0.3 and 12.4±0.2 μmol/kg per min), and plasma glucose concentrations (6.5±0.2 and 6.4±0.2 mM) were also comparable.

In control experiments, the percent of plasma glucose derived from lactate did not change significantly, averaging 7.2±1.0 during the last hour. In contrast, during lactate infusion experiments, the percentage of plasma glucose derived from lactate increased more than threefold to 20.9±1.5 during the last hour. Nevertheless the rate of appearance of glucose and the plasma glucose concentration did not increase and during the last hour of the lactate infusion (10.8±0.5 μmol/kg per min and 5.4±0.4 mM) were not significantly different from respective values observed in control experiments (10.8±0.5 μmol/kg per min and 5.3±0.3 mM).

Plasma alanine kinetics during infusion of lactate (Table II). Since it has been suggested that provision of one gluconeogenic precursor might diminish incorporation of another precursor into glucose (19, 42), [U-^{14}C]alanine was infused, instead of [U-^{14}C]lactate, in three additional subjects during

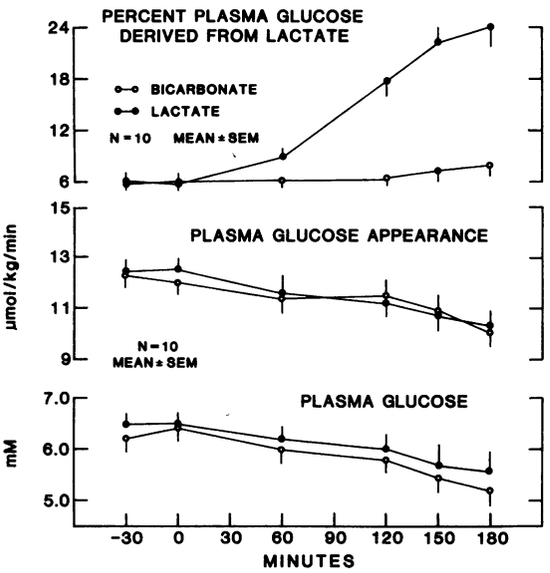

Figure 5. Percent plasma glucose-derived lactate, plasma glucose appearance, and plasma glucose.

Table I. Plasma Glucose ^{14}C Concentration in Control (Bicarbonate) and Lactate Infusion Experiments

	Time					
	min					
Experiment	−30	0	60	120	150	180
	dpm/ml					
Control	395±61	390±51	394±67	401±64	398±63	407±71
Lactate	423±42	425±42	480±44	562±41*	577±47*	598±57*

Values given as mean±SEM. * $P < 0.01$ vs. min 0.

lactate infusions to determine how infusion of lactate affected the rate of alanine conversion to plasma glucose. Data from these experiments are given in Table II.

Plasma insulin, glucagon, growth hormone, C-peptide, bicarbonate, glucose pH, and rates of glucose appearance were not significantly different from those observed in control experiments and in the other lactate infusion experiments.

Plasma lactate and alanine increased to 4.8±0.4 mM and 646±68 μM, respectively, during the last hour of the lactate infusion, comparable to respective values observed in the other lactate infusion experiments (4.5±0.4 mM and 610±33 μM). At baseline rates of appearance and disappearance of plasma alanine were 5.0±0.5 and 5.0±0.5 μmol/kg per min, respectively. During infusion of lactate, plasma alanine appearance increased to 8.0±0.3 μmol/kg per min during the last hour and its disappearance increased to 8.1±4 μmol/kg per min.

The rate of incorporation of alanine into plasma glucose was 1.3±0.1 μmol/kg per min at baseline and increased in all subjects to 2.9±0.3 μmol/kg per min during the last hour of the lactate infusion. The percent of alanine disappearance accounted for by conversion to plasma glucose increased during the lactate infusion (26±4 at baseline vs. 36±3 during the last hour). Alanine conversion to plasma glucose accounted for 5.7±0.5% of glucose appearance at baseline and 14.7±1.1% during the last hour of the lactate infusion. Alanine oxidation was 2.3±0.2 μmol/kg per min at baseline and increased in all subjects to 4.0±0.5 μmol/kg per min during the last 30 min. However, the percent of alanine disappearance accounted for by oxidation did not change (46±4 at baseline; 50±4 during the last 30 min).

Discussion

Substrate supply is generally considered to be an important determinant of gluconeogenesis and overall hepatic glucose output (7, 8). Therefore the observations that patients with NIDDM have increased circulating concentrations of gluconeogenic precursors (6, 11–13) associated with increased rates of gluconeogenesis (6, 14, 15) and increased overall hepatic

Table II. Plasma Hormone and Substrate Concentrations and Plasma Alanine and Glucose Kinetics during [U-^{14}C]Alanine Experiments

	Time					
	min					
Experiment	−30	0	60	120	150	180
Plasma insulin (*pM*)	56±7	59±6	60±3	56±6	52±5	50±5
Plasma C-peptide (*nM*)	0.12±0.03	0.13±0.04	0.09±0.02	0.12±0.04	0.12±0.04	0.11±0.03
Plasma glucagon (*ng/liter*)	245±40	234±26	255±15	229±21	243±10	234±11
Plasma growth hormone (*μg/liter*)	2.2±0.3	1.9±0.3	1.7±0.2	1.6±0.1	1.7±0.1	1.5±0.1
Plasma lactate (*mM*)	1.2±0.1	1.2±0.1	3.7±0.4	4.2±0.3	4.8±0.5	4.9±0.5
Plasma alanine (*μM*)	410±51	417±60	550±50	645±65	643±72	650±71
Blood bicarbonate (*meq/liter*)	22.2±1.9	21.3±2.3	26.6±1.5	30.1±0.9	31.6±0.8	33.2±0.9
pH	7.37±0.00	7.37±0.00	7.41±0.01	7.46±0.00	7.47±0.00	7.46±0.00
Plasma alanine appearance (*μmol/kg per min*)	5.0±0.5	5.0±0.4	6.7±0.4	8.0±0.4	7.5±0.6	8.6±0.5
Plasma alanine disappearance (*μmol/kg per min*)	5.0±0.5	5.0±0.4	6.4±0.6	7.9±0.5	7.5±0.5	8.7±0.5
Alanine conversion to plasma glucose (*μmol/kg per min*)	1.3±0.1	1.3±0.1	1.7±0.1	2.5±0.2	2.8±0.3	3.2±0.3
Glucose Ra (*μmol/kg per min*)	11.3±0.4	11.4±0.5	10.2±0.2	10.5±0.7	9.9±0.5	9.4±0.5
Plasma glucose (*mM*)	6.5±0.3	6.4±0.1	5.7±0.1	5.6±1	5.5±0.1	5.0±0.3
Alanine oxidation (*μmol/kg per min*)		2.3±0.2		3.1±0.3		4.0±0.5

Values given as mean±SEM.

glucose output (6, 14–16) have led to the suggestion that increased delivery of gluconeogenic precursors may be responsible at least in part for the increased hepatic glucose output found in NIDDM (13, 16–18). The present studies were therefore undertaken to test the hypothesis that an increase in gluconeogenic substrate supply could increase overall hepatic glucose output sufficiently to cause fasting hyperglycemia independent of hormonal influences. For this purpose, we infused normal volunteers with lactate, the major gluconeogenic precursor (26), at a rate approximately double the rate of appearance found in patients with NIDDM (18). At the same time we maintained plasma insulin, glucagon and growth hormone concentrations constant using a modification of the pancreatic-adrenocortical-pituitary clamp technique (27). An equimolar infusion of sodium bicarbonate was employed in control experiments to take into account changes in pH expected during the infusion of sodium lactate (43).

Before the lactate infusion, lactate turnover (~ 14 μmol/kg per min) and, the percent of lactate turnover incorporated into plasma glucose (~ 9%) and into CO_2 (~ 55%) were comparable to those reported by other investigators (39, 44, 45). During the lactate infusion, the rate of appearance of plasma lactate increased nearly threefold to ~ 40 μmol/kg per min and the incorporation of lactate into plasma glucose increased more than threefold from 1.3±0.1 to 4.8±0.4 μmol/kg per min. Because of recycling of ^{14}C from the glucose molecule to lactate through glycolysis, the true rate of lactate appearance might have been slightly underestimated. However, plasma glucose ^{14}C specific activities were only 15–20% of plasma lactate specific activities. Therefore, even if all endogenous plasma lactate were derived from plasma glucose, this underestimation could not have been > 10%. At baseline ~ 6% of overall glucose appearance was derived from lactate comparable to values reported by Foster et al. (40) and Lecavalier et al. (5) but somewhat lower than those reported by Kreisberg et al. (44). At the end of the lactate infusion, ~ 25% of overall glucose appearance in plasma was derived from lactate.

These values for lactate incorporation into plasma glucose underestimate the true conversion of lactate into plasma glucose because of Krebs cycle carbon exchange (1, 46, 47). It has been calculated that with [U-^{14}C]lactate, which was employed in the present study, Krebs cycle carbon exchange will cause underestimation of incorporation of lactate into plasma glucose by ~ 55% (1). Correcting our data for underestimation, the lactate incorporation into plasma glucose in the present studies would have increased to 10.4 μmol/kg per min during lactate infusion and would have accounted for ~ 50% of overall glucose appearance in plasma. This value probably still represents an underestimation because the specific activity of [^{14}C]lactate in arterialized plasma was used to calculate lactate incorporation into glucose. Due to dilution of the infused [^{14}C]lactate with lactate formed within the splanchnic bed, the specific activity of liver lactate should be lower than arterial lactate specific activity. Use of a lower lactate specific activity in the equation of Chiasson et al. (41) would result in a greater rate of incorporation of lactate into plasma glucose. Thus, our results probably represent a minimum estimation of the incorporation of lactate into plasma glucose.

Nevertheless, one must consider the possibility that the calculated increase in gluconeogenesis observed during infusion of lactate resulted primarily from the fact that the infusion of unlabeled lactate diluted the intrahepatic lactate specific activity and that no additional carbons from the infused lactate were actually incorporated into plasma glucose. This seems unlikely, however, because in contrast to the control experiment, the total amount of ^{14}C radioactivity in plasma glucose increased during the infusion of lactate. Therefore, the observed increase in lactate gluconeogenesis cannot be ascribed merely to an artefact of the isotopic technique employed.

Despite the marked increase in lactate gluconeogenesis produced by infusion of lactate in the present studies, overall glucose appearance in plasma and the plasma glucose concentration did not increase and were not significantly different from values observed in control experiments. Thus, in the absence of changes in key glucoregulatory hormones, increased supply of a gluconeogenic precursor which markedly increased gluconeogenesis did not increase overall hepatic glucose output nor affect plasma glucose concentrations.

This observation suggests that there is normally some autoregulatory process which can operate independent of hormonal influences and in the absence of a change in plasma glucose concentration to maintain constancy of overall hepatic glucose output. Previous studies of hepatic autoregulation have emphasized the role of changes in glucose concentration on overall hepatic glucose output and gluconeogenesis in the absence of neural and hormonal influences (23–25, 48–54). The present studies extend this concept of autoregulation to include regulation of overall hepatic glucose output occurring in the absence of changes in glucose concentration.

Our results are consistent with those of several previous studies in which gluconeogenic precursor supply was increased (19, 20, 22, 42). Steele et al. (19) infused glycerol into dogs at a rate 5 times its normal rate of appearance and found no change in the rate of glucose appearance despite the fact that the fraction of plasma glucose derived from glycerol increased from 20% to 60%; these investigators postulated that a decrease in the rate of gluconeogenesis from other gluconeogenic precursors was responsible for the lack of increase in overall hepatic glucose output.

Ahlborg et al. (20) infused lactate in normal volunteers at a rate 10 times its normal rate of appearance for 30 min and found no increase in net splanchnic glucose output despite increases in plasma lactate up to 5 mM and an increase in splanchnic lactate uptake; these investigators did not comment on the failure to observe a change in splanchnic glucose output.

Diamond et al. (22) infused alanine into dogs at a rate equal to its normal turnover under hormonal clamp conditions and found no increase in hepatic glucose output although hepatic alanine uptake and alanine conversion to plasma glucose increased approximately twofold; these investigators postulated that the failure to detect an increase in hepatic glucose output was due to the relatively small fraction of hepatic glucose output contributed by alanine.

Most recently, Jahoor et al. (42) infused normal volunteers with glycerol at a rate nine times its normal rate of appearance and found no increase in hepatic glucose output. Although rates of glycerol incorporation into plasma glucose were not measured, the fact that rates of urea production diminished led these investigators, like Steele et al. (19), to postulate that diminution of gluconeogenesis from amino acids was responsible for the lack of increase in hepatic glucose output.

Certain observations of the present study suggest that mere

inhibition of gluconeogenesis from other substrates cannot wholly account for the failure of hepatic glucose output to increase. In the present study, overall hepatic glucose output was ~ 12 μmol/kg per min; normally gluconeogenesis accounts for ~ 25% of this (i.e., 3 μmol/kg per min) (1, 2), and lactate accounts for ~ 50% of gluconeogenesis and 12.5% of overall hepatic glucose output (i.e., 1.5 μmol/kg per min) (26). Indeed in the present study baseline glucose production from lactate (corrected for Krebs cycle carbon exchange) was 1.5 μmol/kg per min accounting for ~ 13% of overall hepatic glucose output in agreement with expected values (1, 2, 26). It follows from these calculations that substrates other than lactate would have been contributing ~ 1.5 μmol/kg per min to overall hepatic glucose output. When lactate was infused in the present study, the amount of plasma glucose derived from lactate increased by 3.7 μmol/kg per min. Thus even if there had been total suppression of gluconeogenesis from precursors other than lactate, 2.2 μmol/kg per min of glucose appearance (3.7 − 1.5 μmol/kg per min), a readily measurable amount, would still be left unexplained.

Furthermore the results of experiments in which we measured alanine turnover and conversion to glucose during infusion of lactate also argue against suppression of gluconeogenesis from non-lactate precursors as an explanation. At baseline, rates of alanine appearance (~ 5 μmol/kg per min), alanine conversion to plasma glucose (~ 1.3 μmol/kg per min) and to CO_2 (2.3 μmol/kg per min) as well as the percent of plasma glucose derived from alanine (~ 6%) and the percent of alanine disappearance from plasma accounted for by conversion to plasma glucose (~ 26%) and CO_2 (~ 46%) were comparable to previously reported values (55–59). During infusion of lactate, plasma alanine concentrations increased. This was due to an increase in the rate of appearance of alanine most likely due to conversion of the infused lactate into alanine (40); a decrease in the rate of alanine disappearance might have occurred had gluconeogenesis from alanine been suppressed but, in fact, alanine disappearance also increased. The actual conversion of alanine into plasma glucose increased during the lactate infusion more than twofold and the percent of alanine disappearance attributable to gluconeogenesis increased almost 50% while the relative oxidation of alanine (and lactate) was not increased. Thus, at least as reflected by alanine, gluconeogenesis from precursors other than lactate was not reduced during infusion of lactate.

Since incorporation of lactate and alanine into glucose released from the liver increased during the lactate infusion, it seems reasonable to consider that a reduction in glycogenolysis might have prevented overall hepatic glucose output from increasing. Net glycogen accumulation from lactate is also suggested by our failure to fully account for lactate disappearance from plasma by its oxidation and conversion to plasma glucose. During lactate infusion, lactate disappearance from plasma averaged 35 μmol/kg per min; of this, 3.5 μmol/kg per min appeared in plasma glucose and 15.3 μmol/kg per min was oxidized; this leaves ~ 16 μmol/kg per min unaccounted for which could represent net glycogen deposition. Perfusion of rat liver with lactate not only increases release of glucose derived from lactate but also increases glycogen formation from lactate (60). This indicates that perfusion with increased lactate increased flux through glucose-6-phosphate. Since the proportion of lactate and alanine turnover that was incorporated into plasma glucose increased in the present study during infusion of lactate, it is probable that infusion of lactate increased the intrahepatic glucose-6-phosphate pool. Glucose-6-phosphate is a well-known inhibitor of phosphorylase and activator of glycogen synthase (61, 62). Therefore, an increase in intrahepatic glucose-6-phosphate could have led to inhibition of phosphorylase and activation of glycogen synthase resulting in a net reduction of glycogenolysis. Such a reduction in net breakdown could have offset an increase in hepatic glucose output due to an increase in gluconeogenesis. This situation would be analogous to the postprandial state in which there is an increase in net glycogen synthesis, an increase in gluconeogenesis and simultaneous suppression of endogenous hepatic glucose output (3, 63).

Whether or not an inhibition of glucose-6-phosphatase is also required to explain the lack of increase in overall hepatic glucose output is unclear. Although insulin and glucose have been reported to suppress glucose-6-phosphatase activity (64), plasma insulin and glucose levels did not increase in the present studies. An inhibitory effect of glucose-6-phosphate on glucose-6-phosphatase activity has not as yet been demonstrated (64). Moreover, the actual increase in hepatic release of glucose derived from the infused lactate argues against inhibition of glucose-6-phosphatase.

One must also consider the possibility that glucagon had not been adequately replaced in the present studies. Similar infusion rates have been used to replace basal glucagon secretion in other studies (27, 48, 65). Moreover, plasma glucagon levels were comparable in control and lactate infusion experiments. Thus unless some critical threshold level of hyperglucagonemia is necessary, inadequate glucagon replacement does not seem a likely explanation. Similarly, overreplacement of insulin is unlikely to have explained our results since plasma insulin concentrations were comparable in control and lactate infusion experiments and the rates of insulin infusion were similar to those used in other studies for physiologic replacement (27, 48, 65).

The demonstration in the present studies that in normal human volunteers, an increase in gluconeogenic substrate supply which increases gluconeogenesis is insufficient in itself to increase overall hepatic glucose output and the plasma glucose concentration implies that there is some autoregulatory process which prevents the substrate-induced increase in gluconeogenesis from increasing overall hepatic glucose output even in the absence of an increase in plasma glucose concentration. Moreover the failure of increased supply of gluconeogenic precursors to increase overall hepatic glucose output suggests that the increased hepatic glucose output found in NIDDM results primarily from an intrahepatic defect. There is evidence that autoregulation of gluconeogenesis is impaired in hepatocytes from streptozotocin diabetic rats (53). The fact that persons with NIDDM have increased gluconeogenic substrate availability (6, 11–13), which is accompanied by an increase in gluconeogenesis (6, 14, 15) and overall hepatic glucose output (6, 11, 14–16) suggests that autoregulation may also be impaired in NIDDM. Since activation of glycogen synthase is impaired in NIDDM (66, 67) and since activation of glycogen synthase may be involved in autoregulation, it is tempting to speculate that this defect in activation of glycogen synthase limits the operation of hepatic autoregulation and contributes to the increased overall hepatic glucose output in NIDDM. Accordingly, impaired activation of hepatic glycogen synthase could play an important role in NIDDM not only

for postprandial hyperglycemia (66) but also for fasting hyperglycemia.

Acknowledgments

We thank the staff of the Clinical Research Center, Carol Korbanic and Lauri Henry, for their excellent technical help; and Laura Brinker and Cathy Butler for superb editorial assistance.

This publication was supported in part by funds received from the NIH/DRR/GCRC grant 5M01 RR00056 and by DEMD/NIDDK/5R37 DK-20411, the Fogarty International Foundation, and the Norwegian Council of Science and the Humanities.

References

1. Consoli, A., F. Kennedy, J. Miles, and J. Gerich. 1987. Determination of Krebs cycle metabolic carbon exchange in vivo and its use to estimate the individual contributions of gluconeogenesis and glycogenolysis to overall glucose output in man. *J. Clin. Invest.* 80:1303–1310.

2. Nilsson, L., and E. Hultman. 1973. Liver glycogen in man: the effect of total starvation or a carbohydrate-poor diet followed by carbohydrate refeeding. *Scand. J. Lab. Invest.* 32:325–330.

3. McGarry, J. D., M. Kuwajima, C. B. Newgard, D. W. Foster, and J. Katz. 1987. From dietary glucose to liver glycogen: the full circle around. *Annu. Rev. Nutr.* 7:51–73.

4. Frizzell, R., G. Hendrick, D. Biggers, D. Lacy, D. Donahue, D. Green, R. Carr, and P. Williams, R. Stevenson, and A. Cherrington. 1988. Role of Gluconeogenesis in sustaining glucose production during hypoglycemia caused by continuous insulin infusion in conscious dogs. *Diabetes.* 37:749–759.

5. Lecavalier, L., G. Bolli, P. Cryer, and J. Gerich. 1989. Contributions of gluconeogenesis and glycogenolysis during glucose counterregulation in normal humans. *Am. J. Physiol.* 256:E844–E851.

6. Consoli, A., N. Nurjhan, F. Capani, and J. Gerich. Predominant role of gluconeogenesis in increased hepatic glucose production in NIDDM. *Diabetes.* 38:550–551.

7. Cherrington, A., and M. Vranic. 1986. Hormonal control of gluconeogenesis in vivo. *In* Hormonal Control of Gluconeogenesis. N. Kraus-Friedmann, editor. CRC Press, Boca Raton, FL. 15–37.

8. Exton, J., L. Mallette, L. Jefferson, E. Wong, N. Friedman, T. Miller, and C. Park. 1970. The hormonal control of hepatic gluconeogenesis. *Recent Prog. Horm. Res.* 26:411–455.

9. Garber, A., D. Bier, P. Cryer, and A. Paglearo. 1976. Hypoglycemia in compensated chronic renal insufficiency substrate limitation of gluconeogenesis. *Diabetes.* 23:982–986.

10. Haymond, M., E. Ben-Galim, and K. Strobel. 1978. Glucose and alanine metabolism in children with maple syrup urine disease. *J. Clin. Invest.* 62:398–405.

11. Hall, S., J. Saunders, and P. Sonksen. 1979. Glucose and free fatty acid turnover in normal subjects and in diabetic patients before and after insulin treatment. *Diabetologia.* 16:297–306.

12. Felig, P., J. Wahren, and R. Hendler. 1978. Influence of maturity onset diabetes on splanchnic glucose balance after oral glucose injection. *Diabetes.* 27:121–126.

13. Reaven, G., C. Hollenbeck, C. Jeng, M. Wu, and Y. Chen. 1988. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24h in patients with NIDDM. *Diabetes.* 37:1020–1024.

14. Zawadski, J., R. Wolfe, D. Mott, S. Lillioja, B. Howard, and C. Bogardus. 1988. Increased rate of Cori Cycle in obese subjects with NIDDM and effects of weight reduction. *Diabetes.* 37:154–159.

15. Comstock, J., J. Ellerhorst, and A. Garber. 1987. Effects of sulfonylurea therapy on glucose-alanine precursor-product interrelationships in NIDDM. *Diabetes.* 36(Suppl. 1):4A.

16. DeFronzo, R., A. Golay, and J. Felber. 1985. Glucose and lipid metabolism in obesity and diabetes mellitus. *In* Substrate and Energy Metabolism. J. Garrow, and D. Hallidaed, editors. John Libbey Press, London. 70–81.

17. Prager, R., P. Wallace, and J. Olefsky. Direct and indirect effects of insulin to inhibit hepatic glucose output in obese subjects. *Diabetes.* 36:607–611.

18. Consoli, A., N. Nurjahan, J. Reilly, L. Mandarino, and J. Gerich. 1988. Gluconeogenesis from lactate is increased in noninsulin-dependent diabetes: importance of overproduction of lactate in nonmuscle tissues. *Diabetes.* 37(Suppl. 1):10A.

19. Steele, R., B. Winkler, and N. Altszuler. 1971. Inhibition by infused glycerol of gluconeogenesis from other precursors. *Am. J. Physiol.* 221:883–888.

20. Ahlborg, G., L. Hagenfeldt, and J. Wahren. 1976. Influence of lactate infusion on glucose and FFA metabolism in man. *Scand. J. Clin. Lab. Invest.* 36:193–201.

21. Dietze, G., M. Wicklmayr, K. Hepp, W. Bogner, H. Mehnert, H. Czempiel, and H. Henftling. 1976. On gluconeogenesis of human liver: accelerated hepatic glucose formation by increased precursor supply. *Diabetologia.* 12:555–561.

22. Diamond, M., R. Rollings, K. Steiner, P. Williams, W. Lacy, and A. Cherrington. 1988. Effect of alanine concentration independent of changes in insulin and glucagon on alanine and glucose homeostasis in the conscious dog. *Metab. Clin. Exp.* 37:28–33.

23. Ruderman, N., and M. Herrera. 1968. Glucose regulation of hepatic gluconeogenesis. *Am. J. Physiol.* 214:1346–1351.

24. Seglen, P. 1974. Autoregulation of glycolysis, respiration, gluconeogenesis and glycogen synthesis in isolated parenchymal rat liver cells under aerobic and anaerobic conditions. *Biochim. Biophys. Acta.* 338:317–336.

25. Shulman, G., W. Lacy, J. Liljenquist, U. Keller, P. Williams, and A. Cherrington. 1980. Effect of glucose, independent of changes in insulin and glucagon secretion, on alanine metabolism in the conscious dog. *J. Clin. Invest.* 65:496–505.

26. Kreisberg, R. 1972. Glucose–lactate inter-relations in man. *N. Engl. J. Med.* 287:132–137.

27. DeFeo, P., G. Perriello, M. M. Ventura, P. Brunetti, F. Santeusanio, J. E. Gerich, and G. B. Bolli. 1987. The pancreatic-adrenocortical-pituitary clamp technique for study of counterregulation in humans. *Am. J. Physiol.* 252:E565–E570.

28. McGuire, E., J. Halderman, J. Tobin, R. Andres, and M. Berman. 1976. Effects of arterial versus venous sampling on analysis of glucose kinetics in man. *J. Appl. Physiol.* 41:565–573.

29. Kelley, D., A. Mitrakou, H. Marsh, F. Schwenk, J. Benn, G. Sonnenberg, M. Archangeli, T. Aoki, J. Sorensen, M. Berger, et al. 1988. Skeletal muscle glycolysis, oxidation, and storage of an oral glucose load. *J. Clin. Invest.* 81:1563–1571.

30. Lowry, O. H., and J. V. Passonneau. 1972. A flexible system of enzymatic analysis. Academic Press, Inc., Orlando, FL. 200 pp.

31. Karl, I., A. Pagliaris, and D. Kipnis. 1972. A microfluorometric enzymatic assay for determination of alanine and pyruvate in plasma and tissue. *J. Lab. Clin. Med.* 80:434–441.

32. Wieland, O. 1974. Glycerol UV method. *In* Methods in Enzymatic Analysis. Volume 3. Second edition. U. Bergmeryer, editor. Academic Press, Inc., New York. 1404–1414.

33. Kreisberg, R., A. Siegal, and D. Crawford-Owen. 1971. Glucose-lactate interrelationship effect of ethanol. *J. Clin. Invest.* 50:175–185.

34. Herbert, V., C. Gottlieb, and S. Bleicher. 1965. Coated charcoal immunoassay of insulin. *J. Clin. Endocrinol. Metab.* 25:1375–84.

35. Faloona, G., and R. Unger. 1974. Glucagon. *In* Methods of Hormone Radioimmunoassay. B. Jaffe and H. Behrman, editors. Academic Press, Inc. New York. 317–330.

36. Peake, G. 1974. Growth Hormone. *In* Methods of Hormone Radioimmunoassay. B. Jaffe and H. Berhruau, editors. Academic Press, Inc., New York. 103–121.

37. Faber, O. K., and C. Binder. 1977. C-peptide response to glucagon: a test for the residual β-cell function in diabetes mellitus. *Diabetes.* 26:605–610.

38. DeBodo, R., R. Steele, N. Altszuler, A. Dunn, and J. Bishop. 1963. On the hormonal regulation of carbohydrate metabolism: studies with C^{14} glucose. *Recent. Prog. Horm. Res.* 19:445–448.

39. Searle, G., and R. Cavalieri. 1972. Determination of lactate kinetics in human analysis of data from single injection vs. continuous infusion methods. *Proc. Soc. Exp. Biol. Med.* 139:1002–1006.

40. Foster, D. M., G. Hetenyi, and M. Berman. 1980. A model for carbon kinetics among plasma alanine, lactate, and glucose. *Am. J. Physiol.* 239:E30–E38.

41. Chiasson, J., J. Liljenquist, W. Lacy, A. Jennings, and A. Cherrington. 1977. Gluconeogenesis: methodological approaches in vivo. *Fed. Proc.* 36:229–235.

42. Jahoor, F., E. Peters, and R. Wolfe. 1988. Gluconeogenic precursor supply and glucose production. *FASEB (Fed. Am. Soc. Exp. Biol.) J.* 2:A505. (Abstr.)

43. Connor, H., and H. Woods. 1982. Quantitative aspects of L (+) −lactate metabolism in human beings. *In* Metabolic Acidosis. R. Portor and G. Lawrenson, editors. *Ciba Found. Symp.* 87:214–234.

44. Kreisberg, R. A., L. F. Pennington, and B. R. Boshell. 1970. Lactate turnover and gluconeogenesis in normal and obese humans. *Diabetes.* 19:53–63.

45. Mazzeo, R. S., G. A. Brook, D. A. Schoeller, T. F. Budinger. 1986. Disposal of blood (1-^{13}C)lactate in humans during rest and exercise. *J. Appl. Physiol.* 60:232–241.

46. Katz, J. 1986. Determination of gluconeogenesis in vivo with ^{14}C-labelled substrates. *Am. J. Physiol.* 248:R391–R399.

47. Hetenyi, G., G. Perez, and M. Vranic. 1983. Turnover and precursor-product relationships of non-lipid metabolites. *Physiol. Rev.* 63:606–667.

48. Bolli, G., P. DeFeo, G. Perriello, S. DeCosmo, M. Ventura, P. Campbell, P. Brunetti, and J. Gerich. 1985. Role of hepatic autoregulation in defense against hypoglycemia in man. *J. Clin. Invest.* 75:1623–1631.

49. Soskin, S., and R. Levine. 1937. A relationship between the blood sugar level and the rate of sugar utilization, affecting the theories of diabetes. *Am. J. Physiol.* 120:761.

50. Glinsmann, W., E. Hern, and A. Lynch. 1969. Intrinsic regulation of glucose output by rat liver. *Am. J. Physiol.* 216:698–703.

51. Bucolo, G., and M. David. 1973. Quantitative determination of serum triglycerides by the use of enzymes. *Clin. Chem.* 19:476–482.

52. Liljenquist, J., G. Muller, A. Cherrington, J. Perry, and D. Rabinowitz. 1979. Hyperglycemia per se (insulin and glucagon withdrawn) can inhibit hepatic glucose production in man. *J. Clin. Endocrinol. Metab.* 48:171–175.

53. Davidson, M. 1978. Autoregulation by glucose of hepatic glucose balance: permissive effect of insulin. *Metab. Clin. Exp.* 30:279–284.

54. Sacca, L., R. Hendler, and R. Sherwin. 1978. Hyperglycemia inhibits glucose production in man independent of changes in glucoregulatory hormones. *J. Clin. Endocrinol. Metab.* 47:1160–1163.

55. Hall, S., J. T. Braaten, J. B. R. McKendry, T. Bozton, B. D. Foster, and M. Berman. 1979. Normal alanine-glucose relationships and their changes in diabetic patients before and after insulin treatment. *Diabetes.* 28:737–745.

56. Chochinov, R. H., H. F. Bowen, and J. A. Moorhouse. 1978. Circulating alanine disposal in diabetes mellitus. *Diabetes.* 27:420–426.

57. Bier, D., K. Arnold, W. Sherman, W. Holland, W. Holmes, and D. Kipnio. 1977. In vivo measurement of glucose and alanine metabolism with stable isotopes. *Diabetes.* 26:1005–1015.

58. Chiasson, J., J. Liljenquist, B. Sinclair-Smith, and W. W. Lacy. 1975. Gluconeogenesis from alanine in normal postabsorptive man: intrahepatic stimulatory effect of glucagon. *Diabetes.* 24:574–584.

59. Chiasson, J., R. Atkinson, D. Cherrington, U. Keller, B. Sinclair-Smith, W. Lacy, and J. Liljenquist. 1979. Effects of fasting on gluconeogenesis from alanine in nondiabetic man. *Diabetes.* 28:56–60.

60. Exton, J., C. Park. 1967. Control of gluconeogenesis in liver general features of gluconeogenesis in the perfused livers of rats. *J. Biol. Chem.* 242:2622–2636.

61. Hems, D., and P. Whitton. 1980. Control of hepatic glycogenolysis. *Physiol. Rev.* 60:1–50.

62. Huijing, F. 1975. Glycogen metabolism and glycogen-storage diseases. *Physiol. Rev.* 609–658.

63. Shikama, H., and M. Vi. 1978. Glucose load diverts hepatic gluconeogenic product from glucose to glycogen in vivo. *Am. J. Physiol.* 235:E354–360.

64. Ashmore, J., and G. Weber. 1959. The role of hepatic glucose-6-phosphatase in the regulation of carbohydrate metabolism. *Vitam. Horm.* 17:91–132.

65. Campbell, P., G. Bolli, P. Cryer, and J. Gerich. 1985. Pathogenesis of the dawn phenomenon in insulin-dependent diabetes mellitus accellerated glucose production and impaired glucose utilization due to nocturnal surges in growth hormone secretion. *N. Engl. J. Med.* 312:1473–1479.

66. Wright, K., H. Beck-Nielsen, O. Kolterman, and L. Mandarino. 1988. Decreased activation of skeletal muscle glycogen synthase by mixed-meal ingestion in NIDDM. *Diabetes.* 37:436–440.

67. Freymond, D., C. Bogardus, M. Okubo, K. Stone, and D. Mott. 1988. Impaired insulin-stimulated muscle glycogen synthase activation in vivo fasting glycogen synthase phosphatase activity. *J. Clin. Invest.* 82:1503–1509.