Metabolic effects of intensive insulin therapy in critically ill patients


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Whyte MB, Jackson NC, Shojae-Moradie F, Treacher DF, Beale RJ, Jones RH, Umpleby AM. Metabolic effects of intensive insulin therapy in critically ill patients. Am J Physiol Endocrinol Metab 298:E697–E705, 2010. First published December 22, 2009; doi:10.1152/ajpendo.00407.2009.—Our aim was to investigate the effects of glycemic control and insulin concentration on lipolysis, glucose, and protein metabolism in critically ill medical patients. For our methods, the patients were studied twice. In study 1, blood glucose (BG) concentrations were maintained between 7 and 9 mmol/l with intravenous insulin. After study 1, patients entered one of four protocols for 48 h until study 2: low-insulin high-glucose (LIHG; variable insulin, BG of 7–9 mmol/l), low-insulin low-glucose (LILG; variable insulin of BG 4–6 mmol/l), high-insulin high-glucose (HIHG; insulin (2.0 mU · kg⁻¹·min⁻¹ plus insulin requirement from study 1), BG of 7–9 mmol/l), or high-insulin low-glucose (HILG; insulin (2.0 mU·kg⁻¹·min⁻¹ plus insulin requirement from study 1), BG of 4–6 mmol/l). Age-matched healthy control subjects received two-step euglycemic hyperinsulinenic clamps achieving insulin levels similar to the LI and HI groups. In our results, whole body proteolysis was higher in patients in study 1 (P < 0.006) compared with control subjects at comparable insulin concentrations and was reduced with LI (P < 0.01) and HI (P = 0.001) in control subjects but not in patients. Endogenous glucose production rate (Rg) glucose disposal, and lipolysis were not different in all patients in study 1 compared with control subjects at comparable insulin concentrations. Glucose Rg and lipolysis did not change in any of the study 2 patient groups. HI increased glucose disposal in the patients (HIHG, P = 0.001; HILG, P = 0.007 vs. study 1), but this was less than in controls receiving HI (P < 0.03). In conclusion, low-dose intravenous insulin administered to maintain BG between 7–9 mmol/l is sufficient to limit lipolysis and endogenous glucose Rg, and increase glucose Rg. Neither hyperinsulinaemia nor normoglycemia had any protein-sparing effect.

The endogenous glucose production rate (Rg) has been shown to be increased by at least twofold in critical illness, compared with healthy controls (45); however, it is not known how effective a variable insulin infusion is at lowering glucose Rg. Insulin administration at a rate of 1 mg·kg⁻¹·min⁻¹ was required to overcome hepatic insulin resistance in septic patients (10), but there was evidence of incomplete suppression (as reflected by high serum insulin-like growth factor-binding protein-1 levels) in surgical patients (36). Accelerated lipolysis is also a consistent feature of critical illness, and elevated fatty acids can contribute to insulin resistance. In health, lipolysis is inhibited by low insulin levels, but in critical illness, higher doses may be required (10). The effect of maintenance of normoglycemia with intensive insulin therapy on lipolysis is also unknown.

Critically ill patients may experience loss of muscle protein approaching 2% per day (19). Patient survival (20), duration of intensive care unit (ICU) admission (12), and time to recovery of normal physiological function (59) are inversely correlated with loss of lean body mass. The effect of insulin on protein metabolism has been contentious, but it appears primarily to inhibit proteolysis (8, 18, 38, 51), although increased protein synthesis has also been reported (6). We hypothesized that the antiproteolytic effects of insulin might explain the benefits of insulin administration to critically ill patients.

We have studied the effect of maintaining normoglycemia with intravenous insulin on the rate of lipolysis, glucose, and protein metabolism in patients requiring tertiary-level support.

CRITICALLY ILL PATIENTS EXHIBIT A STRESS RESPONSE CHARACTERIZED BY LIPOLYSIS, PROTEIN BREAKDOWN, AND HYPERGLYCEMIA. Hyperglycemia was considered to be a beneficial, adaptive response, but recent evidence suggests that it may be detrimental. Maintenance of normoglycemia with either a glucose-insulin-potassium infusion (32) or variable insulin infusion (16, 55, 56) has been reported to improve patient morbidity and mortality. Post hoc analysis suggested that the prevailing glycaemia, rather than insulin dose administered, effects the beneficial outcomes (57). More recently, debate has intensified following the publication of a multicenter study that casts doubt over the benefits of glycemic control in a critical care environment (15). The metabolic effects of such treatment have yet to be characterized.
newly diagnosed HIV. Patients received a variety of vasopressor agents, steroids, and anesthetic agents (Table 1). Severity of illness was determined by the Acute Physiology and Chronic Health Evaluation (APACHE) II score.

All patients were studied on two occasions, 48 h apart. Patients receiving enteral nutrition (Nutrison MultiFibre; Nutricia, Zoetermeer, Holland) had the feed stopped 8 h before study 1. All patients received intravenous 20% dextrose (25 kcal·kg⁻¹·day⁻¹) for at least 8 h before study 1 (range 10–18 h). Study 1 took place within 36 h of ICU admission with blood glucose (BG) concentrations maintained between 7 and 9 mmol/l with intravenous Actrapid insulin (Novo-Nordisk, Bagsvaerd, Denmark). The insulin infusion was adjusted at hourly intervals to maintain BG between 7 and 9 mmol/l for at least 10 h before study 1 (range 10–18 h; Fig. 1). After study 1, patients entered one of four protocols for 48 h until study 2: low-insulin high-glucose (LIHG; variable insulin infusion to maintain BG of 7–9 mmol/l), low-insulin low-glucose (LILG; variable insulin to maintain BG of 4–6 mmol/l), high-insulin high-glucose (HIHG; insulin infusion rate of 2.0 mU·kg⁻¹·min⁻¹ plus insulin requirement from study 1) with additional dextrose, as required, to maintain BG of 4–6 mmol/l; high-insulin low-glucose (HILG; insulin infusion rate of 2.0 mU·kg⁻¹·min⁻¹ plus insulin requirement from study 1) with additional dextrose, as required, to maintain BG of 4–6 mmol/l; Fig. 1).

Study Protocol (Patients)

Study 1. After baseline sampling, a prime of 170 mg [6,6 \(^{2}H_{2}\)glucose, 0.15 mg/kg \([^{2}H_{2}]\)glycerol, 1 mg/kg \([1-^{13}C]\)leucine, and 0.2 mg/kg \([^{13}C]NaHCO_{3}\) (CK Gases, UK, subsidiary of Cambridge Isotope Laboratories, Cambridge, MA) was administered (33) followed by continuous infusion of 1.7 mg/min [6,6 \(^{2}H_{2}\)glucose, 0.61 mg·kg⁻¹·h⁻¹ \([^{2}H_{2}]\)glycerol, and 1 mg·kg⁻¹·h⁻¹ \([1-^{13}C]\)leucine for 180 min. During the 3-h tracer infusion, glucose infusion rate remained constant, and if necessary, intravenous insulin was adjusted every 30 min to maintain BG concentrations. Indwelling arterial and central venous lines were used for blood sampling and for tracer infusion, respectively. BG was determined on heparinized samples using a point-of-care Omni 2 blood gas analyzer (AVL/Roche, Welwyn Garden City, UK).

Table 1. Group characteristics

<table>
<thead>
<tr>
<th></th>
<th>LILG (n = 6)</th>
<th>LIHG (n = 7)</th>
<th>HILG (n = 6)</th>
<th>HIHG (n = 6)</th>
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<tr>
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<td>Age, yr</td>
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<td>66.0 ± 4.4</td>
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<td>BMI, kg/m²</td>
<td>24.8 ± 2.4</td>
<td>25.3 ± 1.8</td>
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<tr>
<td>APACHE II</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Study 1</td>
<td>17.2 ± 2.8</td>
<td>15.9 ± 1.4</td>
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<td>Study 2</td>
<td>15.4 ± 1.5</td>
<td>17.0 ± 1.8</td>
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<td>Plasma glucose, mmol/l</td>
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<td>Study 1</td>
<td>7.94 ± 0.92</td>
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<td>8.02 ± 0.47</td>
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<td>Study 2</td>
<td>5.60 ± 0.43*</td>
<td>8.81 ± 0.41</td>
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<td>4.02 ± 0.85</td>
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<td>Study 2</td>
<td>5.03 ± 1.76</td>
<td>2.09 ± 0.73</td>
<td>13.57 ± 1.61*†</td>
<td>15.48 ± 2.67*†</td>
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<td>Glucocorticoids (n)</td>
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<td>1</td>
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<td>3</td>
</tr>
<tr>
<td>Study 2</td>
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<td>1</td>
<td>3</td>
<td>3</td>
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<tr>
<td>CRP, mg/l</td>
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<tr>
<td>Study 1</td>
<td>118.3 ± 28.7</td>
<td>150.0 ± 48.8</td>
<td>180.8 ± 50.6</td>
<td>72.8 ± 26.0</td>
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<td>Study 2</td>
<td>79.3 ± 24.1</td>
<td>83.7 ± 29.7</td>
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<td>Mean daily caloric intake, kcal/day</td>
<td>1,725 ± 107</td>
<td>1,779 ± 222</td>
<td>2,885 ± 344</td>
<td>4,217 ± 678†</td>
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</tbody>
</table>

Data are means ± SE. APACHE II, Acute Physiology and Chronic Health Evaluation score II; CRP, C-reactive protein; BG, blood glucose; LIHG, low-insulin high-glucose (variable insulin, BG of 7–9 mmol/l); LILG, low-insulin low-glucose (variable insulin, BG of 4–6 mmol/l); HIHG, high-insulin high-glucose [high dose insulin infusion (2.0 mU·kg⁻¹·min⁻¹) plus the insulin requirement from study 1, BG of 7–9 mmol/l], HILG, high-insulin low-glucose [high dose insulin infusion (2.0 mU·kg⁻¹·min⁻¹)] plus the insulin requirement from study 1, BG of 4–6 mmol/l]; *P ≤ 0.01 vs. study 1; †P < 0.001 vs. low-insulin groups.
Blood samples were taken at baseline and at 150, 160, 170, 175, and 180 min (steady state) to measure the isotopic enrichment of glucose, glycerol, and α-keto-isocaprate (α-KIC) and hormone and metabolite concentrations. Samples were stored at −70°C until analysis.

Expired air was collected from the exhaust port of the ventilator, at the same intervals as blood sampling to measure isotopic enrichment, and stored in 250-ml aluminium-coated bags (QuinTron Instrument, Milwaukee, WI), pending transfer into evacuated, septum-capped 10-ml glass tubes (Exetainer; Labco, High Wycombe, Buckinghamshire, UK) for the measurement of $^{13}$CO$_2$ by isotope ratio mass spectrometry. Pulmonary gas exchanges were measured by an open-circuit indirect calorimeter (Deltatrac II metabolic monitor; Datex-Ohmeda). Data acquisition was performed until steady-state values were reached over a 30- to 60-min period, defined as requiring no change in ventilatory settings, no requirement for large volumes of fluids, and institution or cessation of vasopressors. The values of oxygen consumption (V$_{O2}$) and carbon dioxide production (V$_{CO2}$) and the calculated respiratory quotient (RQ) represent an average of the data obtained over 30 min, with a coefficient of variation of <10%.

After study 1, enteral feeding (1 kcal/ml; 16% protein, 49% carbohydrate, and 35% fat) was instituted immediately, unless a clinical decision was made not to do so. The feed was supplemented by intravenous 50% dextrose, as required, to achieve glycemic targets. Patients were fed via nasogastric tube according to a standard feeding protocol. It was the policy of the local ICU to use total parenteral nutrition (TPN) only if specifically indicated, as such, no patient received TPN. Eight hours before the second isotope study the enteral feed was stopped and replaced with isocaloric intravenous 20 or 50% dextrose (depending on fluid requirements).

Study 2. The isotope infusion and sampling protocol of the second isotope study was identical in methodology to study 1.

Study Protocol (Control Subjects)

Five healthy age-matched control subjects (4 males and 1 females) were studied to quantify the normal dose-response effect of insulin on suppression of proteolysis, lipolysis, and glucose production and increase in glucose disposal. All volunteers were in good health and gave informed consent to the study. Subjects were fasted from midnight and were studied throughout in a semi-recumbent position. Following baseline blood sampling, a 9-h, primed, constant infusion of 1.7 mg/min [6,6-$^3$H$_2$]glucose, 0.61 mg·kg$^{-1}$·h$^{-1}$ [$^3$H$_2$]glycerol, and 1 mg·kg$^{-1}$·h$^{-1}$ [1-$^{13}$C]leucine was commenced using an IVAC syringe pump (Alaris Systems, San Diego, CA). Insulin was infused at 0.5 mU·kg$^{-1}$·min$^{-1}$ between 180–360 min and 2.0 mU·kg$^{-1}$·min$^{-1}$ from 360–540 min. Blood samples were taken at baseline, 150, 160, 170, 175, and 180 min (first steady-state); at 330, 340, 350, 355, and 360 min (second steady-state); and at 510, 520, 530, 535, and 540 min (third steady state) (Fig. 2). BG was determined every 5 min (Yellow Springs Instruments, Yellow Springs, OH) and maintained within 5% of 5 mmol/l by adjustment of a 20% glucose infusion (IVAC volumetric pump; Alaris Systems; Ref. 13), spiked with [6,6-$^2$H$_2$]glucose (“hot glucose infusion method,” using 8 mg/g glucose for step 1 and 10 mg/g glucose for step 2).

Experimental Methods

Plasma α-KIC enrichment was measured as a quinoxalinol-tetra-p-trybutoxytrimethylsilyl derivative as previously described (17). With the use of a reciprocal pool model (35), plasma α-KIC enrichment was considered a measure of intracellular leucine enrichment. $^{13}$C enrichment of breath CO$_2$ was measured on a SIRA series II continuous-flow isotope ratio mass spectrometer (VG Isotech, Cheshire, UK) modified with a Roboprep G+ inlet system (Europa Scientific). The isotopic enrichment of plasma glucose was determined by the pent-0-trimethylsilyl-d-glucose-o-methoxamine derivative (46) and glycerol as the Tris-trimethylsilyl derivative (60). Plasma and infusate glucose concentrations were determined using a Clandon Scientific glucose analyzer (Yellow Springs Instruments, Yellow Springs, OH). Blood lactate concentration was measured by a point-of-care AVL/Roche Omni2 blood gas analyzer. Serum nonesterified fatty acids (NEFA) concentrations were determined spectrophotometrically by Cobas analyzer (Roche, Welwyn Garden City, UK) using a kit from Wako Chemicals (Alpha Laboratories, Hampshire, UK). Plasma and infusate glycerol concentrations were measured by direct colorimetry (Randox Laboratories, Crumlin, Antrim, UK) on a Cobas analyzer. Total cholesterol concentration was measured using a kit from ABX Diagnostics (Montpellier, France) on a Cobas analyzer. Serum cortisol concentration was measured by an automated chemiluminescent assay (Bayer Diagnostics, Tarrytown, NY). Serum insulin concentrations were measured by an in-house double-antibody RIA (47). C-peptide concentration was measured using a double-antibody RIA kit (Linco Research, St. Charles, MI). C-reactive protein was measured with an immunoturbidometric assay (Beckman Synchron LX20).
Calculations

The rates of appearance and disappearance of a substrate were calculated from the dilution of labeled substrate in plasma using a monocompartment model and Steele’s equations (2) modified for stable isotopes. During physiological and isotopic steady-state, the production rate (Ra) of unlabeled substrate can be calculated by:

$$Ra (\mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \left( \frac{E_i}{E_p} - 1 \right) \times I$$

where Ei is enrichment of infused [atom %excess (APE)], Ep is enrichment of substrate in plasma (APE), and I is infusion rate (\(\mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}\)). Total glucose Ra was assumed to equal peripheral glucose disposal (Rd). The endogenous glucose production rate was estimated by subtraction of the unlabeled glucose infusion rate from the total glucose Ra. Propofol (Diprivan) contains glycerol at 22.5 mg/ml and soybean oil at 100 mg/ml. We have assumed that 30% of the triglyceride composition of soybean oil is hydrolyzed to glycerol (25). Endogenous glycerol Rd was calculated by subtracting the rate of glycerol appearance from propofol from the total glycerol Ra. Leucine oxidation rate (Ox) was calculated from the CO2 Ra and expired \(^{13}\text{CO}_2\) as previously described (34).

$$Ox (\mu \text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = (E_{\text{CO}_2} \times V_{\text{CO}_2})/E_{\text{KIC}} \times 0.899$$

where E\(_{\text{CO}_2}\) is the APE of \(^{13}\text{CO}_2\) in expired air, E\(_{\text{KIC}}\) is the [\(^{13}\text{C}\)]KIC enrichment, and 0.899 is a recovery factor that accounts for the fraction of \(^{13}\text{CO}_2\) formed on oxidation of the tracer but not released from the bicarbonate pool (53). Nonoxidative leucine disappearance rate (NOLD, a measure of protein synthesis) was calculated by subtracting leucine oxidation rate (Ra) from leucine Rd. Net protein balance was estimated using the equation (NOLD – Rd), with the assumption of 8 g of leucine for 100 g of whole body protein (22).

Commercially available dextrose for intravenous infusion contains carbon with a high natural abundance of \(^{13}\text{C}\) isotope: this could affect calculation of leucine oxidation when first administered. As the patient group received at least 8 h of intravenous dextrose before the \(^{13}\text{C}\)-leucine infusion, the rate of \(^{13}\text{C}\) release by oxidation of exogenous carbohydrate would have equilibrated before \(^{13}\text{C}\) leucine was infused. Basal samples were collected before the isotope infusion, thus allowing correction for this in the subsequent measurement of leucine oxidation. However, in the control group, dextrose was infused after infusion of the isotopes, so the contribution of \(^{13}\text{C}\) isotope to \(^{12}\text{CO}_2\) could not be corrected for and leucine oxidation could not be measured.

Statistical Analysis

The primary endpoint was leucine Ra. With six patients in each group, the study was powered to detect a difference of 20% in leucine Ra between study 1 and study 2 in the patients with a power of 80% at a two-sided 5% level of significance. This was based on previous studies of protein metabolism in ICU in both surgical patients (26) and septic medical patients (5), whereby the SD for leucine Ra was estimated by subtraction of the unlabeled glucose infusion rate from the total glucose Ra. Propofol (Diprivan) contains glycerol at 22.5 mg/ml and soybean oil at 100 mg/ml. We have assumed that 30% of the triglyceride composition of soybean oil is hydrolyzed to glycerol (25). Endogenous glycerol Rd was calculated by subtracting the rate of glycerol appearance from propofol from the total glycerol Ra. Leucine oxidation rate (Ox) was calculated from the CO2 Ra and expired \(^{13}\text{CO}_2\) as previously described (34).

RESULTS

Group Characteristics

The four groups differed significantly only as intended with respect to glucose concentration and insulin infusion rates (group characteristics are shown in Table 1). The age (60.0 ± 1.3 years) and body mass index (25.0 ± 0.9 kg/m\(^2\)) of control subjects were not different from the patients. All subjects survived the duration of the stud, although one patient in the HIHG group subsequently died. Treatment protocols were well tolerated with no short-term adverse effects seen in gas exchange, renal, hepatic, or cardiovascular status. Two glucose measurements were <3 mmol/l during the study, but none were <2.2 mmol/l. The control subjects’ plasma glucose was 5.03 ± 0.14 mmol/l at baseline, 4.34 ± 0.16 mmol/l with LD insulin, and 4.24 ± 0.19 with HD insulin (NS).

Hormone and Plasma Metabolite Concentrations

Serum insulin, plasma C-peptide, and serum cortisol concentrations are shown in Table 2. In the LI study 2 patient groups, insulin concentrations were not different from the LI control study. In the HI study 2 patient groups, insulin concentrations were not different from the HI control study. Plasma lactate, serum NEFA, plasma glycol, and total cholesterol levels are shown in Table 3. Plasma NEFA, glycerol, and total cholesterol concentrations were not different in study 2 compared with study 1 in any of the patient groups. Plasma total cholesterol was significantly lower in all patient groups (at both study 1 and study 2 compared with fasting control subjects; \(P < 0.01\)). Plasma NEFA concentrations were lower in study 1 in all patients (\(n = 25\)) compared with fasting control subjects (NEFA: 0.22 ± 0.04 vs. 0.67 ± 0.11 mmol/l; \(P = 0.02\)) but were higher than in the control study LI step where insulin levels were comparable (NEFA: 0.04 ± 0.005 mmol/l; \(P < 0.001\)).

Kinetic Data

In the control subjects, leucine Rd, a measure of proteolysis (Fig. 3), was reduced from basal levels by 11.6 ± 3.3% with LI...
(from 1.78 ± 0.05 to 1.57 ± 0.03 μmol·μmol·kg⁻¹·min⁻¹; P = 0.01) and by 21.7 ± 0.9% (to 1.39 ± 0.04 μmol·kg⁻¹·min⁻¹) with HI (P < 0.001). In all patients in study 1 (n = 25), leucine Rₐ was not different from the fasting control subjects but was higher than control subjects with comparable insulin concentrations (LI; 2.00 ± 0.13 vs. 1.57 ± 0.03 μmol·kg⁻¹·min⁻¹; P = 0.006). Leucine Rₐ was higher in the HIHG group in study 2 than control subjects receiving HI (2.1 ± 0.15 vs. 1.39 ± 0.40 μmol·kg⁻¹·min⁻¹; P = 0.005). Comparing study 2 to study 1, none of the four treatment regimens altered the rates of leucine Rₐ, NOLD, and leucine oxidation (Fig. 3 and Table 4) with the result that net protein balance was unaffected (Fig. 4). Furthermore, leucine Rₐ of the combined LI groups (n = 13) did not change from study 1 to study 2 (2.07 ± 0.19 vs. 2.13 ± 0.15 μmol·kg⁻¹·min⁻¹); likewise, leucine Rₐ of the combined HI groups (n = 12) was unchanged from study 1 to study 2 (1.94 ± 0.21 vs. 2.28 ± 0.19 μmol·kg⁻¹·min⁻¹). Since the insulin concentration was slightly, but not significantly, higher in the control subjects receiving HI than in the high-insulin patient groups, the change in leucine Rₐ was expressed per unit change in insulin concentration. In the HI patient group, a 1 nmol/l increase in serum insulin concentration lead to a 0.28 ± 0.32 μmol·kg⁻¹·min⁻¹ increase in leucine Rₐ, whereas in the control group it led to a 0.11 ± 0.01 μmol·kg⁻¹·min⁻¹ decrease in leucine Rₐ.

In the control subjects, glucose Rₐ was reduced from basal values with LI (P = 0.07) and was completely suppressed with HI (P = 0.003; NS different from 0; Table 5). Glucose Rₐ was increased with LI and HI compared with basal (P < 0.001). In all patients in study 1 (n = 25), endogenous glucose Rₐ was lower and glucose Rₐ was higher than the control subjects during fasting (P < 0.001) but they were not different from the LI step in the controls that had comparable insulin levels. In study 2, endogenous glucose Rₐ was lower than study 1 in the HIHG group but was no different in the other patient groups. Glucose Rₐ increased in study 2 in both HI groups (P = 0.001 for HIHG; P = 0.07 for HILG) compared with study 1, but this was lower than glucose Rₐ in the control study HI step that had

### Table 3. Plasma metabolite concentration

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<tr>
<th></th>
<th>Lactate, mmol/l</th>
<th>NEFA, mmol/l</th>
<th>Glycerol, μmol/l</th>
<th>Total Cholesterol, mmol/l</th>
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<td>LILG (n = 6)</td>
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<tr>
<td>Study 1</td>
<td>1.63 ± 0.36</td>
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<td>1.67 ± 0.16</td>
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<td>68.72 ± 18.15†</td>
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<td>1.53 ± 0.34</td>
<td>0.22 ± 0.05‡</td>
<td>58.14 ± 7.45†</td>
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<tr>
<td>HIHG (n = 6)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Study 1</td>
<td>1.86 ± 0.32</td>
<td>0.19 ± 0.05</td>
<td>74.14 ± 18.02</td>
<td>2.88 ± 0.46*</td>
</tr>
<tr>
<td>Study 2</td>
<td>2.15 ± 0.17</td>
<td>0.15 ± 0.05‡</td>
<td>101.88 ± 12.93ξ</td>
<td>2.92 ± 0.35*</td>
</tr>
<tr>
<td>Control subjects (n = 5)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>—</td>
<td>0.67 ± 0.11</td>
<td>62.20 ± 5.30</td>
<td>4.88 ± 0.38</td>
</tr>
<tr>
<td>LI</td>
<td>0.04 ± 0.005§</td>
<td>8.42 ± 2.06§</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HI</td>
<td>0.02 ± 0.006§</td>
<td>7.74 ± 1.48§</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are means ± SE. NEFA, nonesterified fatty acids. *P ≤ 0.01 vs. basal control. †P < 0.001 vs. LI and HI control. ‡P < 0.05 vs. HD controls. §P < 0.001 vs. basal control.

### Table 4. Glycerol production rate, leucine oxidation rate, and NOLD

<table>
<thead>
<tr>
<th></th>
<th>Glycerol Rₐ, μmol·kg⁻¹·min⁻¹</th>
<th>Leucine Oxidation, μmol·kg⁻¹·min⁻¹</th>
<th>NOLD, μmol·kg⁻¹·min⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>LILG (n = 6)</td>
<td>1.74 ± 0.28</td>
<td>0.40 ± 0.08</td>
<td>1.57 ± 0.20</td>
</tr>
<tr>
<td>Study 1</td>
<td>1.66 ± 0.24</td>
<td>0.44 ± 0.10</td>
<td>1.61 ± 0.18</td>
</tr>
<tr>
<td>LIHG (n = 7)</td>
<td>1.37 ± 0.21</td>
<td>0.48 ± 0.09</td>
<td>1.69 ± 0.23</td>
</tr>
<tr>
<td>Study 1</td>
<td>1.30 ± 0.21</td>
<td>0.48 ± 0.05</td>
<td>1.63 ± 0.19</td>
</tr>
<tr>
<td>HILG (n = 6)</td>
<td>1.94 ± 0.71</td>
<td>0.24 ± 0.03</td>
<td>1.65 ± 0.08</td>
</tr>
<tr>
<td>Study 1</td>
<td>1.90 ± 0.28</td>
<td>0.35 ± 0.03</td>
<td>1.66 ± 0.12</td>
</tr>
<tr>
<td>HIHG (n = 6)</td>
<td>1.45 ± 0.11</td>
<td>0.29 ± 0.04</td>
<td>1.27 ± 0.08</td>
</tr>
<tr>
<td>Study 1</td>
<td>2.67 ± 0.71</td>
<td>0.34 ± 0.05</td>
<td>1.98 ± 0.31</td>
</tr>
<tr>
<td>Control subjects (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>4.07 ± 0.45</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>LI</td>
<td>1.33 ± 0.17‡</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HI</td>
<td>1.22 ± 0.16*</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Data are mean ± SE. Glycerol Rₐ, glycerol rate of appearance; NOLD, nonoxidative leucine disposal. *P < 0.05 vs. basal control.

---

Fig. 3. Rate of leucine appearance (leucine Rₐ) in the patient group. Data are means ± SE. LILG: n = 7; LILG: n = 6; HIHG: n = 6; HILG: n = 6. LI, low-dose insulin (0.5 mU·kg⁻¹·min⁻¹); HI, high-dose insulin (2.0 mU·kg⁻¹·min⁻¹).
comparable insulin levels ($P < 0.01$ for HILG group study 2; $P < 0.03$ for HIHG group study 2).

Glycerol $R_a$ was reduced from basal by LI in the control subjects ($P < 0.001$), with no further suppression with HI (Table 4). In all patients in study 1, glycerol $R_a$ was not different from control subjects receiving LI and did not suppress further in any group in study 2. The glycerol $R_a$ data were not significantly influenced by whether the patients did or did not receive propofol.

**DISCUSSION**

In this study, we sought to determine the effects of glycemic control and insulin concentration on intermediary metabolism in critically ill medical patients, and for the first time, to measure the effect of insulin on protein turnover in critically ill medical patients. The results demonstrate that low dose intravenous insulin administered to maintain BG between 7 and 9 mmol/l is sufficient to limit lipolysis and endogenous glucose $R_a$ and increase glucose $R_a$ compared with fasting control subjects. Complete suppression of endogenous glucose $R_a$ was only achieved in the HILG group. Neither normoglycemia nor hyperinsulinemia had any effect on whole body protein synthesis or proteolysis.

Protein loss characterizes the catabolic response to critical illness: this is thought to arise both from increased proteolysis (24) and diminished protein synthesis (58). The use of leucine $R_a$ in this study as a measure of proteolysis allows for a more accurate estimation of protein flux than urinary nitrogen balance (31). Our data show that rates of proteolysis in patients in study 1 were higher than in control subjects who received similar insulin doses, even though endogenous glucose production rate was suppressed, which would reduce the requirement for gluconeogenic precursors. This finding is consistent with previous observations in both normal subjects and surgical patients that low-dose glucose administration (causing a doubling of insulin concentration) does not reduce proteolysis (23, 42). Our data also show that the administration of insulin to achieve supraphysiological concentrations did not attenuate protein breakdown and had no effect on net protein balance. This finding was not anticipated. In healthy subjects, insulin inhibits proteolysis in a dose-dependent manner (51), as we confirmed from our control studies. Although this was a small study, the findings suggest that neither tight glycemic control or high doses of insulin are likely to have any clinically significant benefit in terms of protein balance in critical illness.

Stress-induced hyperglycemia of critical illness has been attributed to insulin resistance either in the liver (27) or skeletal muscle (1). While it has been reported that intensive insulin treatment to maintain tight glycemic control between 4.4 and 6.1 mmol/l is unable to overcome hepatic insulin resistance, as assessed by high levels of serum insulin-like growth factor-binding protein-1 in critically ill patients (36), our data show that suppression of hepatic glucose $R_a$ to low levels can occur with an insulin infusion of ~1 mU·kg$^{-1}$·min$^{-1}$. This is in accord with recent studies (10, 52) in critically ill patients that also used kinetic techniques and showed that glycemic control with an insulin infusion is achieved by suppression of hepatic glucose production. In study 2, patients in the HILG group had a level of glycemia and insulinemia that was similar to control subjects receiving HI, enabling a direct comparison of rates of glucose disposal. Glucose $R_a$ was lower than in the control subjects, suggesting the presence of peripheral insulin resistance, a feature that has previously been reported both in sepsis and trauma (40, 43).

As expected, serum cortisol was higher in the patient groups than controls. It is not yet known how important this is for the induction of peripheral insulin resistance associated with critical illness, as over 30 other factors have been proposed (4). These may be grouped as either hormonal responses (for example, glucocorticoid hypersecretion or low circulating IGF-1 (54) or cytokine-mediated responses (including TNFα and IL-6 (4). It is noteworthy that attempts to manipulate the cytokine cascade have not affected the physiological response to illness (11).

Lipolysis is a consistent feature of critical illness (30), making the effect on lipolysis of exogenous insulin an area of interest. In this study, the contribution of exogenous glycerol

**Table 5. Glucose turnover data and $R_Q$**

<table>
<thead>
<tr>
<th></th>
<th>Endog. Glucose $R_a$, mg·kg$^{-1}$·min$^{-1}$</th>
<th>Glucose $R_a$, mg·kg$^{-1}$·min$^{-1}$</th>
<th>$R_Q$</th>
</tr>
</thead>
<tbody>
<tr>
<td>LILG ($n = 6$)</td>
<td>Study 1 0.069 ± 0.54</td>
<td>3.80 ± 0.69</td>
<td>0.83 ± 0.03</td>
</tr>
<tr>
<td></td>
<td>Study 2 0.61 ± 0.40</td>
<td>3.89 ± 0.65</td>
<td>0.88 ± 0.02</td>
</tr>
<tr>
<td>HIHG ($n = 7$)</td>
<td>Study 1 0.61 ± 0.45</td>
<td>4.09 ± 0.34</td>
<td>0.80 ± 0.04</td>
</tr>
<tr>
<td></td>
<td>Study 2 0.87 ± 0.24</td>
<td>4.36 ± 0.20</td>
<td>0.79 ± 0.03</td>
</tr>
<tr>
<td>HILG ($n = 6$)</td>
<td>Study 1 0.51 ± 0.32</td>
<td>4.27 ± 0.68</td>
<td>0.81 ± 0.02</td>
</tr>
<tr>
<td></td>
<td>Study 2 0.10 ± 0.57a,b</td>
<td>7.04 ± 0.80a,c,d</td>
<td>1.02 ± 0.03b,c</td>
</tr>
<tr>
<td>HIHG ($n = 6$)</td>
<td>Study 1 0.67 ± 0.32</td>
<td>4.07 ± 0.46</td>
<td>0.84 ± 0.05</td>
</tr>
<tr>
<td></td>
<td>Study 2 0.20 ± 0.03b</td>
<td>7.46 ± 0.87a,b,c,e</td>
<td>1.02 ± 0.02b,a</td>
</tr>
<tr>
<td>Control subjects</td>
<td>$n = 5$</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>1.89 ± 0.058</td>
<td>1.89 ± 0.058</td>
<td>0.82 ± 0.02c</td>
</tr>
<tr>
<td>LI</td>
<td>0.46 ± 0.59</td>
<td>4.58 ± 0.32f</td>
<td>0.88 ± 0.01c</td>
</tr>
<tr>
<td>HI</td>
<td>$-0.92 ± 0.41f$</td>
<td>$10.15 ± 0.57g$</td>
<td>0.99 ± 0.03</td>
</tr>
</tbody>
</table>

Data are means ± SE. $R_Q$, respiratory quotient; Endog. glucose $R_a$, endogenous glucose rate of appearance; glucose $R_a$, glucose rate of disappearance. $^aP < 0.005$ vs. HILG group in study 1. $^bP < 0.02$ vs. LILG group in study 2. $^cP < 0.03$ vs. HI control. $^dP = 0.05$ vs. LIHG in study 2. $^eP < 0.02$ vs. LIHG group in study 1. $^fP = 0.05$ vs. LI control. $^gP < 0.003$ vs. LI control. $^h$Not significantly different from 0.
was subtracted from the total glycerol $R_a$ to give the endo-
gogenous glycerol $R_a$. Although the contribution of exogenous 
glycerol was small (providing <5% of the total glycerol $R_a$) 
because the rate of release of glycerol from the hydrolysis of 
soybean oil within propofol could not be measured directly, the 
endogenous glycerol $R_a$ is a very close approximation and not 
an exact measurement. In study 1, lipolysis (as measured by 
glycerol $R_a$ and NEFA concentrations) was suppressed by 
insulin and no further suppression occurred with HD insulin or 
tight glycemic control. Cardiac NEFA metabolism requires 
more oxygen per unit energy yield than glucose, which could 
therefore have a deleterious effect on myocardial performance 
in the hypoxic setting (3). Suppression of NEFA concentra-
tions in the presence of increased insulin will result in glucose 
being the prime substrate for myocardial metabolism, which 
may be beneficial. It is of interest that suppression of NEFA 
concentration occurred without the necessity of achieving nor-
meglycemia. Hypocholesterolemia confers a poor prognosis in 
critically ill patients (21). We found that none of the treatment 
regimens normalized cholesterol, although a study period of up 
to 1 wk might have been required to do so (37).

A possible limitation to this study is the difference in the 
study duration of the control and patient groups. Bed rest itself 
may cause insulin resistance after 5–6 days (48), although not 
after 24 h (39): this might have biased the data of the control 
group (recumbent for 9 h). However, it has also been shown that 
the insulin responsiveness of protein metabolism after bed-rest remains intact (44), suggesting that the conclusions 
drawn from the control studies were valid. The 48-h duration 
of clamp (plus an 8-h lead-in to study 1) of the patient groups 
was based on the expected length of stay of such patients in our 
ICU being 3 days, which is similar to reported data (56). The 
effects of insulin on glucose and glycerol turnover in critically 
ill patients have been reported within 500 min (10, 52), but we 
considered that an effect on leucine turnover would take up to 
72 h (7). Although no difference was detected in serum insulin 
centrations between the HI patients and controls, the small 
numbers involved may have lead to a type 2 statistical error. 
However, proteolysis rates increased in the HI patient group 
(per unit change in insulin), whereas a reduction was evident in 
the control subjects.

We did not perform studies in the fed state, as it would have 
required using TPN, which is a means of feeding seldom used 
in our ICU due to infectious complications (29). It is possible 
that amino acid supplementation combined with insulin might 
have increased whole body protein synthesis as reported in 
healthy subjects (8, 51). This was likely to have been a direct 
effect of the amino acids, as in the only study examining the 
action of insulin on protein turnover during an amino acid 
clamp (41), it was found that insulin could suppress leucine $R_a$ 
by 19% but it had no effect on NOLD. However, systemic 
insulin infusion, despite hypoaminoacidemia, is capable of 
suppression of leucine $R_a$ (14), as was also seen in our control 
studies.

Inherent to the study design was that standard treatments be 
provided to obtain a clearer understanding of the effect of 
glycemic control in a setting familiar to physicians and inten-
sivists; this meant that the use of glucocorticoids could not be 
controlled between the patient groups. However, we were 
unable to detect any difference in leucine $R_a$ in either study 1 
or study 2 when patients were categorized as receiving glu-
corticoids or not and neither was any correlation seen be-
tween cortisol concentration and leucine $R_a$. This study was 
of medical ICU admissions only and therefore it remains to be 
seen whether tight glycemic control can exert a protein-sparing 
effect in surgical ICU cases.

To summarize, the use of a variable dose of insulin to 
maintain blood glucose <6 mmol/l in a medical group of 
critically ill patients led to suppression of lipolysis and near full 
reduction of hepatic glucose production and increased periph-
ernal glucose uptake. These effects were also seen with a higher 
level of glyceremia (7–9 mmol/l), suggesting intravenous insulin 
that achieves moderate glucose levels rather than tight glycemic 
control is sufficient to reduce fatty acid levels and over-
production of glucose by the liver. Hyperinsulinemia increased 
peripheral glucose disposal, but this failed to reach values seen 
in health; additionally, hyperinsulinemia could not attenuate 
protein loss. There appears to be a differential effect of resis-
tance to insulin action in skeletal muscle compared with 
adipose tissue or the liver. The intracellular insulin signaling 
network is highly complex and includes cross talk between 
pathways, but the Ras/PI3K pathway has been implicated as 
being responsible for the growth and mitogenic effects of 
insulin (49). It may be that this pathway is particularly suscep-
tible to interruption during critical illness, perhaps by cytokine 
action. Alternatively, it has been shown from studies (28) of 
knockout mice that insulin receptor substrate (IRS)-1 has the 
predominant role in skeletal muscle and IRS-2 in the liver. 
Furthermore, data suggest that IRS-1 knockouts affect protein 
synthesis, whereas IRS-2 knockouts primarily affect glucose 
regulation (49). It may be hypothesized that insulin receptor 
subtypes are differentially affected in critical illness. To con-
clude, this study suggests that any beneficial effect of tight 
glycemic control on morbidity and mortality is not due to 
protein sparing.

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technical assistance with this study. We thank John Smith, Tony Sherry, and 
all the clinical staff at the Intensive Care Unit.

GRANTS

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Charitable Foundation.

DISCLOSURES

No conflicts of interest are declared by the author(s).

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E704

METABOLIC EFFECTS OF INTENSIVE INSULIN THERAPY


