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Protein and substrate metabolism during starvation and parenteral refeeding

KEVIN J. TRACEY, ADRIAN LEGASPI, JAMES D. ALBERT, MALAYAPPA JEEVANANDAM*, DWIGHT E. MATTHEWS, MURRAY F. BRENNAN* AND STEPHEN F. LOWRY*

Department of Surgery, Surgical Metabolism Laboratories, and General Clinical Research Center of the New York Hospital–Cornell University Medical Center, and *Surgical Metabolism Laboratories of the Memorial Sloan-Kettering Cancer Center, New York, U.S.A.

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SUMMARY

1. Healthy male volunteers underwent 10 days of hospitalized protein-calorie starvation and a subsequent 10 day repletion phase with complete intravenous nutritional support (IVF). Non-protein calories were provided as either all D-glucose or as 50% D-glucose/50% lipid.

2. In comparison with starvation, whole-body protein breakdown, as assessed by [^{15}N]glycine, [^{13}C]leucine and urinary excretion of 3-methylhistidine (3-MH), was diminished during IVF. The administration of parenteral nutrition did not specifically suppress peripheral tissue protein breakdown, as measured by extremity 3-MH efflux.

3. Despite the differential insulin response to D-glucose/amino acid (50 ± 6 m-units/ml) as compared with the D-glucose/lipid/amino acid regimen (25 ± 4 m-units/ml), there was no difference in nitrogen retention between the regimens. Indirect calorimetric determinations revealed that oxidation of substrate during IVF was related to the proportion of D-glucose and lipid infusion.

Key words: energy expenditure, [^{15}N]glycine, [^{13}C]leucine, 3-methylhistidine, parenteral nutrition, skeletal muscle protein.

Abbreviations: IVF, intravenous nutritional support; 3-MH, 3-methylhistidine.

INTRODUCTION

Complete intravenous nutritional support (IVF) is currently utilized for patients with involuntary weight loss or cachexia from a variety of aetiologies [1, 2]. The ultimate goal of IVF as a therapeutic modality is restoration of body caloric and protein reserves, but a significant restoration of lean tissue did not occur after IVF in depleted patients, as assessed by neutron activation [3]. Other studies have reported a reduction of perioperative morbidity after 10 days of IVF in a depleted patient population [4, 5], and early IVF appears to favourably influence rehabilitation after trauma [6]. The beneficial impact of parenteral nutrition and the resultant retention of nitrogen represents the repletion of some body-protein compartments, the nature of which remain undefined.

Several reports have demonstrated the kinetics of body-protein regulation during IVF in hospitalized patients [7–9]. However, the interpretation of these protein kinetic observations are confounded by variations of disease, metabolic rate and antecedent nutritional profiles. A prospective controlled study of parenteral nutritional repletion in normal subjects under conditions commonly imposed by hospitalization would allow an evaluation of the impact of IVF on nitrogen retention and protein metabolism in the absence of such variables.

We studied healthy, hospitalized volunteers who were depleted by protein-calorie starvation for 10 days and subsequently repleted by IVF to define (1) the differential impact of a D-glucose versus a D-glucose/lipid nonprotein caloric source on whole-body protein ([^{15}N]glycine) and energy (indirect calorimetry) metabolism, (2) whole-body protein kinetics using both [^{15}N]glycine and L-[1-^{13}C]leucine in a smaller subset of subjects, and (3) simultaneous determination of 24 h urinary excretion and resting peripheral tissue efflux of 3-methylhistidine (3-MH).

METHODS

Subjects

Male volunteers were screened as outpatients by complete physical examination, blood count and serum biochemistries. Eleven subjects (see Table 1), aged 24–34

Correspondence: Dr S. F. Lowry, Department of Surgery, New York Hospital-Cornell University Medical Center, 525 East 68th Street, New York, N.Y. 10021, U.S.A.

Table 1. Subject profile and anthropometric measurements

Subject no.	Age (years)	Height (cm)	Weight (kg)		
			Postabsorptive	Starvation	IVF
D-Glucose					
1	28	176	74.0	67.0	71.5
2	24	176	75.0	69.5	74.0
3	30	176	77.0	72.0	76.5
4	24	182	82.0	77.0	80.5
5	32	177	74.0	69.0	72.5
6	34	174	79.5	76.0	75.0
Mean ± SE	29 ± 2	177 ± 1	77.0 ± 1	72.0 ± 2	75.0 ± 3
D-Glucose/lipid					
7	25	175	66.0	60.5	67.5
8	28	183	70.5	64.5	68.5
9	29	181	77.0	71.0	75.0
10	30	181	83.5	79.0	80.5
11	30	184	84.5	79.5	81.0
Mean ± SE	28 ± 1	181 ± 2	76.0 ± 4	71.0 ± 4	75.0 ± 3

years and within 10% of ideal body weight for sex, age and height (Metropolitan Life Table, 1983), were admitted to the Adult Clinical Research Center of the New York Hospital–Cornell University Medical Center. Informed written consent through a protocol approved by the Institutional Review Board of the New York Hospital–Cornell University Medical Center was obtained.

Study protocol

Upon admission to the Clinical Research Center for 24 consecutive days, subjects were allowed to ambulate within the ward but were restricted from any exercise activity. Subjects were weighed at 06.00 hours after voiding and daily 24 h urine samples were analysed for total Kjeldahl nitrogen. During the first 3 hospital days, each subject was fed orally, with a defined formula diet (Sustacal; Mead-Johnson, IN, U.S.A.) six times a day to provide 0.32 g of N day^{-1} kg^{-1}, 4.8 g of carbohydrate day^{-1} kg^{-1}, and 0.71 g of lipid day^{-1} kg^{-1}. Feeding was discontinued at 20.00 hours on hospital day 3 and at 07.00 hours on day 4, after an overnight fast, the postabsorptive measurement of oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) was performed in the supine position by indirect calorimetry with a Beckman Metabolic Measurement Cart-1 (Sensormedics Corp., Anaheim, CA, U.S.A.). This system contains an OM-11 oxygen analyser and an LB-2 carbon dioxide analyser. The instrument was calibrated before and after each test using a minimum of two standard calibration gases (100% N_2 and 16% O_2/4% CO_2). The system was independently validated against the combustion of methane and proved reliable and stable [10]. Resting energy expenditure was estimated by the Weir formula [11].

From hospital days 4 to 14, a 10 day protein-calorie starvation period was achieved during which oral intake was limited to distilled water and no-calorie soft drink (provided *ad libitum*), and a tablet of sodium chloride (20 mmol/day). On hospital day 14, a percutaneous catheter was inserted into the antecubital vein and was advanced into the superior vena cava (placement confirmed by chest roentgenography).

All subjects received IVF from hospital days 14 to 24 with a commercially available amino acid source (Aminosyn-10%) delivered through the central catheter by constant infusion with a volumetric pump (Abbott, Inc.). The final flow rate of intravenous feeding was achieved by the second day of IVF and maintained at this rate for the duration of the study. Electrolytes, vitamins and trace minerals were added according to previously defined standards [12]. Subjects were randomized to receive one of two IVF regimens which differed in the provision of the non-protein caloric source: group I (D-glucose, $n=6$) received 100% of non-protein calories as D-glucose; group II (D-glucose/lipid, $n=5$) received 50% of calories as D-glucose and 50% as lipid emulsion (Liposyn-10%). The total non-protein calories provided was 1.5 times the measured postabsorptive resting energy expenditure and resulted in amino acid delivery at a rate of 0.29 ± 0.03 g of N day^{-1} kg^{-1} and non-protein calories at a rate of 35 ± 2 kcal day^{-1} kg^{-1}.

Determination of whole-body protein kinetics by the [^{15}N]glycine method, resting energy expenditure and extremity substrate flux was performed in all subjects under three different nutritional conditions. At 08.00 hours on hospital days 3 (enteral), 13 (starvation) and 23 (IVF) a primed, continuous 30 h infusion of [^{15}N]glycine was initiated.

At 07.00 hours on days 4 (postabsorptive), 10 (starvation) and 24 (IVF) resting energy expenditure was determined in the supine position as described above [10, 11]. On these days, after energy expenditure measurements, forearm arteriovenous flux analysis was performed. A radial artery catheter and a deep basilic vein catheter (directed retrograde) were placed to provide simultaneous arterial and deep venous sampling. Forearm blood flow was measured by electrocapacitance plethysmography as previously described [13].

Three subjects also received a primed continuous 6 h infusion of L-[1-^{13}C]leucine beginning at 07.00 hours on hospital days 14 (starvation) and 24 (IVF).

Isotope tracer techniques

[^{15}N]Glycine (99 atom %, KOR Inc, Cambridge, MA, U.S.A.), L-[1-^{13}C]leucine (99 atom %, Tracer Technologies, Inc, Newton, MA, U.S.A.), and $NaH^{13}CO_2$ (90 atom %, Tracer Technologies, Inc) were prepared in sterile saline (150 mmol/l NaCl), filtered through 0.22 μm Millipore filters and were tested for pyrogenicity and sterility before infusion.

Whole-body protein turnover was determined by the [^{15}N]glycine method in all subjects by measurement of isotope enrichment in urinary urea and ammonia [14, 15]. A bolus intravenous injection of [^{15}N]glycine (0.52 mg of ^{15}N/kg given over 10 min) was followed by a continuous 30 h infusion (0.029 mg of ^{15}N h^{-1} kg^{-1}) with a volumetric pump. Urine was collected every 4 h during the first 16 h of the infusion, then every 2 h. Urinary urea and ammonia were isolated in each sample and the ^{15}N enrichments in both end-products were determined in duplicate with a Nier type isotope-ratio mass spectrometer.

Whole-body turnover (Q, g of N day^{-1} kg^{-1}) was determined by stochastic analysis during steady-state conditions [16] and was calculated from the equation:

$$Q = \text{In}/\text{APE}$$

where In is the infusion rate and APE is the atom per cent excess of the mean ^{15}N enrichment of urinary ammonia and urea during steady state. This method has been described previously [14, 15]. Plateau APE was determined from urine samples collected between hours 22 and 30 [14]. A factor of 6.25 is used to convert g of N into g of protein.

The rate of whole-body protein synthesis (S) was calculated from the expression:

$$S = Q - \text{Ex}$$

where Ex is the rate of total nitrogen excretion in the urine. The rate of whole-body protein breakdown (B) was calculated from the equation:

$$B = Q - I_N$$

where I_N is the rate of nitrogen intake. During the postabsorptive study, I_N was determined as the hourly intake averaged over the entire infusion period. During the starvation study I_N was zero, and during the IVF study I_N was calculated from the rate of intravenous nitrogen administration.

Whole-body protein kinetics were also determined by the L-[1-^{13}C]leucine method as previously described [17, 18]. A bolus of $NaH^{13}CO_2$ (0.8 mg/kg) was administered to prime the bicarbonate pool. L-[1-^{13}C]Leucine was administered as a bolus (1 mg/kg) followed by a constant (1 mg of [^{13}C]leucine h^{-1} kg^{-1}) 6 h infusion. Before beginning the [^{13}C]leucine infusion ($t=0$), and at 30 min intervals between $t=4$ and $t=6$ h, aliquots of expired air were collected in a 2 litre anaesthesia bag. Expired CO_2 was trapped by percolation through NaOH for later determination of ^{13}C enrichment. NaOH (19 mol/l) was diluted to a final concentration of 0.01 mol/l in double-distilled water, which had previously been heated to 100°C for 15 min in the presence of H_2SO_4 to eliminate CO_2. Rates of CO_2 production were determined simultaneously by seven to ten 1 min recordings on the Metabolic Measurement Cart. Simultaneous arterial blood specimens were collected for determination of the ^{13}C enrichment of plasma leucine.

Whole-body leucine flux and oxidation were calculated as previously described [17, 18]. During isotopic steady-state leucine flux (Q, μmol h^{-1} kg^{-1}) was calculated from the expression:

$$Q = i \times ([E_i/E_p] - 1)$$

where i is the [^{13}C]leucine infusion rate (μmol h^{-1} kg^{-1}), E_i is the enrichment of the infused [^{13}C]leucine (atom% excess) and E_p is the [^{13}C]leucine enrichment in plasma (atom% excess). The rate of leucine oxidation (O, μmol h^{-1} kg^{-1}) was calculated by the equation:

$$O = F^{13}CO_2 (1/E_p - 1/E_i) \times 100$$

where $F^{13}CO_2$ is the rate of $^{13}CO_2$ production (μmol h^{-1} kg^{-1}) from leucine tracer oxidation. The calculated rate of whole-body protein synthesis (S) is represented by the non-oxidative disappearance of leucine and derived from the equation:

$$S = Q - O$$

The rate of whole-body protein breakdown (B) is determined by the rate of endogenous leucine released from protein and calculated from the equation:

$$B = Q - I_L$$

where I_L is the rate of exogenous leucine intake. During the postabsorptive and starvation studies, the quantity of unlabelled exogenous leucine intake was zero. During the IVF study this rate was calculated from the rate of leucine administered in IVF solution (35.83 μmol/ml).

Analysis of blood and urine

Blood samples for insulin, liver function tests, glucose and 3-MH were collected in tubes containing heparin, centrifuged immediately (3000 *g* at 4°C for 15 min) and the plasma was stored at −70°C until assays were performed. Immunoreactive insulin was measured by radioimmunoassay [19]. Glucose and liver function tests were determined by automated techniques in the core laboratory at the New York Hospital-Cornell University Medical Center.

Plasma for determination of glucagon levels was collected into ethylenediaminetetra-acetate and aprotinin before analysis by radioimmunoassay [20]. Immediately after withdrawal, blood samples (1 ml) were injected into 1 ml of ice-cold 10% perchloric acid, quickly centrifuged (3000 *g* at 4°C for 10 min) and stored at −70°C until later assay for lactate and pyruvate by spectrophotometric

techniques using lactate and pyruvate dehydrogenase [21]. All enzymatic analyses were performed in triplicate.

Determination of plasma 3-MH was performed using deuterated 3-MH internal standards by gas chromatography–mass spectrometry as previously described [22]. Urine was deproteinized (Amicon, Inc., Lexington, MA, U.S.A.) and 3-MH was determined using column chromatography (Beckman Instruments, Fullerton, CA, U.S.A.) by previously described methods [9].

Arteriovenous flux calculations

Extremity flux of 3-MH, glucose, lactate and pyruvate within the plasma compartment were calculated as ([arterial] − [venous]) × blood flow × (1-packed cell volume) [13]. A positive flux represents a relative uptake across the extremity, whereas a negative flux indicates net efflux.

Statistics

All data are expressed as means ± SE grouped by study condition and nutritional regimen. The statistical significance of differences between parameters was estimated by Student's *t*-test for paired or unpaired data as appropriate. Linear correlation was determined by the least squares method.

RESULTS

Weight loss and urinary nitrogen balance (Table 1)

A weight loss of 5.0 ± 0.5 kg occurred by the end of the starvation period. During repletion IVF all subjects gained weight. The difference in net weight gain between the D-glucose and D-glucose/lipid groups was not significant.

During the 10 day starvation period, daily nitrogen intake was zero and daily loss of urinary nitrogen stabilized in the final 3 days of starvation at 98 ± 7 mg of N day^{-1} kg^{-1}. Nitrogen intake during the 10 day IVF period was similar in the D-glucose and D-glucose/lipid groups. The cumulative urinary nitrogen balance taken over the whole 24 day period was positive (136 ± 1 mg of day^{-1} kg^{-1}) in both groups. Provision of 50% of calories as lipid did not significantly alter net nitrogen retention during repletion IVF (98 ± 2 mg of day^{-1} kg^{-1}) as compared with the D-glucose system (92 ± 3 mg of N day^{-1} kg^{-1}).

Laboratory data

Table 2 shows levels of arterial serum albumin, total bilirubin, serum glutamate:oxaloacetate transaminase, alkaline phosphatase and packed cell volume. Albumin levels did not change significantly after either 10 days of starvation or IVF. Bilirubin increased from a mean basal level of 12 ± 2 μmol/l to a starvation level of 17 ± 2 μmol/l ($P < 0.05$) and declined after IVF with D-glucose (9 ± 3 μmol/l) or D-glucose/lipid (5 ± 2 μmol/l). Serum glutamate: oxaloacetate transaminase levels did not change significantly during starvation and refeeding. Alkaline phosphatase levels were increased after IVF in the D-glucose/lipid group (77 ± 4 units/l) as compared with the D-glucose group (50 ± 9 units/l, $P < 0.05$). Based in part on blood sampling for the experimental protocol, packed cell volume declined during the study protocol. Serum electrolytes, calcium, magnesium and phosphorus were maintained within normal limits throughout the study period (data not shown). Renal function, as assessed by blood urea nitrogen, creatinine and urinary creatinine clearance, was unchanged during all nutritional conditions (data not shown).

Hormones

Arterial plasma concentrations of insulin and glucagon are given in Table 3. Insulin levels decreased slightly after starvation (7 ± 1 m-units/ml) but increased significantly in response to IVF. Refeeding with the D-glucose system evoked a significantly higher insulin response (50 ± 6 m-units/ml) as compared with the D-glucose/lipid system (25 ± 4 m-units/ml, $P < 0.05$). Arterial plasma glucagon levels did not change significantly during the starvation and IVF studies.

Table 2. Routine serum biochemical profile and packed cell volume

Results are means ± SE. Statistical significance: *$P < 0.05$ vs postabsorptive; †$P < 0.05$ vs D-glucose IVF; ‡$P < 0.001$ vs postabsorptive.

	Postabsorptive	Starvation	IVF	
			D-Glucose	D-Glucose/lipid
Albumin (g/l)	42 ± 1	40 ± 1	40 ± 1	37 ± 2
Total bilirubin (μmol/l)	12 ± 2	17 ± 2*	9 ± 3	5 ± 2
Serum glutamate: oxaloacetate transaminase (units/l)	30 ± 4	16 ± 2‡	41 ± 13	29 ± 10
Alkaline phosphatase (units/l)	67 ± 2	62 ± 3	50 ± 9*	77 ± 4†
Packed cell volume (%)	44 ± 1	39 ± 1‡	34 ± 2‡	35 ± 1‡

Table 3. Hormone levels and extremity substrate flux

Results are means ± SE. Statistical significance: *$P < 0.05$ vs D-glucose/lipid; †$P < 0.05$ vs starvation; ‡$P < 0.05$ vs postaborptive; §$P < 0.05$ vs D-glucose.

	Postabsorptive	Starvation	IVF	
			D-Glucose	D-Glucose/lipid
Insulin (m-units/ml)	12 ± 1	7 ± 1	50 ± 6*†‡	25 ± 4†‡
Glucagon (ng/l)	102 ± 7	94 ± 7	119 ± 24	103 ± 3
Substrate				
Glucose				
Arterial concn. (mmol/l)	4.9 ± 0.2	3.3 ± 0.1‡	6.0 ± 0.3†‡	5.8 ± 0.2†‡
Flux (μmol min^{-1} 100 ml^{-1} of tissue)	7.3 ± 2.1	4.6 ± 3.1	11.7 ± 4.8	21.9 ± 6.3
Lactate				
Arterial concn. (mmol/l)	0.4 ± 0.1	0.4 ± 0.1	1.0 ± 0.1†‡	0.4 ± 0.1§
Flux (μmol min^{-1} 100 ml^{-1} of tissue)	−0.7 ± 0.2	−0.4 ± 0.1	+0.5 ± 0.2†‡	−0.3 ± 0.2§
Pyruvate				
Arterial concn. (μmol/l)	409 ± 68	284 ± 39	1056 ± 227†‡	607 ± 114§
Flux (μmol min^{-1} 100 ml^{-1} of tissue)	−4.5 ± 4.5	−1.4 ± 3.4	+13.6 ± 4.5	+0.5 ± 0.8

Substrates

Arterial plasma levels and forearm flux of glucose, lactate and pyruvate are shown in Table 3. Arterial glucose declined after starvation (3.3 ± 0.1 mmol/l) as compared with the postabsorptive study (4.9 ± 0.2 mmol/l, $P < 0.001$). Despite differing rates of glucose administration between the IVF regimens and a differential insulin response, the glucose levels after IVF were comparable for the D-glucose (6.0 ± 0.3 mmol/l) and the D-glucose/lipid system (5.8 ± 0.3 mmol/l). These values were significantly increased compared with the levels after starvation ($P < 0.05$) and were also significantly elevated above the postabsorptive basal values ($P < 0.05$).

Arterial lactate levels were not significantly changed after a 10 day fast. However, refeeding with the D-glucose system stimulated a significant increase in arterial lactate (1.0 ± 0.1 mmol/l) as compared with either the basal (0.4 ± 0.1 mmol/l, $P < 0.05$) or starvation state (0.4 ± 0.1 mmol/l, $P < 0.05$). In contrast, the arterial lactate levels were not increased by IVF with the D-glucose/lipid system. Arterial pyruvate declined after starvation, and although this change was not statistically significant, refeeding with the D-glucose system was associated with a significant elevation of arterial pyruvate (1056 ± 227 μmol/l) as compared with the D-glucose/lipid system (670 ± 114 μmol/l, $P < 0.05$).

Forearm uptake of glucose decreased slightly after a 10 day fast as compared with the basal study, but this decline was not significant. Glucose uptake increased after IVF in the D-glucose group as compared with the D-glucose/lipid regimen. In agreement with previous observations, forearm efflux of lactate decreased after starvation and became positive during refeeding with the D-glucose system ($P < 0.05$ vs starvation) which stimulated net uptake of lactate across the forearm [23]. However, the D-glucose/lipid group continued to exhibit lactate efflux during IVF. Forearm pyruvate flux alterations paralleled the lactate flux observations.

Whole-body protein kinetics determined by the [^{15}N]glycine method

Results of protein dynamics determined by the [^{15}N]glycine method (Q, S, B) are shown in Table 4. The determination of whole-body protein turnover in the enteral state (3.81 ± 0.12 g protein day^{-1} kg^{-1}) is in agreement with previous observations [24]. Turnover decreased during the adaptive response to starvation (2.37 ± 0.10 g of protein day^{-1} kg^{-1}, $P < 0.01$ vs basal), but repletion with IVF did not restore the turnover rate to the enteral levels.

Whole-body protein synthesis was significantly reduced after starvation (1.76 ± 0.12 g of protein day^{-1} kg^{-1}) as compared with the enteral state (2.46 ± 0.12 g of protein day^{-1} kg^{-1}, $P < 0.01$). Synthesis was not stimulated by the provision of IVF with either regimen, and remained significantly decreased during the IVF study (1.63 ± 0.15 g of protein day^{-1} kg^{-1}, $P < 0.01$ vs basal). Whole-body protein breakdown increased slightly during starvation (2.36 ± 0.10 g of protein day^{-1} kg^{-1}) as compared with the rate measured during enteral conditions. Refeeding with either the D-glucose (0.94 ± 0.11 g of protein day^{-1} kg^{-1}) or the D-glucose/lipid system (1.10 ± 0.11 g of protein day^{-1} kg^{-1}) did diminish the rate of protein breakdown as compared with starvation ($P < 0.01$), but the difference between refeeding regimens was not statistically significant.

Whole-body protein kinetics determined by the [^{13}C]leucine method

Whole-body leucine flux (Q), rate of appearance into the plasma compartment from protein breakdown (B), rate of leucine disappearance into protein synthesis (S) and oxidation of leucine (O) are shown in Table 4. Leucine flux increased significantly during IVF as compared with the starved state ($P < 0.01$). The observed rate of endogenous leucine appearance during starvation decreased during IVF ($P < 0.01$). Leucine oxidation

increased during the provision of exogenous leucine with IVF as compared with the rate during starvation. The calculated rate of whole-body protein synthesis was elevated during D-glucose IVF as compared with starvation ($P<0.01$).

Urinary excretion and extremity flux of 3-MH

Urinary 3-MH excretion was 3.07 ± 0.20 μmol day^{-1} kg^{-1} during the final day of enteral feeding and increased during unstressed starvation (3.44 ± 0.34 μmol day^{-1} kg^{-1}) (Table 5). Refeeding with IVF suppressed net 3-MH excretion in group I (2.52 ± 0.18 μmol day^{-1} kg^{-1}, $P<0.05$ vs starvation). In group II, IVF was also associated with diminished net 3-MH excretion (2.97 ± 0.39 μmol day^{-1} kg^{-1}, $P<0.05$ vs starvation), but was not significantly different from group I. Under each dietary condition, the rate of whole-body 3-MH excretion correlated with protein breakdown as determined by the [^{15}N]glycine method (Fig. 1).

Forearm skeletal muscle efflux of 3-MH was determined in five subjects (Fig. 2). Efflux declined from 1.25 ± 0.35 nmol min^{-1} 100 ml^{-1} of tissue during the postabsorptive study to 0.75 ± 0.15 nmol min^{-1} 100 ml^{-1} of tissue during starvation, consistent with a slight decrease in net proteolysis in this peripheral tissue bed. During IVF, 3-MH efflux returned to levels (1.21 ± 0.23 nmol min^{-1} 100 ml^{-1} of tissue) similar to that observed in the postabsorptive state. Arterial plasma levels of 3-MH declined sequentially throughout the 24 day study period (Table 5).

Whole-body substrate utilization

The results of the measurement of respiratory gas exchange by indirect calorimetry and the determination of resting energy expenditures are presented in Table 6. Starvation induced a decline of resting energy expenditure, in agreement with previous observations [25]. Repletion IVF with the D-glucose system was associated with an increased resting energy expenditure (24.26 ± 0.9 cal day^{-1} kg^{-1}) as compared with the starvation study (18.11 ± 0.8 cal day^{-1} kg^{-1}, $P<0.05$). The principles of indirect calorimetry were utilized to calculate the relative

Table 4. Whole-body protein kinetics calculated by [^{15}N]glycine and [^{13}C]leucine methods

Results are means ± SE. Statistical significance: *$P<0.01$ vs starvation; †$P<0.01$ vs respective enteral feeding (^{15}N). Numbers in parentheses represent percentage change as compared with the starvation study.

	Enteral ($n=11$)	Starvation ($n=11$)	IVF	
			D-Glucose ($n=6$)	D-Glucose/lipid ($n=5$)
[N^{15}]Glycine				
Q (g of protein day^{-1} kg^{-1})	3.81 ± 0.12	2.37 ± 0.10†	2.75 ± 0.17*† (+16%)	2.89 ± 0.14*† (+22%)
S (g of protein day^{-1} kg^{-1})	2.46 ± 0.12	1.76 ± 0.12†	1.63 ± 0.15† (−7%)	1.70 ± 0.13† (−4%)
B (g of protein day^{-1} kg^{-1})	2.07 ± 0.13	2.36 ± 0.10	0.94 ± 0.11*† (−60%)	1.11 ± 0.10*† (−53%)
[^{13}C]Leucine		$n=3$	$n=3$	
Q (g of protein day^{-1} kg^{-1})	—	3.47 ± 0.1	5.12 ± 0.2* (+48%)	—
S (g of protein day^{-1} kg^{-1})	—	3.08 ± 0.1	4.33 ± 0.1* (+41%)	—
B (g of protein day^{-1} kg^{-1})	—	3.47 ± 0.1	3.02 ± 0.2* (−13%)	—
O (μmol h^{-1} kg^{-1})	—	9.44 ± 1.95	19.26 ± 4.8 (+104%)	—

Table 5. Urinary 3-MH excretion and arterial 3-MH concentration

Results are means ± SE. Values of n are shown in parentheses. Statistical significance: *$P<0.5$ vs starvation; †$P<0.5$ vs postabsorptive.

	Postabsorptive	Starvation	IVF	
			D-Glucose	D-Glucose/lipid
Urinary excretion (μmol day^{-1} kg^{-1})	3.07 ± 0.20 ($n=11$)	3.44 ± 0.34 ($n=11$)	2.52 ± 0.18* ($n=6$)	2.67 ± 0.39 ($n=5$)
Arterial concn. (μmol/ml)	6.07 ± 0.70 ($n=5$)	4.94 ± 1.0 ($n=5$)	3.47 ± 0.32† ($n=3$)	4.63 ± 0.8 ($n=2$)

contribution of protein, fat and carbohydrate substrate to the daily energy requirements (Fig. 3) based on the non-protein respiratory quotient [26]. The D-glucose/IVF regimen was associated with increased whole-body carbohydrate oxidation and net fat synthesis. The provision of lipid as 50% of the non-protein caloric source resulted in net fat oxidation (0.98 ± 0.6 g of fat day^{-1} kg^{-1}). The D-glucose/lipid/IVF group demonstrated oxidation of carbohydrate and lipid in proportion to the relative rate of substrate infusion. There was no significant difference in protein oxidation between the two IVF regimens.

DISCUSSION

This study characterizes the protein-metabolic response to protein-calorie starvation and subsequent repletion by IVF in normal man under conditions imposed by prolonged hospitalization. Furthermore, substrate utilization with a D-glucose system was compared with a D-glucose/lipid system during the unstressed (disease-free) response to IVF repletion. These findings represent the first description of these normal protein-metabolic parameters in this setting and therefore provide a direct control population for comparison studies of intravenous nutritional repletion in patients. The adaptive protein-metabolic responses to starvation are associated with decreased insulin levels and the preservation of peripheral tissue protein stores [27]. In the present study, the repletion of the starvation adapted state with IVF was also associated with a suppression of whole-body protein breakdown as assessed by the [^{15}N]glycine and [^{13}C]leucine methods and by urinary 3-MH excretion.

The limitations of the [^{15}N]glycine method as a precise method of determining whole-body protein turnover have been extensively reviewed [28]. This method of determining protein kinetics may be effected by the route of feeding and the antecedant nutritional status of the subjects

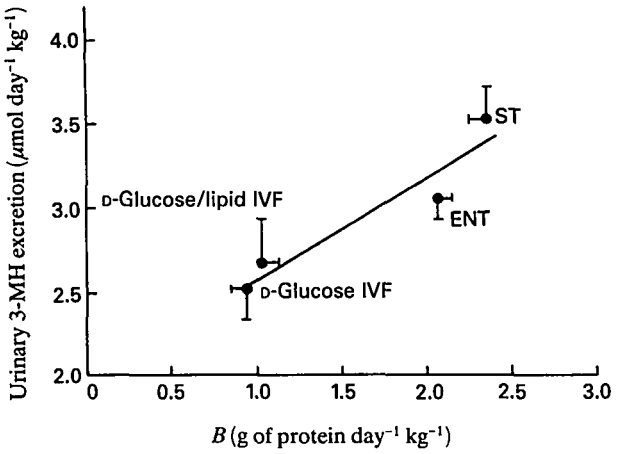

Fig. 1. Relationship of urinary 3-MH excretion to whole-body protein breakdown (B) as determined by the [^{15}N]glycine method. Data were obtained during four different nutritional conditions: high nitrogen enteral feeding (ENT, $n=11$), starvation (ST, $n=11$) and parenteral nutrition [D-glucose IVF ($n=6$) and D-glucose/lipid IVF ($n=5$)]. $r=0.98$, $P<0.05$.

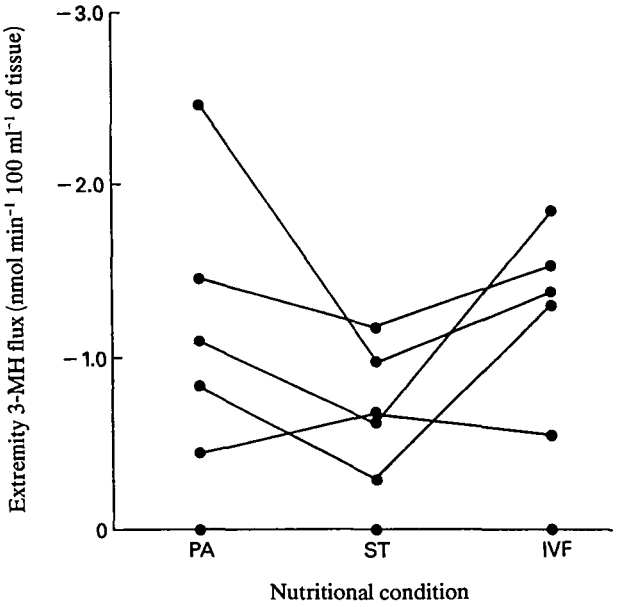

Fig. 2. Isolated resting extremity 3-MH efflux was determined during three different nutritional conditions: postabsorptive (PA), starvation (ST) and parenteral nutrition with the D-glucose regimen (IVF).

Table 6. Indirect calorimetric determination of resting energy expenditure and 24 h urinary nitrogen excretion

Results are means ± SE. Statistical significance: *$P<0.05$ vs starvation; †$P<0.05$ vs postabsorptive; ‡$P<0.05$ vs D-glucose. Abbreviation: REE, resting energy expenditure.

	Postabsorptive	Starvation	IVF	
			D-Glucose	D-Glucose/lipid
Vo_2 (l day^{-1} kg^{-1})	4.48 ± 0.26	3.92 ± 0.17	4.86 ± 0.2	4.33 ± 0.34
Vco_2 (l day^{-1} kg^{-1})	3.46 ± 0.31	2.66 ± 0.15†	4.74 ± 0.17*†	3.61 ± 0.24‡
REE (kcal day^{-1} kg^{-1})	21.50 ± 1.34	18.11 ± 0.8	24.26 ± 0.9*	21.34 ± 1.78
Urinary N (g day^{-1} kg^{-1})	0.113 ± 0.006	0.098 ± 0.007	0.178 ± 0.016*	0.188 ± 0.014*

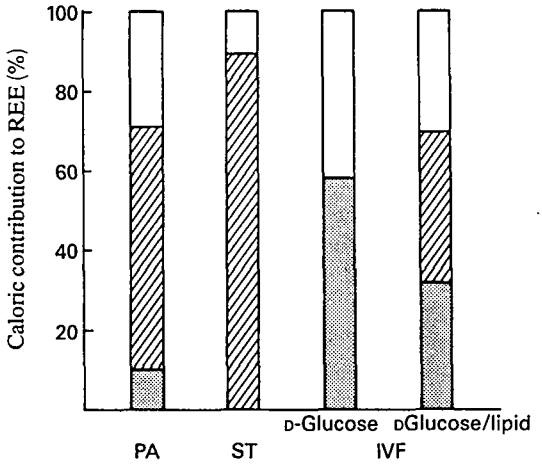

Fig. 3. Estimation of substrate caloric contribution to resting energy expenditure (REE) as calculated from the non-protein respiratory quotient. The percentage contribution of protein (□), fat (▨) and of carbohydrate (▩) to REE is shown. During D-glucose IVF, 6% of the glucose oxidized was utilized for fat synthesis (not shown). Abbreviations: PA, postabsorptive; ST, starvation.

[28]. By this method, parenteral feeding is associated with decreased whole-body protein turnover, synthesis and degradation [24]. Sim *et al.* studied protein turnover using [^{15}N]glycine in normal subjects and demonstrated a decrease in Q measured during IVF (2.79 g of protein day^{-1} kg^{-1}) as compared with enteral feeding (3.39 g of protein day^{-1} kg^{-1}). The reductions of both synthesis and breakdown during IVF were primarily attributed to diminished turnover of gastrointestinal lining cells and enzyme secretion [24]. However, that study was discontinuous and not designed to assess the impact of hospitalization and repletion IVF on protein kinetics. Fern *et al.* [29] and Garlick *et al.* [30] suggest that during parenteral feeding the partitioning of unlabelled nitrogen between urea-producing and ammonia-producing metabolites is altered as compared with enteral feeding. Thus we cannot exclude the possibility that the ^{15}N isotope methodology may contribute to the differences in protein flux between the groups fed by the enteral or parenteral route.

Both the [^{13}C]leucine and [^{15}N]glycine methods demonstrated significant decreases in breakdown in response to IVF but differed in determination of synthesis. The net anabolic effect of insulin in skeletal muscle occurs through an inhibition of protein breakdown and stimulation of synthesis [31]. Recently, Fukagawa *et al.* infused insulin and [^{13}C]leucine in postabsorptive subjects and observed an insulin-mediated dose-dependent reduction of leucine flux which occurred through a reduction of whole-body protein breakdown [32]. In the present study, the provison of D-glucose and amino acids resulted in a physiological hyperinsulinaemia, increased leucine flux, suppression of breakdown and stimulation of [^{13}C]-leucine-determined synthesis. However, the rate of synthesis determined using [^{15}N]glycine was not increased and nitrogen accrual occurred primarily through a decrease in whole-body protein breakdown during parenteral nutrition. Whole-body protein balance (calculated as $S - B$) during IVF differed between the [^{13}C]leucine (1.3 g of protein day^{-1} kg^{-1}) and the [^{15}N]glycine (0.7 g of protein day^{-1} kg^{-1}) methods. While the precise aetiology of this difference cannot be determined on the basis of the available data, methodological differences of plasma flux and end-product analysis would be anticipated. In part, the isotopes may more closely reflect different body pools, with the leucine method weighted towards skeletal muscle [33] and the glycine method weighted towards splanchnic or hepatic protein metabolism [28, 29]. These findings suggest the need for future investigation of organ-specific amino acid nitrogen kinetics.

3-MH is an amino acid derived from the breakdown of actin and myosin which is not subsequently oxidized or reincorporated into protein [34]. Extremity plasma flux of 3-MH has been used to assess peripheral tissue myofibrillar protein breakdown, whereas urinary 3-MH excretion represents the weighted average of 3-MH excretion from all myofibrillar-containing tissues (e.g. skeletal and smooth muscle, gut and dermis) [35]. We observed a correlation of urinary 3-MH excretion with whole-body protein breakdown as determined by [^{15}N]glycine, but no correlation of isolated extremity 3-MH flux with urinary 3-MH excretion. Our findings support earlier observations that urinary 3-MH excretion contains a significant non-skeletal muscle protein component [35, 36]. Rennie *et al.* observed no correlation of urinary 3-MH excretion with changes of extremity 3-MH efflux in postabsorptive patients after major surgery [35]. The present data extend these results: there is no apparent correlation of urinary 3-MH excretion with extremity 3-MH efflux during three different nutritional regimens. Moreover, the provision of adequate circulating nutrient and the associated hyperinsulinaemia does not specifically suppress peripheral tissue protein erosion as determined by extremity 3-MH flux. The mechanisms that mediate this persistent breakdown of peripheral tissue remain uncertain, but the failure of IVF to diminish peripheral tissue myofibrillar protein breakdown may be related to the effects of hospitalized immobility [27] or to the release of endogenous mediators during parenteral nutrition.

The limitations of indirect calorimetry as a method of estimating whole-body substrate oxidation during IVF have been reviewed [26, 37]. Resting energy expenditure and substrate utilization were significantly different between the D-glucose and D-glucose/lipid regimens (Fig. 3). During adapted starvation, fat utilization provided the primary caloric requirements. The provision of glucose as the non-protein caloric source during IVF stimulated an increase in $\dot{V}O_2$ and $\dot{V}CO_2$ in agreement with previous reports [37]. As might be expected, the preferential oxidation of glucose during hyperinsulinaemia is in contrast with the D-glucose/lipid group which utilized lipid and glucose in equicaloric amounts. These findings suggest that during unstressed intravenous nutritional repletion, substrate oxidation is dependent upon availability as well as the prevailing insulin levels during IVF.

In summary, this report characterizes the normal protein-metabolic response to parenteral repletion after hospitalized starvation. The present findings are consistent with a reduction of whole-body protein breakdown during repletion with parenteral nutrition. IVF administered during hospital ward conditions, however, did not preferentially suppress skeletal muscle protein breakdown, which suggests a potential role for additional anabolic stimuli during the delivery of parenteral nutrition.

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