Hyperglucagonemia and Insulin-mediated Glucose Metabolism

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Abstract

The effect of chronic physiologic hyperglucagonemia on basal and insulin-mediated glucose metabolism was evaluated in normal subjects, using the euglycemic insulin clamp technique (+ 50, + 100, and + 500 μU/ml). After glucagon infusion fasting glucose increased from 76±4 to 93±2 mg/dl and hepatic glucose production (HGP) rose from 1.96±0.08 to 2.25±0.08 mg/kg · min ($P < 0.001$). Basal glucose oxidation after glucagon increased ($P < 0.05$) and correlated inversely with decreased free fatty acid concentrations ($r = -0.94$; $P < 0.01$) and decreased lipid oxidation ($r = -0.75$; $P < 0.01$). Suppression of HGP and stimulation of total glucose disposal were impaired at each insulin step after glucagon ($P < 0.05$–0.01). The reduction in insulin-mediated glucose uptake was entirely due to diminished nonoxidative glucose utilization. Glucagon infusion also caused a decrease in basal lipid oxidation and an enhanced ability of insulin to inhibit lipid oxidation and augment lipid synthesis. These results suggest that hyperglucagonemia may contribute to the disturbances in glucose and lipid metabolism in some diabetic patients.

Introduction

Diabetes mellitus has been described as a bihormonal disorder, where both insulin deficiency and absolute or relative glucagon excess contribute to the metabolic disturbances (1). Defects in insulin-mediated glucose metabolism have been found in Type II (noninsulin dependent) and Type I (insulin dependent) diabetic patients (2–14). Several counterregulatory hormones including epinephrine (15), cortisol (16, 17), and growth hormone (16, 18) have been shown to impair the insulin-mediated glucose metabolism and have been implicated in the deterioration of glucose tolerance under certain metabolic conditions. In contrast, little information is available concerning the effect of hyperglucagonemia on insulin action, particularly on peripheral tissues. Early studies by Pozefsky et al. (19), using the forearm perfusion technique, failed to demonstrate any effect of glucagon on glucose disposal and since then it has become widely accepted that glucagon has no effect on insulin-mediated glucose metabolism by peripheral tissues. Although numerous studies (20–26) have documented that glucagon can oppose the suppressive effect of insulin on hepatic glucose production (HGP),[1] the pathogenetic role of hyperglucagonemia in insulin-dependent diabetes mellitus has been questioned because the stimulation of HGP by glucagon has been claimed to be only a transient phenomenon that wanes within hours (26–29). A more recent study (30) has suggested that the evanescent hepatic effect of glucagon results from an initial but brief stimulation of glycogenolysis. Interestingly, this transient effect on glycogenolysis was followed by a later onset, progressive rise in gluconeogenesis (30).

In the present investigation, we have examined the effects of a chronic elevation in plasma glucagon concentration on HGP and on insulin-mediated glucose metabolism by peripheral tissues in normal subjects.

Methods

Subjects. 14 healthy volunteers, ranging in age from 18 to 38 yr (mean±SEM = 24±3 y), were studied. There were 10 males and 4 females. Their ideal body weight (based on medium-frame individuals from the Metropolitan Life Insurance Tables, 1959) ranged from 90 to 115% (mean = 103±2%). Their mean height and weight were 175±2 cm and 72±3 kg, respectively, and the body mass index (kilograms per meter^2) was 24.7±0.5. None of the subjects had any family history of diabetes mellitus and none were taking any medication. Participants consumed a weight-maintaining diet that contained at least 250–300 g of carbohydrate/d for 3 d before each study. Before their participation the nature, purpose, and risks of the study were explained to all subjects and their voluntary, informed, written consent was obtained. The experimental protocol was approved by the Human Investigation Committee of Yale University School of Medicine.

Experimental protocol

All studies were performed in the recumbent position at 0800 h after a 10–12-h overnight fast. Before study, a Teflon catheter was inserted into an antecubital vein for the infusion of all test substances. For blood sampling, a second catheter was inserted retrogradely into a wrist vein and kept patent with an infusion of isotonic saline. The hand was then inserted into a heated box (70°C) to achieve arterialization of venous blood. 120 min before beginning the insulin clamp studies, a priming dose of D-[3-^{3}H]glucose (25 μCi) was rapidly injected and a constant infusion (0.25 μCi/min) was begun and continued throughout the insulin clamp studies. 60 min before beginning the insulin clamp studies, continuous respiratory exchange measurements were also begun and continued until the end of the study.

Subjects participated in four studies as described below. Nine subjects participated in both studies I and II and five of these same nine subjects participated in study III. An additional five subjects (different from those in studies I–III) participated in study IV.

Study I: control study. After the 120-min equilibration period for tritiated glucose, nine subjects underwent a stepwise euglycemic insulin clamp as described below. Indirect calorimetry measurements were continued throughout the insulin clamp period.

Study II: postglucagon study. Within 3–8 d after the initial insulin clamp study, the same nine subjects were admitted to the Yale Clinical Research Center. At 0800 h the morning after admission, a continuous glucagon infusion was started at the rate of 3 ng/kg · min. Glucagon (Eli Lilly & Co., Indianapolis, IN) was dissolved in a 0.3% human albumin solution and continuously delivered through a pump (Flow-Gard 6,000; Baxter Travenol Laboratories, Inc., Deerfield, IL). A fresh glucagon solution was prepared every 3 h. 48 h later, at 0800 h, a prime-continuous tritiated glucose infusion and indirect calorimetry measurements were

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1. *Abbreviations used in this paper:* FFA, free fatty acids; HGP, hepatic glucose production; NPRQ, nonprotein respiratory quotient.

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begun as previously described. After 2 h of equilibration, a stepwise insulin clamp study was performed. The glucagon infusion was continued throughout.

During the 2½-d hospitalization, all subjects consumed a weight-maintaining diet containing 50% carbohydrate, 35% fat, and 15% protein. All subjects remained fasting after 2200 h on the evening before study. Samples for metabolites were collected in the postabsorptive state, before, and 24 and 48 h after starting the continuous glucagon infusion.

Study III: glucagon/intralipid study. Because the glucagon infusion resulted in a significant reduction in plasma free fatty acid (FFA) concentration (see Results), five of the nine subjects participated in a second 48-h glucagon infusion protocol that was performed 1–2 wk after study II. During this study, in addition to the glucagon (3 ng/kg · min) infusion, Intralipid (10% triglyceride emulsion; Cutter Laboratories, Inc., Berkley, CA) also was administered at the rate of 0.7–1.0 ml/min for 48 h. No heparin was added. The Intralipid infusion was discontinued at the same time that the insulin clamp study was initiated.

Study IV: hyperglycemic hyperinsulinemia study. After glucagon infusion in normal subjects, HGP is stimulated, plasma glucose concentration rises, and plasma insulin concentration may increase. To examine whether the modest increases in plasma glucose and insulin after glucagon infusion could explain any of the observed changes in glucose metabolism, five additional subjects received a two-step euglycemic insulin (+ 50 and + 100 μU/ml) clamp study in combination with [3-^{3}H]glucose. Within 3–7 d after this initial study, subjects were admitted to the Clinical Research Center, where they received a 48-h glucose infusion (2.8 mg/kg · min), designed to elevate the plasma glucose concentration to levels comparable to those observed during glucagon infusion and to produce modest hyperinsulinemia. The two-step euglycemic insulin clamp was then repeated. Indirect calorimetry was not performed in these studies.

Euglycemic insulin clamp study. After the 120-min equilibration period for tritiated glucose and after baseline respiratory exchange measurements, a stepwise euglycemic insulin clamp study was performed as previously described (31). Briefly, a stepwise prime-continuous infusion of crystalline porcine insulin (0.5, 1.0, and 5.0 mU/kg · min) was administered to acutely raise and maintain the plasma insulin concentration by ~ 50, 100, and 500 μU/ml above the basal concentration. The three insulin infusion steps lasted 100, 90, and 80 min respectively. In study IV only the 0.5 and 1 mU/kg · min insulin infusions were carried out. Plasma glucose concentration was held constant at the basal preinfusion level by determination of the plasma glucose concentration every 5 min and appropriately adjusting a 20% glucose solution based on a negative feedback principle (31). In studies II, III, and IV the basal plasma glucose concentration was slightly higher than in the control study (study I). Therefore, after starting the insulin infusion, the plasma glucose concentration was allowed to drop to the same level as in the control study. Under steady-state conditions of constant euglycemia, all of the infused glucose must be taken up by tissues. Thus, the exogenous glucose infusion rate, when added to the endogenous rate of residual HGP, provides a measure of the total amount of glucose metabolized by the entire body. During the basal period and throughout the insulin clamp, plasma samples were drawn at 10–15-min intervals for determination of plasma hormone and substrate levels and every 5–10 min for tritiated glucose specific activity.

Respiratory exchange measurement. For 60 min before and throughout the stepwise insulin clamp study, continuous indirect calorimetry was performed as previously described (32). A transparent plastic, ventilated hood was placed over the head of the subject and made airtight around the neck. A slight negative pressure was maintained in the hood to avoid loss of expired air. Ventilation was measured by means of a dry gas meter (American Meter Div. of the Singer Co., Philadelphia, PA). A constant fraction of the air flowing out of the hood was automatically collected for analysis. The oxygen content was continuously measured by electrochemical analysis (model S-3A oxygen analyzer; Applied Electrochemistry, Inc., Sunnyvale, CA). CO_2 content was measured by an infrared analyzer (model CD-3A CO_2 analyzer; Applied Electrochemistry, Inc.).

The amount of protein oxidized during the basal state was calculated from the urinary nitrogen excretion (32), determined on an overnight urine specimen. A second urine was collected during the entire insulin clamp study and the urinary nitrogen excretion during this period was used to calculate protein oxidation.

At nonprotein respiratory quotients (NPRQs) > 1.0, the equations for the calculation of substrate utilization remain valid. However, because lipid oxidation is surpassed by lipid synthesis, the rate of lipid oxidation becomes negative by a numerical value equivalent to the amount of fat synthesized (33). The values so obtained are identical to those calculated by Rapport et al. (34), who used an entirely different method. These authors computed that 1 ml of CO_2 eliminated in excess of the oxygen consumed corresponds to an increase of 1.7 mg in the body's fat content. This calculation has recently been reviewed and verified by Frayn (35).

Analytical procedures. Plasma glucose concentration was determined in duplicate by the glucose–oxidase method on a glucose analyzer (Analyzer II; Beckman Instruments Inc., Fullerton, CA). Methods for the determination of plasma-tritiated glucose specific activity (36), plasma immunoreactive insulin (37), glucagon (38), and plasma FFA (39) have previously been described. Glucagon was measured using the COOH-terminal 30-kD antibody of Unger. Urinary nitrogen excretion was determined by the method of Kjeldahl (40).

Calculations. During the stepwise insulin clamp studies, the glucose infusion rate was determined by calculating the mean value observed during selected time intervals. For data presentation, the mean of the last 60 min of each insulin infusion step was used. Total glucose metabolism was calculated by adding the mean rate of endogenous glucose production during the last 60 min of each insulin infusion step to the mean glucose infusion rate during the same time period.

In all studies, a steady-state plateau of tritiated-glucose specific activity was achieved during the 30-min period before starting the insulin clamp. Glucose production in the basal state was determined by dividing the [3-^{3}H]glucose infusion rate (counts per minute) by the steady-state plateau of [3-^{3}H]glucose specific activity (counts per milligram) achieved during the last 30 min of the preinsulin infusion control period. After insulin–glucose administration (euglycemic insulin clamp), a nonsteady-state condition in glucose specific activity exists and HGP was calculated by Steele's equations in their derivative form (41); this permits the evaluation of continuous changes in the rates of glucose turnover. The value of 0.65 was used as the pool fraction in the present calculations (42). The determination of glucose turnover by the prime-constant infusion and pool-fraction technique has recently been validated for both steady and nonsteady states (43). The rate of endogenous glucose production was calculated by subtracting the glucose infusion rate from the rate of glucose appearance as determined by the isotopic tracer technique. Negative numbers for HGP were assumed to be zero.

Glucose oxidation, lipid oxidation, and net lipid synthesis were calculated from calorimetric measurements at 5-min intervals throughout the last 60 min of each insulin infusion step and were expressed in mg/kg · min. Nonoxidative glucose disposal was calculated by subtracting the rate of glucose oxidation from the rate of total body glucose uptake during the last hour of each insulin step.

All data are presented as the mean±SEM. Statistical comparisons within a group were performed by paired *t* test analysis (44). When all three groups were compared, analysis of variance was used. Coefficients of variation were determined by standard formulae (44).

Results

Effect of glucagon infusion on basal plasma substrate and hormone concentrations

Fig. 1 shows the plasma concentrations of glucagon, insulin, glucose, and FFA before and after 24 and 48 h of glucagon infusion (3 ng/kg · min). The continuous glucagon infusion resulted in a stable increase in plasma glucagon concentration to 414±24 pg/ml. This represents an increase above baseline of

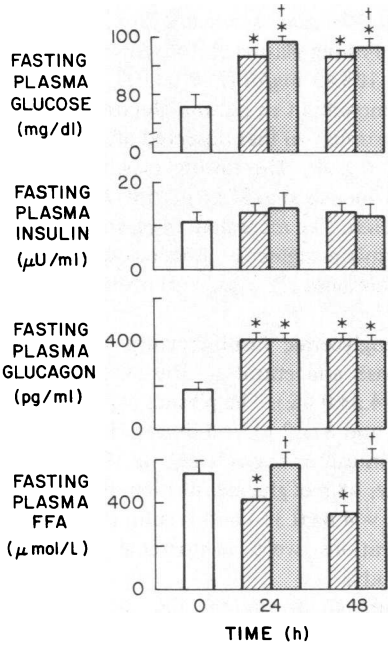

Figure 1. Fasting plasma concentrations of glucose, insulin, glucagon, and FFA before (*open bars*) and after 24 and 48 h of glucagon (*hatched bars*) and glucagon/Intralipid (*stippled bars*) infusion. All values represent the mean±SEM. *$P < 0.05$–0.001 vs. basal; †, $P < 0.05$–0.001 vs. glucagon infusion.

242±14 pg/ml. The fasting plasma glucose concentration was significantly higher after 24 and 48 h of glucagon infusion (from 76±4 to 93±3 and to 93±2 mg/dl respectively; $P < 0.001$). The fasting plasma insulin concentration (11±2 μU/ml) did not change significantly after 24 h (13±2 μU/ml) or 48 h (13±2 μU/ml) of glucagon infusion. Fasting plasma levels of FFA progressively declined from 530±58 to 410±47 and to 354±33 μmol/liter (both $P < 0.05$) after 24 and 48 h glucagon infusion, respectively. To prevent the drop in plasma FFA levels associated with the glucagon infusion, five out of the nine subjects received a repeat 48 h glucagon infusion in combination with Intralipid (Fig. 1). In this study, the fasting FFA level remained constant throughout the 48-h period of glucagon infusion. Concomitant Intralipid infusion did not affect the plasma levels of insulin or glucagon (Fig. 1). However, the increase in plasma glucose concentration after 24 h (98±2 mg/dl, $P < 0.05$) and 48 h (96±3 mg/dl, $P < 0.05$) of glucagon/Intralipid infusion was significantly greater than when glucagon was infused alone.

Stepwise euglycemic insulin clamp

Plasma substrate and hormone concentrations. After 48 h of glucagon infusion, basal plasma glucose concentration was significantly increased (93±2 vs. 76±4 mg/dl, $P < 0.001$). Therefore, in the repeat insulin clamp study the plasma glucose concentration was allowed to decline slightly. In the control studies, the mean steady-state plasma glucose was 84±2 mg/dl with a coefficient of variation of 3.8±0.3%. In the postglucagon study, the steady-state plasma glucose was 87±3 mg/dl with a coefficient of variation of 3.5±0.4%. The steady-state plasma insulin concentrations during each step of the insulin clamp studies performed before (52±3, 96±6, and 629±55 μU/ml) and after 48 h of glucagon infusion (52±5, 97±8, and 600±69 μU/ml) were similar. Basal glucagon concentration was 148±19 pg/ml in the control study and declined to 128±17, 122±17, and 118±17 pg/ml during the three steps of the insulin clamp. After 48 h of glucagon infusion, the plasma glucagon was 414±24 pg/ml and during the stepwise euglycemic insulin clamp study declined to 381±20, 337±19, and 336±15 pg/ml. Thus, in the postglucagon study, hyperglucagonemia was maintained in spite of hyperinsulinemia. Fasting plasma FFA concentrations were significantly lower after 48 h of glucagon infusion, 354±33 vs. 530±58 μmol/liter ($P < 0.05$). However, during the insulin clamp studies, plasma FFA levels declined to the same extent during each of the three hyperinsulinemic steps (control study: 142±14, 130±9, and 122±10 μmol/liter; postglucagon study: 144±13, 131±10, and 113±8 μmol/liter) (Fig. 2).

In the five subjects who received glucagon plus Intralipid infusion, basal plasma glucose concentration, 96±3 mg/dl, was higher ($P < 0.05$) than in the control and initial glucagon studies (Fig. 1). The basal plasma insulin concentration, 13±2 μU/ml, was similar to the control and initial glucagon studies (Fig. 1). During the stepwise insulin clamp study, the mean steady-state plasma glucose (83±1 mg/dl, coefficient of variation = 3.5±0.6%) and insulin (51±6, 93±5, and 558±67 μU/ml) concentrations did not differ from the previous two clamp studies. Similar plasma glucagon concentrations were attained during glucagon (414±19 pg/ml) and glucagon/Intralipid (403±31 pg/ml) infusion. During the three-step insulin clamp study, plasma glucagon fell to 394±45, 369±24, and 358±23 pg/ml. After 48 h of combined glucagon/Intralipid infusion, the basal plasma FFA concentration, 594±31 μmol/liter, was similar to that observed in control study, 530±58 μmol/liter, and higher than after 48 h of glucagon infusion alone, 354±33 μmol/liter ($P < 0.05$) (Fig. 1). Even though the Intralipid infusion was stopped at the very beginning of the insulin clamp study, the plasma FFA concentration during each step of the insulin clamp was higher than during control and glucagon clamp studies (284±18, 263±19, and 221±11 μmol/liter during the 0.5, 1.0, and 5.0 mU/kg·min insulin infusion) (Fig. 2).

Glucose metabolism (Fig. 3). In the postabsorptive state, total body glucose utilization equals the rate of endogenous glucose appearance as calculated by tritiated-glucose kinetic analysis. In the control study, total body glucose uptake was 1.96±0.08 mg/kg·min and during the 0.5, 1.0, and 5.0 mU/kg·min insulin clamp steps increased to 4.58±0.40, 8.29±0.36, and 12.59±0.32 mg/kg·min. After 48 h of glucagon infusion, total glucose uptake in the postabsorptive state was significantly ($P < 0.001$) increased, 2.25±0.08 mg/kg·min, compared with the control study. However, insulin-stimulated glucose disposal was lower at each of the three insulin clamp steps compared with the control study (3.88±0.43, $P < 0.05$; 6.84±0.37, $P < 0.01$; 11.57±0.24 mg/kg·min, $P < 0.01$). When the plasma FFA concentration was

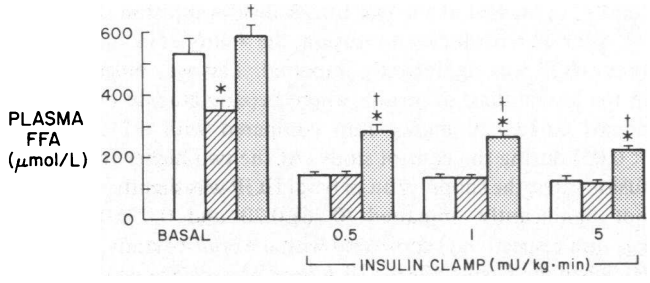

Figure 2. Plasma FFA concentration in the basal state and during the three-step (0.5, 1.0, and 5.0 mU/kg·min) insulin clamp study before glucagon (*open bars*), after glucagon (*hatched bars*), and after combined Intralipid/glucagon (*stippled bars*) infusion. All values represent the mean±SEM. *, $P < 0.05$–0.001 vs. control study; †, $P < 0.05$–0.001 vs. glucagon infusion.

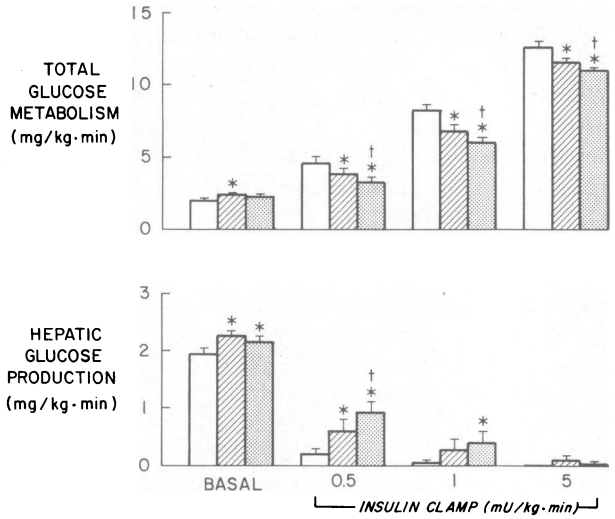

Figure 3. Total body glucose metabolism and HGP in the basal state and during the three-step (0.5, 1.0, and 5.0 mU/kg·min) insulin clamp study performed before (*open bars*) and after glucagon (*hatched bars*), and after combined Intralipid/glucagon (*stippled bars*) infusion. All values represent the mean±SEM. *, $P < 0.05$–0.001 vs. control study; †, $P < 0.05$–0.001 vs. glucagon infusion.

held constant by combined Intralipid/glucagon infusion (study III), basal glucose uptake, 2.14±0.10 mg/kg·min, was intermediate between the control and glucagon alone studies. During the stepwise insulin clamp study performed after combined glucagon/Intralipid infusion, total body glucose metabolism was slightly less than in the same five subjects studied after glucagon infusion alone (3.34±0.25; 6.09±0.42, and 11.02±0.19 mg/kg·min, $P < 0.05$).

HGP (Fig. 3). During the control study, HGP averaged 1.96±0.08 mg/kg·min. After 48 h of glucagon infusion, this increased to 2.25±0.08 mg/kg·min, $P < 0.001$. The basal metabolic clearance rate of plasma glucose (2.40±0.11 and 2.43±0.10 ml/kg·min) was unchanged by 48 h of glucagon infusion. When glucagon was infused with Intralipid to maintain basal FFA levels constant, HGP, 2.14±0.10 mg/kg·min, was similar to glucagon infusion alone and slightly greater than in the control studies ($P < 0.05$).

During the stepwise insulin clamp study carried out before glucagon, 90% inhibition of the HGP (0.21±0.09 mg/kg·min) was observed with the lowest insulin infusion rate. HGP was totally suppressed at the two higher insulin infusion steps (Fig. 3). After 48 h of glucagon infusion, the ability of insulin to suppress HGP was significantly impaired. This was most obvious at the lowest dose of insulin where hepatic glucose output averaged 0.61±0.20 mg/kg·min compared with 0.21±0.09 ($P < 0.05$) during the control study. At the two higher insulin infusion rates, the suppression of basal HGP was slightly, although not significantly, impaired (0.26±0.20 and 0.09±0.08 mg/kg·min respectively) compared with the control study. In study III, when the fasting plasma FFA level was maintained constant by the concomitant infusion of Intralipid with glucagon, a slightly greater impairment in the ability of insulin to suppress HGP was observed during the first two insulin steps (0.92±0.19 and 0.41±0.24 during the 0.5 and 1.0 mU/kg·min insulin infusion rates, respectively; both $P < 0.05$). HGP during the 5.0 mU/kg·min insulin clamp was 0.01±0.01 mg/kg·min.

Plasma glucose and insulin, glucose metabolism, and HGP after 48 h glucose infusion. Fasting plasma glucose concentration, 79±5 mg/dl, increased to 103±3 mg/dl ($P < 0.001$) after 48 h of glucose infusion. The increment in plasma glucose concentration (24±4 mg/dl) was similar to that observed after 48 h of glucagon infusion (17±1 mg/dl). The fasting plasma insulin concentration, 8±1 μU/ml, increased to 31±6 μU/ml ($P < 0.005$) after the 48 h glucose infusion. The increment in plasma insulin concentration (23±6 μU/ml) postglucose infusion was significantly greater than the increment (2±1 μU/ml) observed after glucagon infusion.

During the control euglycemic insulin clamp study, the steady-state plasma glucose concentration was 85±2 mg/dl (coefficient of variation = 4.5±0.4%). The plasma insulin levels were maintained at 50±3 and 87±2 μU/ml during the 0.5 and 1.0 mU/kg·min insulin infusion, respectively. In the insulin clamp study performed after 48 h of glucose infusion, the plasma glucose (85±2 mg/dl; CV = 4.6±0.3%) and insulin (60±8 and 92±10 μU/ml) concentrations were comparable to those achieved in the control study.

Total body glucose uptake during the 0.5 mU/kg·min (5.30±0.98 vs. 6.03±0.65 mg/kg·min) and 1.0 mU/kg·min (8.77±1.01 vs. 8.57±0.73 mg/kg·min) insulin clamp studies were similar before and after 48 h of glucose infusion. Basal HGP during the control study was 1.89±0.09 mg/kg·min and decreased to 0.07±0.07 and 0.03±0.03 mg/kg·min, respectively during the two-step insulin clamp. During the insulin clamp study performed after 48 h glucose infusion, suppression of HGP (0.16±0.10 and 0.14±0.11 during the 0.5 and 1.0 mU/kg·min insulin clamps, respectively) was similar to the control study.

Indirect calorimetry measurements (Fig. 4). Fig. 4 summarizes the results obtained from continuous indirect calorimetric measurements performed before and during the stepwise insulin clamp studies carried out before and after 48 h of glucagon infusion. The basal NPRQ was higher after glucagon infusion (0.88±0.02 vs. 0.78±0.02, $P < 0.01$). During the control study, each insulin step was associated with a progressive rise in NPRQ to 0.87±0.03, 0.92±0.03, and 0.99±0.04, respectively. During the stepwise insulin clamp performed after glucagon, the NPRQ was significantly greater ($P < 0.05$–0.01) at each insulin infusion rate. Notably, during the lowest dose insulin clamp study (0.5 mU/kg·min) the NPRQ already exceeded 1.00, indicating net lipid synthesis. When Intralipid was infused with glucagon to maintain the basal plasma FFA constant, the postabsorptive NPRQ (0.83±0.02) was returned to a value similar to that observed during the control study. Similarly, during the three-step insulin clamp, the NPRQ during each insulin infusion was similar to that in the control study and less than that observed after glucagon infusion ($P < 0.05$).

After glucagon infusion basal glucose oxidation (1.27±0.21 mg/kg·min) was higher than in the control study (0.76±0.17 mg/kg·min, $P < 0.05$). The increment in basal glucose oxidation was inversely correlated both to the reduction in basal FFA plasma concentration ($r = -0.94$; $P < 0.01$) and to the change in lipid oxidation ($r = -0.75$, $P < 0.01$) (Fig. 5). Glucose oxidation remained elevated during the lowest insulin infusion step (2.09±0.15 vs. 1.34±0.23 mg/kg·min; $P < 0.05$), However, it was not significantly different at the two higher insulin clamp steps (1.91±0.26 vs. 2.26±0.14 mg/kg·min and 2.70±0.25 vs. 2.69±0.21 mg/kg·min, P = NS). After combined Intralipid/glucagon infusion, basal glucose oxidation (1.10±0.16 mg/kg·min) was reduced to control levels. However, during each

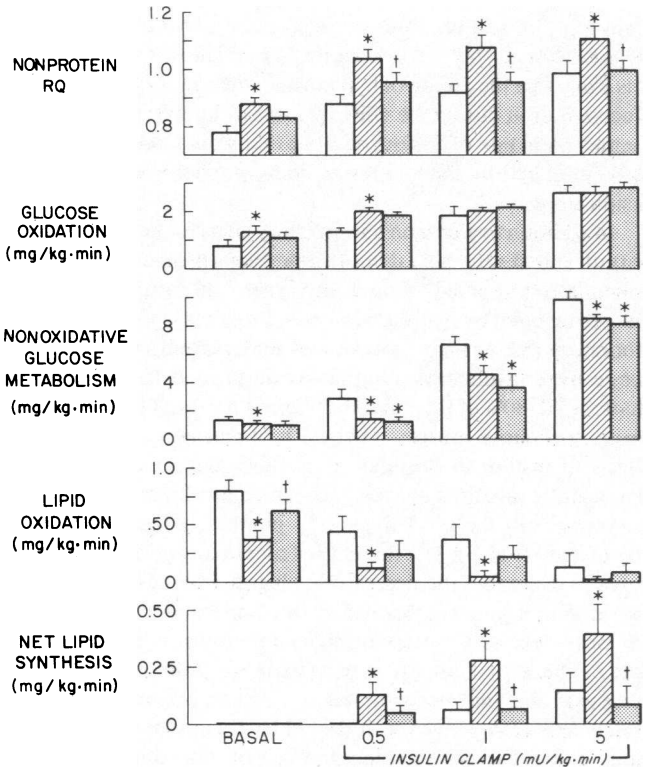

Figure 4. NPRQ, glucose oxidation, nonoxidative glucose metabolism, lipid oxidation, and net lipid synthesis in the basal state and during the three-step (0.5, 1.0, and 5.0 mU/kg · min) insulin clamp study performed before (*open bars*) and after glucagon (*hatched bars*) and after combined Intralipid/glucagon (*stippled bars*) infusion. All values represent the mean±SEM. *, $P < 0.05$–0.001 vs. control study; †, $P < 0.05$–0.001 vs. glucagon infusion.

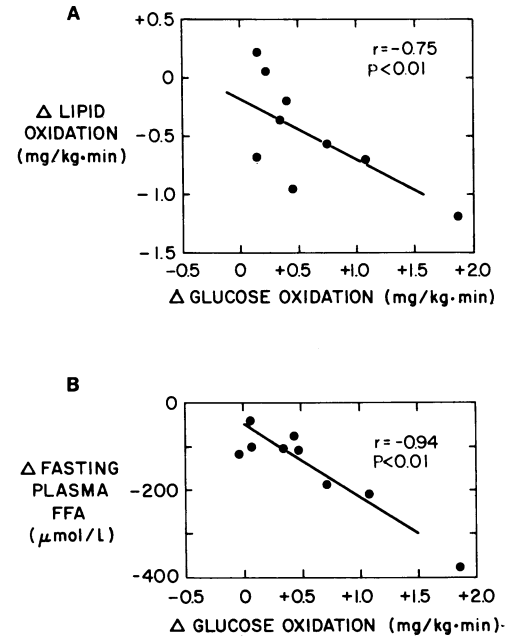

Figure 5. Correlation between the decrease in fasting plasma FFA level and the increase in glucose oxidation (*B*) and between the decrease in lipid oxidation and the increase in glucose oxidation (*A*) after 48 h of glucagon infusion.

insulin infusion step the rate of glucose oxidation was similar to that observed in the same five subjects during the insulin clamp study performed after glucagon infusion alone.

Decreased nonoxidative glucose metabolism was primarily responsible for the impairment in total body glucose metabolism after glucagon infusion. Thus, after glucagon infusion, both basal as well as insulin-stimulated nonoxidative glucose metabolism was significantly lower than in the control studies (basal = 1.38±0.19 vs. 1.05±0.18 mg/kg · min; $P < 0.05$; insulin-stimulated = 2.91±0.34 vs. 1.47±0.35, $P < 0.01$; 6.33±0.26 vs. 4.33±0.30, $P < 0.005$; 9.90±0.46 vs. 8.66±0.18, $P < 0.05$). After combined Intralipid/glucagon infusion, the decrease in nonoxidative glucose disposal was similar to that observed with glucagon alone.

Basal lipid oxidation was reduced after glucagon infusion (0.81±0.12 vs. 0.38±0.08 mg/kg · min, $P < 0.05$). This reduction inversely correlated to the increase in basal glucose oxidation ($r = -0.75$; $P < 0.01$) (Fig. 5). During the stepwise insulin clamp studies performed before and after glucagon, lipid oxidation progressively decreased. However, fat oxidation was significantly lower after glucagon at the two lower insulin clamp steps ($P < 0.01$). When Intralipid was infused with glucagon, basal lipid oxidation (0.63±0.10 mg/kg · min) was significantly greater ($P < 0.05$) than with glucagon alone but was not completely restored to control values. During each of the three insulin clamp steps, lipid oxidation was slightly, although not significantly, increased above that observed with glucagon alone.

It is noteworthy that during the insulin clamp studies performed after glucagon infusion, the NPRQ reached and overcame the unity value at the lowest insulin infusion step (Fig. 3). On the contrary, in the control study, an average NPRQ of one was observed only at the highest insulin clamp step. A NPRQ > 1 indicates net lipid synthesis. During the insulin clamp studies performed after glucagon infusion, net lipid synthesis occurred during all three insulin clamp steps (0.13±0.05; 0.28±0.10; and 0.40±0.14 mg/kg · min) (Fig. 3). During the control study net lipid synthesis was not observed during the lowest insulin clamp step and was significantly lower than during the postglucagon study at the two highest insulin steps (0.06±0.03 and 0.17±0.08 mg/kg · min, both $P < 0.05$). Intralipid infusion with glucagon reduced lipid synthesis to values observed during the control study.

Discussion

Unger and Orci (1) were the first to propose that diabetes mellitus was a bihormonal disease in which both insulin deficiency and glucagon excess (absolute or relative) contributed to the disturbance in glucose metabolism. Since this initial hypothesis, however, considerable controversy has been generated concerning the precise role of glucagon in the pathogenesis of diabetes mellitus (45). Positive evidence favoring a role for glucagon includes: the absolute or relative hyperglucagonemia found in human diabetes (46) and the improvement in diabetic control after glucagon suppression with somatostatin (47, 48). In contrast, antagonists of the glucagon hypothesis have cited the transient hyperglycemic effect of the hormone (26–29). In the present paper we have examined the impact of prolonged hyperglucagonemia on glucose metabolism in healthy young subjects. Our results indicate that hyperglucagonemia is associated with a significant, albeit modest, impairment in glucose disposal.

When hyperglucagonemia (414±24 pg/ml) was created for

48 h, a significant rise in the fasting plasma glucose concentration (from 76 to 93 mg/dl, $P < 0.001$) was observed at both 24 and 48 h. This hyperglycemic effect occurred without any significant change (actually, a slight increase) in the fasting plasma insulin concentration. In the postabsorptive state, the fasting plasma glucose concentration is determined by two variables: the rate of HGP, and the rate of glucose removal by all the tissues of the body. To examine which of these mechanisms was responsible for the increase in fasting plasma glucose level, [3-^{3}H]glucose was used to quantitate HGP and glucose disposal. The results of these studies demonstrated that the hyperglycemic effect of glucagon was entirely accounted for by an enhancement in HGP. In accordance, the rate of glucose disposal by the entire body during the postabsorptive state was significantly enhanced (Fig. 3) and the glucose clearance remained unchanged. These results provide conclusive evidence that the increase in fasting plasma glucose concentration after glucagon is entirely due to an augmentation in endogenous HGP.

As previously discussed, several reports (26–29) have indicated that the stimulatory effect of glucagon on HGP in healthy subjects wanes with time. However, in all of these reports, glucagon infusion lasted no more than 6 h and no information is available concerning the effect of more prolonged glucagon stimulation on endogenous glucose production. Boden et al. (49) studied a patient with the glucagonoma syndrome as an example of chronic hyperglucagonemia, but could not demonstrate any increase in total hepatic glucose output. However, they did note that the rate of gluconeogenesis was markedly enhanced and accounted for essentially all of HGP during the postabsorptive state. It should be noted that the glucagonoma patient described by Boden et al. (49), in contrast to our subjects, had a markedly elevated fasting plasma insulin concentration (20–60 μU/ml) and this could have masked the true stimulatory effect of glucagon on HGP. With regard to the mechanism(s) contributing to the increased glucose production, glucagon has been shown to stimulate both glycogenolysis and gluconeogenesis (30). Cherrington et al. (30) demonstrated in dogs that the decline in glucose production during continuous glucagon infusion is due to an attenuation of glycogenolysis. On the contrary, after 3 h of glucagon infusion, the rate of gluconeogenesis was markedly enhanced. Although not directly measured in our studies, it is likely that the increased rate of endogenous glucose production seen at 24 and 48 h primarily reflects a persistent stimulatory effect of glucagon on hepatic gluconeogenesis (30, 50).

Both insulin (51) and hyperglycemia (52) are known to suppress HGP. Therefore, the finding of an increased hepatic glucose output in the face of hyperglycemia and a normal to slightly elevated plasma insulin concentration suggests that prolonged hyperglucagonemia causes the liver to become resistant to the suppressive effects of insulin on basal hepatic glucose output. This hepatic insulin resistance also was evident during the insulin clamp studies. In the control study, HGP was promptly inhibited by 90% during the lowest dose insulin infusion step (0.5 mU/kg · min) and was completely suppressed during the two highest insulin doses. In contrast, after 48 h of sustained hyperglucagonemia, suppression of HGP during the 0.5 mU/kg · min insulin infusion step was impaired by 50%. Suppression of HGP was also less complete during the two higher insulin infusion steps. Our results do not delineate whether the impaired suppression of HGP during the insulin clamp study is due to an incomplete inhibition of glycogenolysis vs. gluconeogenesis. However, because the stimulatory effect of glucagon on glycogenolysis is transient (30) and because very high plasma insulin concentrations (> 200 μU/ml) are needed to shut off gluconeogenesis (53), it is likely that the failure of hyperinsulinemia to suppress hepatic glucose output during the stepwise insulin clamp reflects an augmented basal rate of gluconeogenesis that would not be expected to be inhibited by the two lower, more physiologic, insulin infusion steps.

In addition to glucagon's stimulatory effect on hepatic glucose output, our results also demonstrate that chronic hyperglucagonemia is associated with an impairment in insulin-mediated glucose disposal by peripheral tissues. Previous studies from our laboratory (32, 54) have shown that under conditions of euglycemic hyperinsulinemia, similar to those used in the present study, > 70–80% of the infused glucose is removed by peripheral tissues, primarily muscle. After 48 h of glucagon infusion, the ability of insulin to stimulate total body glucose disposal was significantly impaired at each of the three insulin infusion steps, spanning both the physiologic and pharmacologic range (50–600 μU/ml) (Fig. 3). The inability of maximally effective insulin doses to overcome the decrease in glucose uptake is most consistent with a postreceptor defect in insulin action (55) but by no means excludes an abnormality in insulin binding. These data might, at first, appear to be at variance with the commonly accepted belief that glucagon has no effect on peripheral glucose metabolism (19). However, it should be noted that in these previous studies exposure to glucagon was of short duration, < 1–2 h. More recently, Schneider et al. (56), using the forearm perfusion technique, have demonstrated that acute exposure to glucagon (~ 600 pg/ml) causes a 30% reduction in insulin-mediated glucose uptake across both the superficial and deep venous beds. These results are in agreement with those of Rizza et al. (57) who found that hyperglucagonemia causes a significant decline in the metabolic clearance rate of glucose. Cherrington et al. (30) also noted that hyperglucagonemia in dogs caused a significant fall in the glucose clearance, which was reversed upon restoration of normal basal glucagon levels.

In our studies, chronic hyperglucagonemia was associated with a 35% decline in the fasting plasma FFA concentration. Because FFA are known to impair glucose use (58), the true inhibitory effect of glucagon on total body glucose metabolism may have been underestimated. To examine this question, subjects were restudied with a combined glucagon/Intralipid infusion, which was designed to maintain the plasma FFA concentration constant at basal levels. When the stepwise insulin clamp study was repeated, an even greater decline in insulin-mediated glucose disposal was observed. The mean decline, 30±3%, was similar to the percent decrease in insulin-mediated forearm glucose uptake reported by Schneider et al. (56). Furthermore, the results of these authors (56) indicate that part of the inhibitory effect of glucagon must be exerted on peripheral tissues. Although it is possible that decreased splanchnic glucose uptake could contribute to the defect in total body glucose uptake, this seems unlikely. We have previously shown that basal splanchnic glucose uptake is in the order of 0.5 mg/kg · min and that this is not enhanced by insulin levels as high as 500–1000 μU/ml (54).

The indirect calorimetry results provide some information concerning the cellular mechanisms responsible for the insulin resistance induced by prolonged hyperglucagonemia. Basal glucose oxidation was significantly elevated after glucagon and was either increased (0.5 mU/kg · min insulin step) or normal (1.0 and 5.0 mU/kg · min insulin steps) during the insulin clamp. It is noteworthy that the fasting plasma FFA concentration was

reduced (by 180 μmol/liter) after hyperglucagonemia. It seems unlikely that an increased plasma insulin concentration can explain the decline in FFA levels because the increment (2 μU/ml) was small and not statistically significant. Previous reports have described an FFA lowering effect of glucagon (59–61) without any change in the plasma insulin level. The reduction in plasma FFA concentration also might be explained by the higher plasma glucose level. Shulman et al. (62) have demonstrated in dogs that hyperglycemia lowers the plasma FFA concentration, probably by an inhibitory effect on lipolysis. The lower plasma FFA level is likely to contribute to the increase in basal glucose oxidation after glucagon administration. This is supported by the finding that basal FFA levels and lipid oxidation were diminished and these changes were inversely correlated with an increased rate of basal glucose oxidation (Fig. 5). Similarly, during the lowest dose insulin clamp step, the decline in lipid oxidation was also less after glucagon. In the two highest insulin clamp steps, glucose oxidation was similar in the pre- and postglucagon studies, while the decline in plasma FFA and lipid oxidation were not significantly different. Further support for the important interaction between FFA and glucose oxidation is afforded by the combined Intralipid/glucagon infusion protocol. In this study, maintenance of basal FFA levels returned both the decreased rate of basal lipid oxidation and elevated basal rate of glucose oxidation to normal. Thus, our results are in agreement with the so called glucose–fatty acid cycle proposed 20 yr ago by Randle et al. (63) and more recently confirmed by several studies in humans (58, 64, 65).

Because glucose oxidation was either increased or unchanged during each of the three insulin infusion rates, yet total body glucose utilization was significantly reduced at each step, it is clear that the entire impairment in insulin action must be accounted for by a defect in the nonoxidative pathways of glucose metabolism. Consistent with this, a very strong positive correlation ($r = 0.97$, $P < 0.001$) was observed between total body and nonoxidative glucose disposal when the pre- and postglucagon studies are considered together (Fig. 6). From the quantitative standpoint, the major nonoxidative pathway of glucose disposal is glycogen formation (32). Some of the glucose that is not oxidized may be converted to lipid (66). However, a defect in this pathway is unlikely to account for the impairment in nonoxidative glucose metabolism. In fact, the indirect calorimetry measurements documented an increase in net lipid synthesis during all three steps of the insulin clamp study performed after glucagon infusion. Thus, prolonged hyperglucagonemia seems to primarily affect insulin-mediated glycogen synthesis. However, the cellular mechanism(s) via which glucagon can oppose insulin action, particularly at the level of peripheral tissues, remains to be clarified.

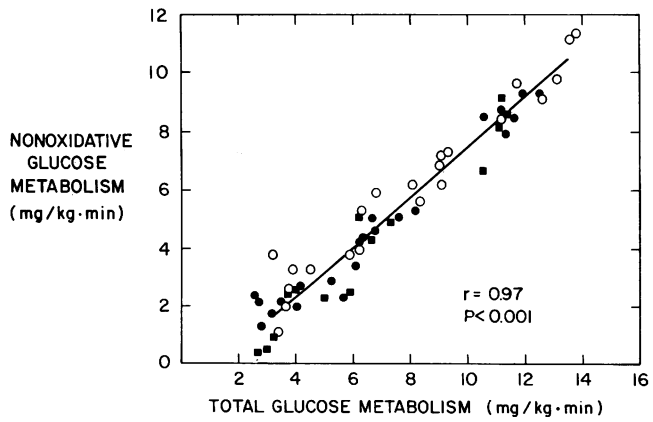

Figure 6. Correlation between nonoxidative glucose disposal and total body glucose uptake during each of the three-step insulin clamp studies performed before (*open circles*) and after (*filled circles*) glucagon and after combined Intralipid/glucagon (*filled boxes*) infusion ($r = 0.97$, $P < 0.001$).

Some comment is warranted concerning a possible role for insulin in the development of insulin resistance after hyperglucagonemia. Rizza et al. (67) have shown that 40 h of sustained hyperinsulinemia (25–35 μU/ml), produced in normal subjects by continuous insulin infusion, resulted in a modest (15%) reduction in insulin-mediated glucose disposal. Because insulin binding to monocytes was not diminished, the authors concluded that the development of insulin resistance was due to a postreceptor defect in insulin action. In the present study, basal insulin concentration was not found to be increased after glucagon administration. However, plasma insulin response to meals was not measured and increased portal concentration of the hormone can not be excluded. Thus, an elevated insulin secretory rate after glucagon can not be entirely excluded. To evaluate whether or not endogenous hyperinsulinemia can affect glucose metabolism, five normal volunteers were studied before and after 48 h of glucose infusion. Despite the maintenance of plasma glucose levels comparable to those found during glucagon infusion and despite the presence of moderate hyperinsulinemia (31 μU/ml), the ability of insulin to enhance glucose uptake and to suppress HGP was not impaired. These observations are in agreement with recent data obtained in animals (68, 69), and support a direct effect of hyperglucagonemia to inhibit insulin-mediated glucose metabolism by peripheral tissues.

After glucagon infusion, net lipid synthesis was observed during each of the three insulin clamp steps (Fig. 4). Of note, the steady-state plasma insulin concentration during the lowest insulin infusion step was only 52±5 μU/ml. Glucagon by itself is not known to be a lipogenic hormone. In fact, both in vitro and in vivo studies indicate that glucagon stimulates lipolysis in adipose tissue (70) and promotes fatty acid oxidation, not fat synthesis, in hepatocytes (71). However, none of these studies has examined the combined effect of hyperinsulinemia and hyperglucagonemia on fat synthesis. After 48 h of glucagon infusion nonoxidative glucose disposal is impaired and, as discussed earlier, this most likely reflects diminished insulin-mediated glycogen synthesis. In humans, the primary site of de novo fatty acid synthesis is the liver (66), and both lactate and pyruvate are excellent precursors for lipogenesis (72). Because glucose oxidation is not impaired, glucose flux through the glycolytic pathway must be intact, and a normal or even increased intracellular lactate/pyruvate pool would be expected. It is possible, therefore, that during the insulin clamp a significant amount of the infused glucose might be shunted from glycogen to lipid formation.

Lastly, some potential clinical implications of our work are worthy of comment. Both insulin-dependent and noninsulin dependent diabetic individuals, as well as obese nondiabetics, are characterized by insulin resistance (2–14). All three of these clinical conditions are often associated with elevated plasma glucagon (45, 46) and FFA (73) levels. Both of these metabolic disturbances are known to be associated with insulin resistance and may contribute to the diminished tissue sensitivity to insulin

observed in obesity and diabetes. In contrast, patients with diabetes secondary to pancreatectomy or pancreatic disease are characterized by hypoglucagonemia (74, 75) and normal plasma FFA concentrations (16); they also have higher tissue sensitivity to insulin (76–78). Our observations also may have relevance to the frequent occurrence of fatty liver and steatosis in obesity and noninsulin-dependent diabetes mellitus (79, 80). In both of these common clinical conditions, elevated basal glucagon levels, fasting hyperinsulinemia, and an augmented plasma insulin response to glucose are frequently observed. The present results provide evidence that the combination of hyperinsulinemia and hyperglucagonemia is markedly lipogenic.

In summary, prolonged hyperglucagonemia produced in normal subjects by continuous glucagon infusion for 48 h: (*a*) induces a sustained increase in HGP and opposes the suppressive effect of insulin on HGP; and (*b*) impairs the ability of insulin to increase glucose utilization, primarily by reducing nonoxidative glucose disposal, and enhances the lipogenic effect of insulin. These glucagon-induced alterations in glucose and lipid metabolism may contribute to the alterations in insulin sensitivity and lipid metabolism observed in diabetes mellitus and obesity.

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